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ABSTRACT BOOK

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1

SAFETY AND EFFICACY OF CO-ADMINISTERED DIETHYLCARBAMZINE, ALBENDAZOLE AND IVERMECTION DURING MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN HAITI

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Efficacy studies have shown that a single co-administered dose of diethylcarbamazine (DEC), albendazole (ALB) and ivermectin (IVM) is more effective eliminating *Wuchereria bancrofti* microfilariae (Mf) from the blood than DEC and ALB, which is currently used for mass drug administration (MDA). In Haiti, 23 communes still require MDA to eliminate lymphatic filariasis (LF) as a public health problem. In a clinical trial, we compared the safety and efficacy of a single dose of IVM (200 µg/kg) + DEC (6 mg/kg) + ALB (400 mg) vs. a single dose of DEC (6 mg/kg) +ALB (400 mg) administered to participants ≥ 5 years of age during an MDA in Haiti. Ten localities were randomized into two treatment arms. Participants were monitored for adverse events (AE), parasite antigenemia, and Mf. Antigen positive participants were tested for Mf and included a year later in an efficacy study. Overall, 3007 participants were included in the 3-drug arm and 3009 in the 2-drug arm. Fewer participants in the 3-drug arm (10.6%, 321/3007) experienced at least one AE compared to the 2-drug arm (16.3%, 491/3009, p<0.001). In both arms, most AEs were mild and consisted of headaches, dizziness and abdominal pain. Three participants included in the 2-drug arm developed serious AE that resolved within 48 hours. Before treatment, 8.0% (240/3006) of the participants in the 3-drug arm and 11.5% (346/3005, p<0.001) in the 2-drug arm were antigen positive. Of those, 17.6% (42/239) in the 3-drug arm and 21.5% (73/340, p=0.25) in the 2-drug arm were Mf positive. One year after treatment, 5.5% of the participants initially Mf positive in the 3-drug arm were still microfilaremic compared to 23.7% (p=0.02) in the 2-drug arm. Antigenemia persisted in 79.1% (159/201) of the participants tested in the 3-drug compared to 76.4% (217/291, p=0.24 in the 2-drug arm. In conclusion, the 3-drug therapy was more effective in eliminating Mf from the blood and fewer AEs were reported in the 3-drug arm than in the 2-drug arm during the study in Haiti. Effective MDA coverage with 3-drug therapy could accelerate the elimination of LF as a public health problem in the 23 communes still requiring MDA in Haiti.

2

FIRST COHORT STUDY TO INVESTIGATE THE ASSOCIATION BETWEEN ONCHOCERCIASIS AND EPILEPSY PROVIDES STRONG EVIDENCE FOR A CAUSAL RELATIONSHIP

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Previous meta-analyses on the relationship between onchocerciasis and epilepsy suggested that the two conditions are closely associated both at community and individual level. To tackle criticisms about the fact that these studies were cross-sectional, and that confounding factors might explain the relationship, we conducted a prospective cohort study to assess the incidence of epilepsy according to the initial *Onchocerca volvulus* microfilarial density (*Ov*-MFD). The study was conducted in the Mbam valley (Cameroon) where *Ov* MFD was measured in 1991-1993 in children by examination of skin snips. In 2017, seven villages with variable initial community microfilarial loads (CMFL) were revisited to gather information on the 858 children aged 5-10 years who had been examined in these communities in 1991-1993. Information on the occurrence of epilepsy between the initial parasitological survey and 2017 was collected from the subjects themselves or from their relatives, using a standardized "5-questions" questionnaire. Data on the history of epilepsy could be obtained from 85.2% of the 858 targeted subjects. In 2017, the overall incidence rate of epilepsy in the cohort subjects was 350 per 100,000 persons-years. Multivariable analyses taking into account age, sex, initial individual *Ov*-MFD, and initial CMFL in the village of residence, were performed. Using the individuals who had no *Ov* microfilariae (mf) in their skin in 1991-1993 as the reference group, the relative risks of having developed an epilepsy were 7.07, 11.26, 12.90, 20.00, 22.58, and 28.50 in subjects with initial *Ov*-MFD of 1-5, 6-20, 21-50, 51-100, 101-200, and >200 mf/skin snip, respectively. Individual *Ov* MFD at childhood was found to be strongly associated with the risk of developing subsequently epilepsy. This study adds a temporal dimension and demonstrates a dose-response effect supporting a causal relationship between onchocerciasis and epilepsy.

3

THE BURDEN OF SKIN DISEASE AND EYE DISEASE DUE TO ONCHOCERCIASIS IN AFRICA FOR 2015 AND 2025

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Onchocerciasis is now targeted for elimination through ivermectin mass drug treatment (MDA), after long-term morbidity control by the African Programme for Onchocerciasis Control (APOC: 1995-2015). Here we assess how this affected the prevalence and associated disease burden for 2015 and will affect that for 2025. We used the expanded disease framework within the individual-based mathematical model ONCHOSIM to estimate the prevalence of various onchocerciasis-associated clinical manifestations, focusing on onchocercal skin disease (OSD: severe itch, reactive skin disease (RSD), hanging groin, atrophy, and depigmentation) and onchocercal ocular disease (OOD: visual impairment, blindness). The model was quantified by reproducing pre-control associations between prevalence of disease and infection and age patterns. The distribution of pre-control mean mf prevalence per APOC administrative area ("project") was extracted from a published pixel-level onchocerciasis infection map. We simulated trends in prevalence of manifestations for various mf prevalences in 157 projects, accounting for project-level treatment history. Numbers of cases and disability-adjusted life years (DALYs) lost due to onchocerciasis were then calculated for each clinical manifestation per APOC project, each time point, and stratified by age, sex, and endemicity. The total number of cases with any type of OSD was estimated to be approximately 3,110,000 for 2015 (335,900 DALYs), and 370,500 for 2025 (40,000 DALYs). The total number of cases with vision loss was estimated to be approximately 234,700 for 2015 (18,500 DALYs), and 51,700 for 2025 (2,900 DALYs). Out of all OSD cases in 2025, 15% from a

chronic disease, and 85% from an acute clinical manifestation (i.e. severe itch, RSD). By 2025, vision loss remains mainly a problem for savanna areas (96% of all vision loss). By 2025, most of the onchocerciasis burden is expected to be in areas of DRC and Nigeria (39% of total DALYs). These areas might benefit most from alternative strategies or more efficient targeting of patient-based treatments, given the limited human and financial resources available.

4

LYMPHATIC FILARIASIS ELIMINATION IN AMERICAN SAMOA - HOUSEHOLD CLUSTERING OF SEROPOSITIVE PERSONS AND IMPLICATIONS FOR SURVEILLANCE STRATEGIES

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Under the Global Programme to Eliminate Lymphatic Filariasis, American Samoa made significant progress toward interrupting transmission of lymphatic filariasis (LF) by conducting seven rounds of mass drug administration (MDA) from 2000-2006, and passing WHO recommended Transmission Assessment Surveys (TAS) of Grade 1 & 2 school children in 2011 & 2015. As antigen (Ag) prevalence drops to very low levels at the end stages of elimination efforts, detection of residual infected persons and/or *Foci* of ongoing transmission becomes increasingly challenging. WHO has therefore highlighted the need for cost-effective and innovative post-MDA surveillance strategies for identifying residual infections and high-risk groups. In 2016, we conducted a TAS of Grade 1 & 2 (mostly 6-7 year old) school children in American Samoa, in parallel with a cluster survey of community members aged ≥8 years in 30 villages. To determine if household members of Ag-positive school children were a high-risk group, and therefore a potential focus for targeted surveillance, we compared Ag prevelance in household members aged ≥8 years with results from the community survey. The Alere Filariasis Test Strip™ was used to detect circulating filarial Ag. Of 1143 school children and 2507 participants in the community survey, 9 (0.8%, 95% CI 0.4-1.5%) and 102 (4.1%, 95% CI 3.3-4.9%) were Ag-positive, respectively. Of the 65 eligible household members, 58 (89%) were tested and 12 (20.7%, 95% CI 11.2-33.4%) were Ag-positive; Ag-positive household members were identified in 5 (55.6%) of the 9 households. Our findings indicate that household members of Ag-positive school children have a significantly higher Ag prevalence than the general community (chi^2 test, $p<0.001$) and should be considered for targeted surveillance. Considering the significant household-level clustering and the highly focal nature of LF transmission, future studies should explore the utility of snowball sampling of household members and near neighbours of Ag-positive persons, including those identified through opportunistic surveillance strategies such screening programs at work sites or health clinics.

5

RIVER BLINDNESS IN TOGO: PERFORMANCE OF THE OV-16 RAPID DIAGNOSTIC TEST AND THE ELISA IN PREVIOUSLY HYPERENDEMIC *FOCI*

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Decisions to stop mass drug administration (MDA) to eliminate onchocerciasis are based on evidence of interruption of parasite transmission, such as absence of antibody to Ov-16 in children. In 2014, a rapid diagnostic test (RDT), became commercially available that can be performed in the field using blood from a finger prick and can provide real-time results, compared to the currently accepted enzyme-linked immunosorbent assay (ELISA). Here we compare performance of the RDT to ELISA in two previously hyperendemic districts in Togo that underwent >20 years of MDA. In each selected village in Kozah and Yoto districts, we randomly sampled households. For each household, we selected 1 individual from ≤5, 6-10, 11-15, 16-20, and ≥21 year-old age groups, with a goal of obtaining 200 individuals per age group in each district. All participants provided blood specimens tested for Ov16 antibody by RDT (SD Bioline) and ELISA (CDC protocol). We adjusted seroprevalence estimates with sampling weights accounting for non-response and numbers of individuals by age group per household. We enrolled 1015 participants in Kozah and 980 in Yoto. The adjusted proportion of seropositive participants i n the youngest three age groups (0-15 years old) ranged from 0-1% by RDT and 0-7% by ELISA in Kozah and 0-2% by RDT and 0-3% by ELISA in Yoto. In the two oldest age groups, respective proportions were 1% and 9% by RDT, 4% and 28% by ELISA in Kozah; 5% and 9% by RDT, 7% and 22% by ELISA in Yoto. The overall adjusted proportion positive was 3.0% (95% confidence interval [CI] 0.7-5.3%) by RDT and 10.5% (95% CI 6.8-14.2%) by ELISA in Kozah and 4.8% (95% CI 2.0-7.7%) by RDT and 11.1% (95% CI 7.1-15.2%) by ELISA in Yoto. Thus, RDT sensitivity ranged from 28.6% to 43.2% in various age groups. We observed lower estimated seroprevalence using RDTs compared to ELISA, suggesting that the RDT may under-detect the number of individuals with exposure to Onchocerca volvulus in low prevalence settings. Negative results by RDT would need to be confirmed by ELISA or sample sizes would need to be substantially increased to compensate for the low sensitivity in these settings.

6

DETECTION OF LYMPHATIC FILARIASIS ELIMINATION THRESHOLDS IN PAPUA NEW GUINEA

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This study was designed to evaluate human markers of lymphatic filariasis infection and transmission in 14 communities in East Sepik Province of Papua New Guinea during a period when transmission was expected to cease. The site had received 5 rounds of mass drug administration (MDA) in the 1990's, vector control with insecticidal bednets from 2008 to present, and renewed MDA starting in 2015. This study collected finger prick blood samples between 10pm and 2am before and after annual MDA from 2015 to 2018. Samples were processed for microscopy to detect circulating microfilaria (MF) and serological assays. The site was characterized by western communities with historically higher entomological indicators of transmission and eastern communities with lower transmission (and mathematically predicted to achieve transmission cessation). MF prevalence decreased from 18.0% to 8.1% and 2.0% after one and two MDAs in western communities and from 1.4% to 0.8% and 0.4% in the eastern communities. Among the 56 MF-positive individuals followed longitudinally, 22 and 48 converted to MF-negative after one MDA and two MDAs, and only one individual followed longitudinally converted to MF positive after receiving MDA. An additional 10 MF-positive individuals were detected in annual surveys following the start of community-wide MDA. Only one sentinel-age child (<10 years of age) remained MF positive after two MDAS. However, antigen prevalence detected by Filarial Test Strips (FTS) remained high across the study site with 40.6% FTS positive in western communities and 7.4% positive in

eastern communities. Furthermore, 63.4% and 26.9 % of the FTs positive individuals exhibited moderate (>1+) and 26.9%high antigenemia (3+) on the semi-quantitative value of FTS result. These results suggest that elimination targets based on epidemiological criteria of MDA success (< 1% microfilaremia or < 2% antigenemia) have not yet been met across this study site. Additional serological assays (BM14, WB123, and Og4C3) are currently being processed to decipher markers of exposure and infection across these subpopulations before and during this MDA program.

7

TIME FOR DIAGNOSTIC TOOLS THAT ACCURATELY DETECT *ONCHOCERCA VOLVULUS* IN ONCHOCERCIASIS ELIMINATION PROGRAMS IS NOW

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Simulium fly dissections is still being used as a method of determining infection and infective rates of *Onchocerca volvulus*. However, these same *Simulium* vectors are likely to be infected with other domestic or wild animals *Onchocerca* species such as *O. ochengi or O. ochengi 'Siisa'* type in cattle, and *O. ramachandrini* in Warthogs as demonstrated by Unnasch (2017) in a study in northern Uganda. Dissections can therefore lead to overestimation of *O. volvulus* in *Simulium* flies. For this reason, entomological assessment in the 2016 WHO guidelines for verification of onchocerciasis elimination require the use of specific O-150 polymerized chain reaction (PCR) tests for *O. volvulus*. The national onchocerciasis elimination program in Uganda used *Simulium* dissections in parallel with PCR in Kiryandongo District, Uganda. This gave us an opportunity to compare those results, together with and OV16 ELISA data from the same areas. A total of 830 *Simulium* flies from two fly collection sites along the River Nile were collected. Dissection results showed a parous rate of 45% (374), and an infection rate among the parous flies of 7 % (infective rate, 6%) indicating a very high transmission rate of onchocerciasis. However, PCR (20 pools, 200 flies) was negative (upper bound of the 95% CI 1.9/2000 flies carrying L3). Dry blood spots (DBS) from 3,302 young children analyzed with OV 16 were also negative. We concluded that the positive results from dissected flies were likely of *Onchocerca* species associated with domestic or wild animals. In the current elimination era, it is imperative to apply diagnostic tools that accurately detect *Onchocerca volvulus*. This calls for endemic countries to establish means for serological and PCR testing for accurate verification of onchocerciasis elimination.

8

SPECIFIC GUT MICROBIAL PROFILES ARE ASSOCIATED WITH ROBUST IMMUNE RESPONSES TO KILLED, WHOLE-CELL CHOLERA VACCINE

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Killed, whole-cell cholera vaccines elicit immune responses of variable amplitude and kinetics. Limited data suggests that the gut microbiome may explain variation to mucosal vaccine responses. To evaluate for this interaction in an endemic area, we used 16S rRNA sequencing of stool and serum vibriocidal titers to examine the gut microbiome and immune responses to vaccination at day 0, 7, 17 and 44 in adult vaccine recipients in Dhaka, Bangladesh. Vibriocidal titer magnitude and kinetics were used to classify participants. Gut microbial diversity and bacterial phylogenetic groups were not significantly changed after vaccination. Within 17 days of vaccination, 86/89 (96%) adults developed a 4-fold rise in vibriocidal titer. The speed to seroconversion (fourfold increase in vibriocidal titer by day 3 after vaccination) was correlated to individuals with greater bacteria from the genus Prevotella (multivariate analysis using linear models, q value 0.04). The gut microbiome of participants with higher peak vibriocidal titers was characterized by increased Prevotella (3% vs <0.1% of the total microbiome, p<0.001 unpaired t test, linear discriminant analysis score >3.5), particularly the species *Prevotella copri* (p<0.001, unpaired t test, linear discriminant analysis score >3.5). In animal models, lipopolysaccharide from mucosally derived Prevotella species have been found to increase vaccination-associated antigen-specific antibody titers and influence antigen specific CD4 T cell clonal expansion. The biological interaction between mucosal vaccines and the gut microbiota, and potential modulation of immune responses, warrants further study.

9

LONG-TERM EFFECTIVENESS OF KILLED BIVALENT WHOLE CELL ORAL CHOLERA VACCINE IN HAITI - FOUR YEAR DATA

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The two long-term efficacy trials of vaccination with a killed oral cholera vaccine (OCV) reported conflicting results. In addition, no study of long-term protection following killed OCV has been conducted outside of the historically cholera-endemic areas of South Asia. Gavi, the vaccine alliance has evaluated the investment case for OCV based on a need to revaccinate after two years. We examined the duration of protection of the standard two-dose regimen and a single-dose regimen up to four years following vaccination with OCV in Haiti. In the setting of two-dose vaccination campaigns with a killed bivalent whole cell (BivWC) OCV, we conducted a case-control study from October 2012 through November 2016. Cholera cases had a positive stool culture and were recruited from cholera treatment centers. Community controls were matched to cases by age group, time, and neighborhood. We conducted adjusted matched regression analyses to calculate vaccine effectiveness and examine heterogeneity in effectiveness over time. In analyses including 178 cases and 706 controls, we found no evidence that two-dose effectiveness decreased during follow-up. In adjusted analyses, the average cumulative four-year effectiveness for two doses was 76% [95% CI: 59% to 86%]. In contrast, single-dose effectiveness decreased over time in a log-linear fashion, with a predicted vaccine effectiveness of 79% at the end of 12 months [95% CI: 43% to 93%], which declined to zero before the end of the second year. In a setting of epidemic and newly endemic cholera, we found consistent, lasting protection with two doses of BivWC OCV up to four years post-vaccination, supporting its critical role in a comprehensive public health response to cholera, and suggesting that vaccine investment analyses should longer duration of protection in cost-effectiveness analyses.

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PROJECTING THE HEALTH IMPACT AND COST-EFFECTIVENESS OF ORAL CHOLERA VACCINE INTRODUCTION IN AFRICA

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The World Health Organization's Global Task Force on Cholera Control (GTFCC) declared a commitment to reduce worldwide cholera deaths by 90% by 2030. The proposed Global Roadmap presents a multi-sectoral

strategy for intervening in cholera transmission hotspots with water sanitation and hygiene (WaSH), improvements to infrastructure and surveillance, community engagement, and routine mass oral cholera vaccine (OCV) campaigns. Building upon previously published high-resolution maps of cholera incidence, we projected the potential impact and cost-effectiveness of multiple OCV vaccination campaign targeting strategies in 43 countries in sub-Saharan Africa from 2018-2030. Realistic strategies targeted high incidence districts every three years with estimates of global vaccine availability based on forecasts from manufacturers and recent trends; best- and worst-case targeting strategies (targeting high incidence 20x20 km grid cells and no targeting, respectively) were also performed to demonstrate the upper and lower limits of impact. We then estimated the averted cases, deaths, disability-adjusted life-years, and cost-effectiveness of each strategy using phenomenological models that account for the direct and indirect vaccine effects and population projections over time. Sensitivity analyses for baseline cholera incidence projections, vaccine effectiveness and waning immunity, and frequency of campaigns in a given location were used to identify the factors most important for health impact. In models with 23-58 million vaccines available annually, we found that an untargeted strategy performed barely better than a model without vaccination. On the other hand, strategies targeting high incidence districts may avert 344,000-366,000 cholera cases from 2018-2030, representing 25-43% of the impact of the optimal, yet impractical, best-case targeting strategy. Averting a single cholera case with this strategy required 650-750 individuals to be fully vaccinated. Our findings may inform policy characterizing the health impact and cost-effectiveness of cholera interventions in advance of implementation.

11

IMPACT OF CD4 T CELL RESPONSES ON CLINICAL OUTCOME IN A HUMAN CHALLENGE MODEL OF *VIBRIO CHOLERAE*

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Vibrio cholerae (*V. cholerae*) is the causative agent of cholera and remains a significant public health threat resulting in an estimated 1.3 to 4 million cases of diarrhea and greater than 100,000 deaths annually. Furthermore, cholera can occur in large, explosive outbreaks and pandemics resulting in considerable strain on health systems. Although there are vaccines licensed to combat cholera, including Vaxchora in the US, there remains limited information regarding the immunological mechanisms by which they confer protection. Due to the non-invasive nature of this pathogen, antibodies are presumed to be a major determinant of protection. However, the T cell responses that are associated with providing B cell "help" and inducing long-term humoral responses have not been explored. Using peripheral blood mononuclear cells (PBMC) from volunteers immunized with Vaxchora and/or challenged with wild-type *V. cholerae*, we have investigated the potential role of CD4 T cells, in particular circulating T follicular helper cells (cT_{fh}), in clinical outcome. We identified the presence of activated cT_{fh} (expressing the activation molecule CD154 and/or producing IL-2) with gut-homing potential (expressing integrin $\alpha4\beta7$) prior to immunization. Moreover, higher percentages of *V. cholerae*-responsive cT_{fh} with gut-homing potential prior to immunization were associated with lower cumulative stool output following challenge. We further demonstrate that volunteers who did not develop moderate-to-severe cholera diarrhea had significantly higher production of IL-2 by both gut-homing cT_{fh} and CD4 T effector memory cells (T_{EM}) prior to challenge. In conclusion, these results indicate a likely role for cT_{fh}, particularly those with gut-homing potential, in the induction of protection against moderate-to-severe cholera diarrhea.

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HUMANS RECOVERING FROM CHOLERA IN BANGLADESH DEVELOP ANTIBODIES AGAINST THE O-SPECIFIC POLYSACCHARIDE (OSP) OF *VIBRIO CHOLERAE* O1 THAT INHIBIT *V. CHOLERAE* MOTILITY: A POSSIBLE MECHANISM OF PROTECTION AGAINST CHOLERA

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The mechanism of protection against cholera afforded by previous illness or vaccination is currently unknown. We have recently shown that antibody responses targeting the O-specific polysaccharide (OSP) of *V. cholerae* correlate highly with protection against cholera. *V. cholerae* is a motile bacterium that has a single polar flagellum, and motility of *V. cholerae* highly correlates with virulence. The *V. cholerae* flagellum is sheathed and therefore covered by OSP. We hypothesize that protection against cholera may be mediated by anti-OSP antibodies that inhibit *V. cholerae* motility. To address this, we purified IgG, IgA, and IgM fractions from 10 humans recovering from cholera in Bangladesh. We also analyzed human monoclonal anti-OSP antibodies recovered from plasmablasts of patients with cholera. We characterized these fractions and antibodies for anti-OSP, vibriocidal, and agglutinating activity. Using high-speed video microscopy, we showed that at subagglutinating and agglutinating antibody concentrations that these antibodies impede *V. cholerae* motility, that this effect is blocked by adsorbing convalescent sera with purified OSP, and that Fab fragments of these antibodies did not affect *V. cholerae* motility, suggesting a requirement for antibody mediated crosslinking in antimotility action. Using *in vivo* competitive index studies in mice and a motile rough *V. cholerae* mutant lacking OSP, as well as a non-motile *V. cholerae* mutant expressing OSP, we showed that antibodies impeded survival of *V. cholerae in vivo*, and that this impact was OSP- and motility-dependent. These results are the first to show that humans recovering from cholera develop an antibody response that blocks *V. cholerae* motility. Since *V. cholerae* motility is required for virulence, our results strongly suggest a mechanism of protection against cholera.

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EPIDEMIOLOGY OF CHOLERA IN ZANZIBAR: IMPLICATIONS FOR THE ZANZIBAR CHOLERA CONTROL AND ELIMINATION PLAN

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Zanzibar is a semi-autonomous region of the United Republic of Tanzania consisting of two main islands, Unguja and Pemba. Frequent cholera epidemics pose a major threat to Zanzibar's economic development since at least 1978 and have repeatedly disrupted local public health services. To inform a multi-sectoral cholera elimination plan currently under development, we summarize the epidemiology of cholera in Zanzibar from 1997 to 2017 using detailed epidemiologic, environmental, and social data. From 1997 to 2017, 11,921 suspected cholera cases were reported across 87% of Zanzibar's shehias representing an average incidence rate of 4.4 per 10,000/year. The geographic distribution of cases across outbreaks was highly variable although a number of high-burden areas were identified. We found that on both islands, relative risk of cholera infection in a single outbreak was poorly correlated with the average relative risk across all other outbreaks (0.13 in Unguja and 0.18 in Pemba), making it difficult to target cholera control activities on the neighborhood level. If shehias are sequentially targeted based on historic cumulative incidence, targeting just 4.7% (IQR 1.6-10.5 in hold-out analyses) of Pemba's population living in 4 (IQR 2-11) shehias with an ideal cholera prevention package could avert 50% of future cases. However, 20.8% (IQR 15.5-24.6) of Unguja's population coming from 36 (IQR 20-36) shehias would need to be perfectly targeted in order to avert 50% of future cases on the island. Using flexible regression models, we found that outbreaks were highly seasonal, providing a clear window for annual preparedness activities. The risk of reporting cases at the start of two rainy periods in May and December was 2.6 times (95% CI 1.6-4.1) and 1.8 times (95% CI 1.1-3.0) higher than the mean risk throughout the year. Rainfall in the previous seven days had a significant positive relationship with daily reproductive number on both islands.

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BIOMARKERS OF ENVIRONMENTAL ENTEROPATHY ASSOCIATE WITH IMMUNOGENICITY OF THE BIVALENT ORAL CHOLERA VACCINE IN HAITIAN CHILDREN

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Environmental enteropathy (EE) is a condition that is prevalent among children in low-income countries with chronic exposure to intestinal pathogens due to poor sanitation and water security. The disorder refers to inflammation and structural changes of the gut, leading to intestinal permeability and lack of nutrient absorption. The presence of EE in children associates with lower immunogenicity of the oral polio and rotavirus vaccines. Currently, a lack of interventions and non-invasive diagnostic tests limits our understanding and treatment of this condition. To determine whether there is an association between EE and immunogenicity of the bivalent whole cell oral cholera vaccine ((BivWC)), Shanchol, we examined a cohort of 98 Haitian children who received two doses of BivWC 14 days apart. We measured plasma antibody responses to cholera antigens at baseline and 7 days after each dose. We measured the following EE markers at baseline (Day 0): plasma C-reactive protein (CRP), alpha-1-acid glycoprotein (AGP), ferritin, soluble CD14 (sCD14), intestinal fatty acid binding protein (I-FABP) and endotoxin-core antibody (EndoCAb). Through a multivariate linear regression analysis, we identified associations between vibriocidal antibody titer after vaccination to markers of EE. Age and blood-type O were included in the model as possible confounders. There was no collinearity between the EE markers. We found significant associations between markers for EE to vibriocidal fold change after vaccination; Vibriocidal Inaba D21-AGP (P=0.002); Vibriocidal Ogawa D21- AGP (P=0.04); Vibriocidal Ogawa D21- CD14 (P=0.04); Vibriocidal Ogawa D21- Ferritin (P=0.03). Our findings suggest that EE impacts the immunogenicity of the BivWC oral cholera vaccine.

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ENDOTOXIN AT THE MATERNAL-FETAL INTERFACE IS ASSOCIATED WITH ELEVATED EXPRESSION OF TISSUE INHIBITOR OF METALLOPROTEINASES

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Tightly regulated invasion of trophoblast cells into maternal decidua is essential for normal placentation in humans. We have previously shown trophoblasts to be responsive to schistosome egg antigen, demonstrating increased cytokine production and decreased invasion rates. We have also demonstrated that schistosomiasis is associated with elevated endotoxin at the maternal-fetal interface (MFI). We hypothesized that expression of proteins involved in extracellular matrix remodeling, namely tissue inhibitor of metalloproteinases (TIMPs) may be reduced at the MFI when complicated by schistosomiasis. Herein, we utilized samples from pregnant women who participated in a trial designed to evaluate the safety of praziquantel treatment during pregnancy. All women were positive for *S. japonicum* at enrollment and randomized to receive praziquantel or placebo. We examined levels of endotoxin, cytokines and TIMPs in blood from the MFI taken immediately following placental bed biopsy. Neither praziquantel nor infection with soil transmitted helminths (*A. lumbricoides*, *T. trichiura*, hookworm) impacted expression of TIMPs in blood from the MFI. High endotoxin expression was associated with elevated TIMP-1 and TIMP-3 at the MFI ($P<0.01$), after controlling for gestational and maternal age. Pro-inflammatory cytokine production (e.g. IL-1, IFNγ, IL-8, TNFα) was also correlated with elevated TIMP levels at the MFI ($P<0.02$). Further, TIMP-1, -2 and -3 levels were lower in mothers with iron deficiency anemia ($P<0.01$), in keeping with studies that have demonstrated increased trophoblast invasion among anemic women. In summary, an expression pattern of heightened TIMPs, observed in the context of elevated endotoxin and/or pro-inflammatory cytokines, suggests decreased invasion at the MFI, and may help explain the observed association between placental endotoxin and small-for-gestational-age newborns we have previously demonstrated in this cohort. These data provide a candidate mechanism by which chronic inflammation at the MFI may impact the invasion characteristics of trophoblasts and thus the establishment of a healthy placenta.

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INTRODUCING *SCHISTOSOMA MANSONI* AMP-ACTIVATED PROTEIN KINASE (AMPK): DEVELOPMENTAL REGULATION, ACTIVITY, AND ROLE IN SCHISTOSOME ENERGY METABOLISM

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Schistosomes are obligate parasites and auxotrophic for many critical biomolecules, such as such as fatty acids and cholesterol, which must be acquired from the host in order to sustain parasite growth and development. Furthermore, schistosome development requires other signals from the host, as parasite growth and reproductive capacity are significantly attenuated in certain lines of gene-targeted mice. We previously showed that impaired growth and reproduction of *Schistosoma mansoni* in immunodeficient mice correlated with reduced expression and activity of the parasite cAMP-dependent protein kinase (PKA), a cellular protein involved in metabolic regulation. As schistosomes use a combination of glycolysis, fatty acid oxidation and oxidative phosphorylation to meet their bioenergetic needs and support the daily production of hundreds of eggs, we hypothesized that schistosomes from immunodeficient mice might also exhibit alterations in signaling

pathways and enzymes that more directly regulate energy metabolism. First, we show that adult schistosomes express an AMP-activated protein kinase (AMPK), a heterotrimeric enzyme that is central to regulating energy metabolism in other eukaryotes. Second, we provide evidence that expression of the catalytic alpha subunit is developmentally regulated during the parasite's life cycle. While there is evidence of AMPK in all developmental stages, our data indicate that transcription of the AMPK alpha gene is shut off in cercariae and is re-initiated once inside the mammalian host. Third, we show that schistosome AMPK activity is sensitive to changes in the worm's environment, suggesting a mechanism by which schistosome metabolism may be responsive to host factors. Finally, we provide evidence that AMPK expression is attenuated in parasites isolated from immunodeficient mice, suggesting the host exerts significant influence over the regulation of schistosome energy metabolism. Elucidating how schistosome energy metabolism is regulated and how host factors influence parasite energy metabolism may reveal opportunities to disrupt transmission of these important pathogens.

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TARGETING THE REDOX NETWORK FOR DRUG DISCOVERY FOR SCHISTOSOMIASIS

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Praziquantel is the only drug available for schistosomiasis treatment. The FAD/NAD linked enzyme thioredoxin glutathione reductase (TGR) is central to the redox network of these worms and has been determined to be essential and druggable. We have screened >500,000 small molecules for TGR inhibition and have identified >10,000 actives. Progress to identify potential lead compounds in these actives will be discussed. 3D structures of small molecules in complex with TGR has identified an allosteric binding site for inhibitor development. Compounds binding this site perturb the catalytic mechanism. Related FAD/NAD linked reductases, targets for malaria, trypanosomiasis, leishmaniasis, and cancer drug development, have similar catalytic mechanisms and binding pockets. The topology/charge distribution in this binding site is not highly conserved, suggesting ways to develop enzyme-specific inhibitors for drug development for many diseases in addition to schistosomiasis.

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USING A GLYCAN MICROARRAY TO IDENTIFY DOMINANT ANTI-GLYCAN IGE RESPONSES IN GHANAIAN SCHOOL-CHILDREN LIVING IN A HELMINTH-ENDEMIC AREA

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In helminth-endemic areas, elevated levels of cross-reactive IgE to environmental and food allergens are often observed that do not translate into allergy symptoms. In Ghanaian children, this cross-reactivity has been shown to be associated with *Schistosoma haematobium* infection and dominated by elevated levels of IgE against the sugar structures on glycoproteins known as cross-reactive carbohydrate determinants. The aim of the current study was to characterize the specific carbohydrate motifs involved in this IgE recognition in Ghanaian children using a synthetic glycan microarray. The target population was schoolchildren living in rural and urban areas with different socioeconomic status (SES). Sera from children attending a rural school (n=20), an urban low SES school (n=20) and an urban high SES school (n=20) were used to assess IgE responses against 128 synthetic N-glycans and short oligosaccharides by microarray. The highest levels of IgE to glycan structures were observed among rural children followed by urban low SES and urban high SES children. Analysis of specific N-glycan entities showed that IgE responses were predominantly directed to structures with a core α-1,3-fucose motif alone and to a lesser extent, to those with core xylose only. *S. haematobium* infection was mainly observed in urban low SES subjects and was significantly associated with elevated IgE to structures with core xylose. Glycan microarray technology represents a novel approach to characterize IgE responses to carbohydrate motifs that could complement existing *in vitro* immunoassay technologies and clinical evaluation.

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UROGENITAL SCHISTOSOMIASIS IS ASSOCIATED WITH WIDESPREAD IMPACTS ON THE ADOLESCENT INTESTINAL MICROBIOME

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Urogenital schistosomiasis is a neglected parasitic disease endemic to Nigeria, affecting millions across Africa. Given previous work indicating that helminth infection can impact immune responses and even the composition of the intestinal microbiome, we examined the impacts of infection with *Schistosoma haematobium* on the gut microbiome of adolescents in Kebbi State in Nigeria. We sequenced the hypervariable V4 region of the 16S rRNA gene of the faecal microbiome of infected and uninfected participants, and analyzed whether there were diversity or taxonomical differences in the microbial community composition. While the overall richness and evenness of the infected individuals was similar to controls, we detected an increased number of observed taxa, decreased phylogenetic diversity, and differential beta diversity in infected individuals resulting from decreased prevalence of Firmicutes and increased prevalence of Proteobacteria. More specifically, we detected a number of changes in taxa reminiscent of changes seen in inflammatory bowel disease in adolescents infected with *S. haematobium*, including increases in members of Bacteroides, Veillonellaceae, Pasteurellaceae, and Desulfovibrio and decreases in Clostridiales; these changes were confirmed using qPCR. Functional potential analysis also revealed an enrichment in urease producers, which past studies have linked to dysbiosis and inflammation.Overall, our analysis indicates that *S. haematobium* infection state is associated with widespread perturbations in the gut microbiota.

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BETA GLUCAN, IRI-1677, MODULATES TH2 INDUCED PATHOLOGY, INFLAMMATION AND FIBROSIS IN A MURINE MODEL OF SCHISTOSOMIASIS

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Beta glucans are Pathogen Associated Molecular Pattern, or PAMP, molecules that trigger a coordinated immune response to pathogens. They can induce a variety of responses, including the ability to shift an overstimulated Th2 immune response to a Th1 response. Because Schistosoma mansoni (Sm) infection in mice is associated with a Th2-dominated inflammatory response that drives liver pathology (and fibrosis)

we examined the effects of a purified yeast beta 1,3/1,6 glucan, IRI-1677, for its modulatory effects on pathology, inflammation, and concomitant fibrosis in murine Sm infection. C57BL/6 mice were infected with (Sm) cercariae. Five weeks after infection, mice were treated with biweekly doses of IRI-1677 for a total of 4 weeks and then sacrificed. IRI-1677, in a dose dependent manner, was able to dramatically suppress the expression of genes (assessed by Nanostring) in the liver that target IL-13 (a key cytokine regulating fibrosis in murine schistosomiasis). In particular, IRI-1677 -treated Sm-infected mice were able to down regulate IL-13Ra2, Fizz 1 and Col6a1 while upregulating Th1/Th17-associated cytokines compared to IRI-1677-untreated Sm-infected mice. The IRI-1677-induced changes in cytokines in Sm-infected mice were paralleled by alterations in the makeup of liver tissue inflammation with the beta glucan driving neutrophil and inflammatory monocyte infiltration compared to the eosinophil and M2-macrophage rich environment seen in untreated Sm-infected mice. Gross pathology was also markedly different between IRI-1677-treated and -untreated Sm-infected mice. We are currently assessing markers (tissue hydroxyproline) of fibrosis, inflammation and pathology to understand whether balancing the effect of chronic robust TH2 responses in this parasite model with IRI-1677 administration can be used to limit the pathologic consequences of infection with Sm.

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THE INTERLEUKIN-4 INDUCING PRINCIPLE FROM *SCHISTOSOMA MANSONI* EGGS (IPSE) EXACERBATES UTI-INDUCED PAIN AND SUPPRESSES ANTI-MICROBIAL PEPTIDE PRODUCTION

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Numerous parasitic infections can increase susceptibility to bacterial co-infections. This appears to be true for urogenital schistosomiasis and bacterial urinary tract co-infections (UTI). In a 2014 Infection and Immunity paper, we reported that this co-infection is immunologically facilitated by *Schistosoma haematobium* eggs triggering interleukin-4 (IL-4) production, which in turn ablates NKT cell activation-associated bacteria clearance. We sought to determine how *S. haematobium* eggs induce this IL-4-dependent response. The interleukin-4 inducing principle from *Schistosoma mansoni* eggs (IPSE) is one of the most abundant schistosome egg-secreted proteins, and is a molecule that binds to IgE on the surface of basophils and mast cells to trigger IL-4 release. IPSE is also able to translocate into host nuclei using a nuclear localization sequence (NLS) to modulate host gene transcription. We hypothesized that IPSE is the factor responsible for the ability of *S. haematobium* eggs to worsen UTI pathogenesis. Mice were intravenously administered a single 25ug dose of recombinant *S. haematobium*-derived IPSE, an NLS mutant of IPSE or PBS and 24 hours later challenged with the uropathogenic *E. coli* strain UTI89 by urethral catheterization. We verified that a single intravenous dose of IPSE induced IL-4 production by basophils. Unexpectedly, we did not observe significant differences in urine bacterial CFU. Even more surprisingly, IPSE worsened UTI-induced pain. The NLS mutant induced even worse outcomes. Moreover, IPSE administration resulted in significant reduction in the expression of anti-microbial peptides. Our data show that IPSE may play a major role in *S. haematobium* induced UTI exacerbation, albeit in an unexpected fashion. These findings also indicate that IPSE either works in concert with other IL-4-inducing factors to increase susceptibility of *S. haematobium*-infected hosts to bacterial co-infection, or does not play a role in these co-infections.

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EVIDENCE CONSISTENT WITH SCHOOL BASED TRANSMISSION OF *TAENIA SOLIUM* CYSTICERCOSIS IN PRIMARY SCHOOLS, SOUTHWEST CHINA

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Cysticercosis is caused by larval forms of the pig tapeworm, *Taenia solium*, invading human tissue. The disease is spread person-to-person via a fecal oral route from tapeworm carriers shedding eggs in their feces. We characterized exposure and the relationship of infection to social networks in primary school children in southwest China. We surveyed 3,038 students across 27 schools in southwest China, drawing blood for *T. solium* cysticercosis IgG antibody testing by ELISA. We collected social network data in one school study site (362 children), speciating tapeworms in taeniasis cases and detailing social networks by having students identify peers sharing their same classroom, dorm, and dining table as well as frequent playmates and children with whom they frequently shared food. 6% of children (180/2867) had serologic evidence of cysticercosis IgG antibodies, the proportion of seropositive children in different schools ranged from 0% to 22%. 11% (283/2606) of children reported having worms or worm segments in their feces, with a range of 0% to 28% in different schools. In the social network study, 4% of the school population had *T. solium* GI carriage (16/362), with the highest prevalence found in 9 year olds (3rd, graders, 15%, 6/39) and 8 year olds (2nd graders, 8%, 3/38). 22% (9/41) of seropositive children had identifiable school based close contacts with tapeworm carriers consistent with school based transmission. Students attending schools with a higher proportion of self-reported taeniasis were more likely to have serologic evidence of cysticercosis antibodies ($p = 0.017$; odds of seropositivity increases 1.13 [95% CI 1.01 - 1.25] with a 5% increase in school prevalence). Social connections between infectious tapeworm carriers and students with cysticercosis antibodies as well as the association between seropositivity and the proportion of children with taeniasis suggests potential transmission of cysticercosis within schools. School based interventions including improving school hygiene measures as well as school based treatment of taeniasis carriers may play a role in decreasing disease burden in school-aged children.

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INTRODUCING CYSTIAGENT: AN AGENT-BASED MODEL TO SIMULATE *TAENIA SOLIUM* TRANSMISSION IN PERU

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Persistent endemic transmission of the pork tapeworm, *Taenia solium*, causes widespread neurological disease (neurocysticercosis) and a significant economic burden on low-income rural populations across Latin America, Asia and Africa. The past 20 years have seen important advances in the development of effective control interventions against *T. solium*, including mass anti-helminthic treatment, pig vaccination, and geographically targeted ring interventions. Despite this progress,

few strategies have been systematically evaluated, and there is a critical lack of evidence on which to base recommendation for control in different geographic settings. To address this evidence gap, we introduce CystiAgent, an agent-based model designed to simulate *T. solium* transmission. CystiAgent can be used to compare the effectiveness of available control strategies in a variety of endemic settings, and prioritize those most likely to be successful for evaluation in prospective studies. While previous transmission models for *T. solium* have been developed, CystiAgent is the first spatially-explicit model, and therefore the first with the ability to model clustered transmission patterns while accounting for risk heterogeneities caused by open human defecation, pig roaming, and travel patterns. It is also the first model to be validated with data collected from prospective control and elimination interventions. This presentation includes visual demonstration of CystiAgent operation and output, and provides validation results using prospective intervention data from two large multi-year studies (Cysticercosis Elimination Project and Optimizing Ring Strategy) that span 10 unique interventions and 40 villages (pop. ~20,000) in northern Peru. Validation results include the accuracy of model-predicted reductions in the prevalence of taeniasis and porcine cysticercosis, and sensitivity analyses demonstrating parameter uncertainty and opportunities for future model improvements. This oral presentation represents a unique opportunity to share a much-anticipated tool with the cysticercosis research community.

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EVALUATING POTENTIAL FOR AN UNADDRESSED URBAN RESERVOIR OF *TAENIA SOLIUM* TAENIASIS IN PERU

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Neurocysticercosis is a leading cause of adult onset epilepsy. In 2012, the causative parasite, *Taenia solium*, was eliminated from 107 rural villages in the region of Tumbes, Peru. Urban centers were not included in the elimination project given that the conditions for established transmission (free ranging pigs and open human defecation) are uncommon in these settings. However, a recent study found active transmission of the parasite in two of the villages that had been previously cleared. These villages experience both frequent travel and commerce with the nearby city of Zarumilla, as well as immigration from other rural regions outside the elimination zone. The objective of this study was to determine the prevalence of taeniasis in the urban center of Zarumilla, and to explore the plausibility of an urban reservoir of taeniasis as a potential threat for reintroduction of *T. solium* into the elimination area. We conducted random cluster sampling at the block level within the city limits of Zarumilla (population ~ 20,000). Participants were asked about the frequency, duration and sites of travel to rural areas. They were also offered oral niclosamide as presumptive treatment for tapeworms, and were asked to provide a stool sample for diagnosis of taeniasis using coproantigen ELISA. 636/ 1113 (67%) of households approached had one or more participants. A total of 2340 residents provided survey data, of which 2061(88 %) accepted treatment, and 1621 (69%) provided a stool sample. Four participants (0.25% [95%CI: 0.08, 0.64]) were diagnosed with taeniasis; all had traveled to a nearby rural community and three reported having defecated outside while there. While it is possible that urban taeniasis carriers could have contributed to reintroduction of *T. solium* to the rural elimination area, the low prevalence of urban taeniasis compared to the typical prevalence of 1-2% in rural areas, suggests that other sources are more likely. Alternative sources for reintroduction such as rural-rural migration and persistent parasite eggs in the environment should be considered.

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RELATIONSHIP BETWEEN PLASMA LEVELS OF ALBENDAZOLE SULFOXIDE AND ANTIPARASITIC EFFICACY IN THE TREATMENT OF NEUROCYSTICERCOSIS

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The efficacy of albendazole therapy to destroy brain cysticercosis cysts is incomplete. Plasma levels of albendazole sulfoxide (ABZSO), the active metabolite of albendazole, are extremely variable among patients. We assessed whether high plasma ABZSO levels during albendazole therapy are associated with increased antiparasitic efficacy. Plasma ABZSO levels were measured at treatment day 7 in 118 patients with intraparenchymal neurocysticercosis enrolled in a trial of antiparasitic efficacy. Drug levels were analyzed in regard to the likelihood of complete cyst clearance in the patient, and the proportion of baseline cysts that resolved by therapy (evaluated by brain MRI 6 months after treatment). A trend towards high cure rates in patients with increasing quartiles of ABZSO levels was observed (40%, 48%, 50%, and 60% for Q_1 [≤245 ng/ml], Q_2 [>245 to ≤385 ng/ml], Q_3 [>385 to ≤650 ng/ml], and Q_4 [>650 ng/ml] respectively; *P*-value = 0.059). A similar trend was also observed for the proportion of resolved cysts with increasing quartiles of ABZSO levels (46%, 60%, 58%, and 77% for Q_1, Q_2, Q_3, and Q_4 respectively; *P*-value = 0.093). In patients with ≥3 baseline brain cysts, the regression models adjusted by praziquantel co-administration, and the type of anti-epileptic drug used showed higher cure rates and proportion of resolved cysts when comparing ABZSO levels in the 4th versus the 1st quartile (risk ratio = 3.17 [95% CI: 1.12-8.99] for cure rates, and risk ratio = 2.0 [95% CI: 1.03-3.93] for the proportion of resolved cysts); in patients with 1-2 baseline cysts not effect between ABZSO levels and antiparasitic efficacy was observed. There is a direct relationship betwen increasing levels of ABZSO in plasma and treatment efficacy, although the strength of the association is mild to moderate, and other factors including baseline brain cyst burden, and antiparasitic drug scheme may also impact in treatment efficacy.

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DESIGN AND VALIDATION OF A SENSITIVE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION, LAMP ASSAY, TARGETED *T. SOLIUM COX 1* GENE TO IMPROVE THE DIAGNOSTIC OF NEUROCYSTICERCOSIS USING CEREBROSPINAL FLUID

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Taenia solium neurocysticercosis (NCC) is a common cause of epileptic seizures and other neurological morbidity in several low-income countries including Madagascar. The neuroimaging diagnosis remains challenging and expensive especially for the population living in remote areas. Here we describe a sensitive and affordable NCC molecular diagnosis test based on LAMP method using cerebrospinal fluid (CSF). Patients presenting epileptic

seizures and/or unusual progressive headache, with neuroimaging lesions, recruited at the Neurology Department of Befelatanana Hospital were included in this study. In-depth CT-Scan analysis was used as reference test to ascertain the NCC diagnosis. LAMP-NCC technique detecting *T. solium* cytochrome C oxydase subunit 1 gene was first validated on 7 patients with confirmed NCC (presence of scolex in CT-Scan) and 4 patients with negative NCC. Immunoblot test (EITB) using purified *T. solium* metacestode glycoproteins were realized on blood and CSF. The LAMP-NCC presented a detection limit of 2.5pg using DNA extracted from *T. solium* metacestodes and showed no amplification with *T. saginata*, *T. asiatica* and *Loa loa* DNA. Using CT-Scan as reference method, the sensibility of LAMP-NCC on CSFs without any DNA extraction step is higher compared to the immunoblot (70% vs 40%) when the specificity for both tests was similar (80%). The validation of the LAMP-NCC is on-going on a biobank of CSFs samples from patients with confirmed NCC diagnosis or with other neurological pathologies. The encouraging preliminary results obtained indicates that LAMP-NCC is a promising molecular Point-Of-Care tool to strengthen the NCC diagnosis in Madagascar.

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ALVEOLAR ECHINOCOCCOSIS IN GERMANY, 1992-2017: PREVALENCE, SURVEILLANCE AND SPATIAL AUTOCORRELATION

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Alveolar echinococcosis (AE) caused by the parasite *Echinococcus multilocularis* is a rare disease in Germany. The aim of this analysis was the detection of high risk areas and the determination of prevalence of AE in Germany. EpiInfo ™ was used for cartographic representation and visualization of the penetration position. The Moran's I geoanalysis by clusters and risk areas was performed with GeoDa™. SAS Version 9.2 was used for statistical analysis. The analysis of the AE cases (N=566) revealed a concentration of diseases in Baden-Württemberg and Bavaria. Analysis based on Moran's I gave a positive spatial autocorrelation for Germany (I=0.215887, Z=33,1461, p<0.001). The prevalence for Germany in the period 1992-2017 resulted in 0.69 cases per 100,000 inhabitants. For Baden-Württemberg, the estimated prevalence was 2.42 cases and in Bavaria 1.64 cases per 100,000 inhabitants. The analysis on spatial autocorrelation and possible risk areas shows the direct neighborhood of "high-high" risk areas in the southeastern regions of Baden-Württemberg (I=0.203845, Z=11,6582, p<0.001) and in the southwestern part of Bavaria (I=0.153844, Z=11,4593, p<0.001). Striking are clusters of illnesses in the area of the Swabian Alb uplands, the Alps and the foothills of the Alps. By informing the population in high risk areas, AE cases can possibly be prevented or diagnosed at an early stage.

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PERFORMANCE OF A POINT-OF-CARE RAPID DIAGNOSTIC TEST FOR THE SERODIAGNOSIS OF CYSTIC ECHINOCOCCOSIS IN AN ENDEMIC SETTING: A FIELD STUDY IN PERÙ

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Cystic Echinococcosis (CE) is a zoonosis caused by the infection with the larval stage of the dog tapeworm *Echinococcus granulosus*. 1.2 million people are estimated to be infected worldwide. Peruvian highlands are endemic for CE. Ultrasound (US) is the gold standard for the diagnosis of abdominal CE, while serology has a complementary role. However, in rural endemic areas, expertise in US is often scant and conventional serology is unavailable. We studied the performance of a commercial Rapid Diagnostic Test (RDT) (VIRapid HYDATIDOSIS - Vircell, Spain) for the serodiagnosis of CE during an US-based screening in 3 districts in the central Peruvian highlands. Of the 546 volunteers scanned by abdominal US (227 in Ondores, 213 in Corpacancha and 106 in Tomas), 37 (6.7%; 95%CI 4.8-9.2%) had CE cysts. The highest prevalence was observed in Corpacancha (13,6%). A history of surgery for CE was reported by 6% of the subjects. We found 68 cysts: 28 (41%) were active (CE1, CE2, CE3b), 8 (12%) transitional (CE3a) and 32 (47%) inactive (CE4, CE5). Blood samples were taken on the same day of US examination from 34 patients with CE cysts, 23 patients with a history of CE surgery and 57 US-negative controls matched for age and sex. Sensitivity and specificity were calculated using individuals with CE as cases and individuals with negative US exam (including those reporting past surgery for CE) as controls. RDT results were positive for 25 individuals with abdominal CE (73,5%), 8 subjects with no visible abdominal CE (14%), and 9 individuals with a history of surgery for CE (39%). Sensitivity and specificity were 73.5% and 78.8% (75% and 84.5% when subjects reporting past surgery for CE were excluded from the analysis). Although a proportion of positive serology results in subjects with negative US may be due to lung CE until proven otherwise (a chest X-ray examination is planned for these subjects), our initial findings show that the RDT we tested has a low specificity when used on field samples from an endemic area.

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PHOSPHOETHANOLAMINE-N-METHYLTRANSFERASE IS A POTENTIAL BIOMARKER FOR THE DIAGNOSIS OF *PLASMODIUM KNOWLESI*, *P. FALCIPARUM* AND *P. VIVAX* MALARIA

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Plasmodium knowlesi is recognised as the main cause of human malaria in Southeast Asia. *P. knowlesi* is primarily diagnosed by PCR as it is difficult to diagnose by microscopy. Prompt diagnosis and treatment of *P. knowlesi* infections is required as this species can rapidly cause severe disease. There are currently no biomarkers for antibody based rapid diagnostic tests (RDT) to detect a *P. knowlesi* infection. We aimed to identify a protein biomarker with potential for the diagnosis of *P. knowlesi*, *P. falciparum* and *P. vivax* malaria. Phosphoethanolamine-N-methyltransferase (PMT), a protein involved in malaria lipid biosynthesis and not expressed in humans was selected. Peptides on the surface of PMT that were either common (*P*LENNQYTDEGVKC) or unique to *P. knowlesi* (*Pk*LYPTDEYNSLKDC), *P. falciparum* (*Pf*CEVEHKYLHENKE) and *P. vivax* (*Pv*VYSIKEYNSLKDC) PMT were selected. Polyclonal antibodies against all of the peptides and recombinant *P. knowlesi*, *P. falciparum* and *P. vivax* PMT proteins, were raised in chickens and affinity purified. The antibodies against each recombinant PMT protein and the common PMT epitope (*P*LENNQYTDEGVKC) each detected all three recombinant proteins. Antibodies raised against unique peptides selectively detected the parent protein in western blots and in spiked blood lysates. The antibodies detected a 29 kDa protein in a *P. falciparum* culture lysate. PMT, like the pan-specific malaria LDH biomarker used in RDTs is soluble, present at similar concentrations to LDH, is essential to parasite survival and represents a promising antimalarial drug target. Unlike LDH, PMT is not present in the human proteome. PMT has the potential as a biomarker for malaria and in particular to detect *P. knowlesi* parasites.

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A NOVEL QUANTITATIVE SUSPENSION ARRAY TOOL FOR HIGH SENSITIVE QUANTIFICATION OF HRP2 AND PLDH IN BLOOD FROM *PLASMODIUM FALCIPARUM* AND *P. VIVAX*-INFECTED PATIENTS IN PERU AND NIGERIA

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Quantification of protein antigens is increasingly important for the assessment of ultrasensitive diagnostic tools for infectious diseases. We developed a quantitative suspension array technology (qSAT) for multiplexed quantification of HRP2 and pLDH malaria antigens in patient blood samples, and assessed its performance as part of a malaria RDT evaluation study in 4489 patients in Peru and Nigeria. Patient samples underwent on-site and off-site reference testing (with malaria RDTs, microscopy, real-time PCR, and sequencing for *Plasmodium* species determination). qSAT testing was performed on samples positive for *P. falciparum* (845/4489, 18.8%) and *P. vivax* (892/4489, 19.9%) by real-time PCR and sequencing. Analysis of HRP2 detection was conducted on Nigerian samples only because of *hrp2* gene deletions known to be prevalent in Peru. The limit of quantification of the qSAT was 7.2 pg/mL for HRP2 and 47.8 pg/mL for pfLDH. Preliminary results on 791 *P. falciparum* samples showed that the qSAT assay could detect and quantify HRP2 and pfLDH antigens in 49.0% (123/251) and 36.2% (147/406) of samples, respectively, which were PCR-positive but negative by microscopy and RDT, confirming its increased sensitivity over microscopy and RDT. When the qSAT is used as the reference method, the sensitivity and specificity of the HRP2 test line of the SD Bioline Malaria Ag Pf/Pan (Alere, USA) used in Nigeria was 70.3% [CI95%: 66.1-74.6%] and 87.2% [CI95%: 81.8-92.6%], respectively, while the panLDH test line of the Carestart Pf/PAN (pLDH) Antigen test (Access Bio, USA) used in Peru was 85.3% sensitive [CI95%: 79.7-90.1%] and 96.6% specific [CI95%: 91.9-100.0%]. This compares closely to PCR as a reference method, and confirms that this new qSAT assay can successfully be used as a reference test for detection and quantification of HRP2 and pLDH in *P. falciparum* and *P. vivax* samples. Preliminary and first-of-its-kind data also show good quantification of pLDH in *P. vivax* samples, which is extremely important given concerted efforts by the global health community to improve the sensitivity of *P. vivax* diagnostics to support elimination of all malaria species.

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SCREENING FOR *PFHRP2/3*-DELETED *PLASMODIUM FALCIPARUM*, NON-FALCIPARUM, AND LOW-DENSITY MALARIA INFECTIONS USING A MULTIPLEX ANTIGEN ASSAY

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Detection of *Plasmodium* antigens from human malaria parasites has broad applications for diagnostics, surveillance, and laboratory research. A bead-based multiplex assay was developed to simultaneously detect the pan-*Plasmodium* aldolase (pAldo), pan-*Plasmodium* lactate dehydrogenase (pLDH), and *P. falciparum* histidine rich protein 2 (PfHRP2) antigens. The multiplex assay was validated against recombinant antigens and 239 mono-species malaria infections. Limit of detection for the antigens in human blood samples was determined for pAldo at 128pg/mL, Pf-pLDH at 4,425pg/mL, Pv-pLDH at 4,435pg/mL, PfHRP2(A) at 6.7pg/mL, PfHRP2(B) at 8.8pg/mL, and PfHRP2(C) at 15.8pg/mL. Samples from Angolan patients attending outpatient clinics (n=1267) were assayed, and the most common antigen profiles were PfHRP2+/pAldo+/pLDH+ (167, 36%), PfHRP2+/pAldo-/pLDH- (163, 35%), and PfHRP2+/pAldo+/pLDH- (130, 28%) with the number of detected antigens being predictive of qRT-PCR positivity and parasite density as well as patient febrile status. Of the 466 (36.8%) Angolan samples positive for any antigens, eight had either no or very low levels of PfHRP2, but were positive for pan-*Plasmodium* antigens. Molecular analysis confirmed three were *P. ovale* infections, and two represented *P. falciparum* parasites lacking *Pfhrp2* and/or *Pfhrp3*. The findings suggest HRP2-based rapid diagnostic tests are still an appropriate tool for confirmation of *P. falciparum* diagnosis in Angola. High-throughput multiplex antigen detection can inexpensively screen for low density *P. falciparum*, non-falciparum, and *Pfhrp2/3*-deleted malaria parasites to provide population-level antigen estimates and identify specimens requiring further molecular characterization.

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CAN MEDICAL-DETECTION DOGS IDENTIFY PEOPLE WITH MALARIA PARASITES?

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The task of malaria elimination would be simpler if a non-invasive method was available for detecting infected individuals in populations where the parasite prevalence is low; infected individuals could then be treated with antimalarials. Dogs have a highly developed sense of smell and may be able to detect volatiles released from people carrying malaria parasites. We carried out a pilot study to determine whether trained dogs could detect malaria infections in Gambian children aged 5-13 years old. Samples of skin odour were collected from 600 school children. Samples from malaria-infected children and uninfected children were used to train medical-detection dogs in the UK. After four months of training a double-blinded study was undertaken to assess the diagnostic accuracy of the dogs. To assess the reaction of local people to medical-detection dogs, a well-disciplined dog with a 'medical-detection dog' coat was introduced to residents of a Gambian village. Focus group discussions assessed the benefits and challenges of using medical-detection dogs for malaria surveillance in Gambian villages. The results of this study will be reported at the meeting. If successful, this diagnostic approach could be used for mass screening of malaria cases at ports of entry.

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CELL FREE DNA AS A MARKER OF CEREBRAL MALARIA

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Cerebral malaria (CM) is a life-threatening manifestation of *Plasmodium falciparum* infection that disproportionately affects young children living in sub-Saharan African. Pediatric CM is characterized by parasitemia and coma, but additional tests are required to identify those most at risk for an adverse or fatal outcome. Retinal funduscopy, to identify malarial retinopathy (Ret+CM), and quantification of histidine rich protein 2 (Pfhrp2), a surrogate measure of total parasite biomass, assist in identifying pediatric CM cases at highest clinical risk, but are not widely available. Recent work identified cell free parasite DNA concentration in the plasma as a correlate of disease severity in children with malaria. Host neutrophils and circulating neutrophil extracellular traps, comprised of host DNA, are reported to be activated in malaria. Thus, we hypothesized that total plasma cell free DNA (host & parasite) may also correlate with CM classification. Here, we assayed the plasma of Malawian children ages 1-12 years with uncomplicated malaria (UM) (n=37) or CM (total n=61; Ret-CM n=15; Ret+CM n=46), without extraction or enrichment steps, for total cell free DNA (cfDNA) level using the Qubit 2.0 Fluorometer, a compact benchtop analyzer with high sensitivity and reproducibility. Total cfDNA in the plasma distinguishes children with UM or Ret-CM from children with Ret+CM (UM ROC: 0.85, p<0.0001; Ret-CM ROC: 0.69, p=0.03). Amongst Ret+CM cases, cfDNA differentiates fatal cases (n=7) from children with resolution of coma and clinical disease (n=39) (ROC: 0.87, p=0.02). The ability to distinguish case classifications by total cfDNA level paralleled that of Pfhrp2, and both measures were superior in distinguishing cases relative to cell free parasite DNA levels only. Current studies are underway to determine the relative contributions of host vs parasite DNA to cfDNA. As expected, plasma cfDNA level declined by 30 days post-infection. The simple methodology and high assay sensitivity suggest that Qubit plasma cfDNA quantification may be useful in identifying CM cases in field settings where resources and trained staff are highly limited.

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AI SCOPE - OPEN SOURCE AUTOMATED MICROSCOPY USING MACHINE LEARNING FOR MALARIA DIAGNOSIS

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Access to accurate microscopy diagnosis still represents a cornerstone for delivering treatment for many global killers. Currently, machine learning reached a point where deep learning scripts (convolutional neural networks) can help to improve the accuracy of microscopy diagnosis by recognizing pathogens automatically, quickly and accurately, even with low image resolution. The study aim was to develop an open source automated microscope and assess its diagnostic accuracy for falciparum malaria. The Ai Scope was developed as an automated, low cost (under 50$), portable, user-friendly and multiplatform, do-it-yourself microscope. It was designed to allow to plug in any of the standard smartphones. The scripts were developed using deep learning frameworks TensorFlow (for the first models) and MXNet (latest version). Also, it allows further applications such as real-time surveillance, big data analytics, and telemedicine. The mobile app connects via Bluetooth to the microscope motors for slides focusing and mapping. To train the *Plasmodium falciparum* malaria deep learning script we used a total of 1,317 images from Giemsa-stained thick blood smears. Of these, we obtained 5,492 images from infected erythrocytes with ring-stage trophozoites and 5,492 images from nearby uninfected erythrocytes as controls. We randomly extracted 70% from each subset to train the neural network during 150 epochs using technics of data augmentation such as rotations and symmetries. We used the remaining 30% to assess its diagnostic accuracy of the algorithm. Compared to controls, the Ai scope correctly classified 98.2% of the positive images, with an estimated sensitivity of 97.2-98.6% (95% CI), a specificity of 97.7-98.9%, a positive predictive value of 97.6-98.9%, a negative predictive value of 97.3-98.6%, and a ROC area of 97.7-98.9%. To sum up, the Ai Scope showed promising results by diagnosing ring-stage trophozoites accurately from falciparum malaria under laboratory conditions. Further improvements will allow the Ai Scope to offer the diagnosis of other infectious diseases such as tuberculosis and diarrhea caused by intestinal parasites.

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ASSESSMENT OF ULTRASENSITIVE DIAGNOSTICS FOR ASYMPTOMATIC MALARIA IN A HIGH TRANSMISSION AREA OF ETHIOPIA

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To accomplish malaria elimination, it is necessary to address the asymptomatic reservoir which contributes to perennial transmission. Microscopy and rapid diagnostic tests (RDT) are feasible, but they have a relatively high limit of detection. Hrp2/3 gene deletions may also contribute to false negative RDT results. Molecular methods such as polymerase chain reaction (PCR) are highly sensitive but remain too complex. There is an urgent need for ultrasensitive diagnostics able to identify low-level infections and parasites with Hrp2/3 deletions at the community level. We performed a community-based, cross-sectional study in Gambella, Ethiopia at four kebeles in the district of Abobo between November 2017 and March 2018. Individuals were screened to determine baseline medical information and epidemiological data. If afebrile, blood was collected by venipuncture in EDTA blood tubes. Two RDTs (SD Bioline PfHrp2/PfLDH and Carestart PfHrp2/PvLDH) were performed on site. Ultra-sensitive RDT (Alere PfHrp2) was also performed from stored blood samples. Dried blood spots (DBS) were collected on site and hemoglobin measured using HemoCue® HB 201+ strips. DBS was evaluated by ultrasensitive loop mediated isothermal amplification (usLAMP) and real-time PCR (RT-PCR). We collected 940 blood samples from afebrile community members. The majority were female (526, 56%), adult (676, 72%), living in rural areas (800, 85.1%) and farmers (545, 58%). Blood testing revealed that 225 (23.9%) were anemic. The prevalence of *P. falciparum* malaria among the subjects studied was 4.3% (40), 2.64% (25) and 8.1% (76) as detected by Carestart PfHrp2, SD Bioline PfHrp2/PfLDH and ultrasensitive Alere PfHrp2 RDTs, respectively. Preliminary results with usLAMP suggest 10 out 46 (21.7%) are positive for malaria. Our data demonstrate that the ultrasensitive RDT targeting PfHrp2 can detect between 1.9 to 3.1 times more cases of *P. falciparum* malaria than traditional RDTs, whereas usLAMP can detect 5.0 to 8.2 fold higher asymptomatic individuals in this high transmission setting. Current efforts are focused on completing usLAMP and RT-PCR on all specimens.

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META-ANALYSIS OF *ANOPHELES GAMBIAE* POPULATIONS FROM ACROSS AFRICA IDENTIFIES POTENT NEW PYRETHROID RESISTANT MECHANISMS

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Vector control represents the single largest contributor to the reduction in human malaria cases since the year 2000 with pyrethroid treated bednets estimated to be responsible for nearly two thirds of the malaria cases averted. Resistance to pyrethroid insecticides is now widespread in African malaria vectors and there is increasing evidence that this is reducing the efficacy of bednets. In order to understand more about the causes and consequences of pyrethroid resistance for malaria control, we searched all available transcriptomic data on insecticide resistant populations across Africa to identify the major resistance mechanisms. Previous approaches for selecting potential resistance mechanisms have largely focused on *a priori* candidates such as those associated with insecticide detoxification. By integrating 31 geographically disparate datasets, representing collections over 5 years (2009-2014) and three species of the *Anopheles gambiae* complex, and using gene silencing to confirm the association between transcriptomics profiles and resistance phenotype we have: (a) Confirmed the association between over expression of a small subset of cytochrome P450s and pyrethroid resistance; (b) Identified novel gene families representing potent resistance mechanisms across much of Africa; (c) Identified potential transcriptional regulators of insecticide resistance; and (d) Employed correlation networks to identify putatively co-regulated transcripts and hence, elucidate transcript function. A public access, easy to use ShinyR web-based application (IR-TEx), has been developed to facilitate integration of future data on insecticide resistance and to enable the geographic distribution of resistance genes to be visualised.

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SEXUAL SELECTION AS A POSSIBLE DRIVER OF CUTICULAR INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* POPULATIONS FROM WEST AFRICA

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Insecticide resistance is now widespread across Africa and threatens progress in vector control efforts against *Anopheles* mosquitoes, the vectors of malaria. Several mechanisms are known to convey insecticide resistance, including target site mutations, metabolic resistance, behavioural changes, and cuticular resistance. Cuticular resistance is characterized by a thickening of the cuticle, including an increase in the quantity of Cuticular hydrocarbons (CHCs) present in the epicuticle. Cuticular resistance has been only recently described in *Anopheles* mosquitoes, and the extent of its contributions to resistance in natural populations is not yet understood. We therefore investigate the presence of cuticular resistance in natural populations of *Anopheles* mosquitoes in Burkina Faso. Additionally, we hypothesize that an increase in CHC production may play overlapping roles with mate selection, given known roles of CHCs in mating behavior of other *Dipterans*. To address these questions, we exposed mosquitoes derived from natural breeding sites in Burkina Faso to permethrin, using time to knock-down to classify individuals into groups of high, medium, and low permethrin resistance. We measured quantities of CHCs in these mosquitoes using hexane extraction of hydrocarbon compounds followed by Gas Chromatography Mass Spectrometry. We demonstrate for the first time, to our knowledge, that higher quantities of CHCs are correlated with higher levels of insecticide resistance in natural *Anopheles* populations. We further investigate the association of CHCs with mating success by comparing CHCs of males caught in copula from natural mating swarms with CHCs of males who were not mated during the time of the swarm. We find higher levels of CHCs in mated males compared to their unmated counterparts, indicating that increased CHC production is associated with mating success, as well as insecticide resistance. This provides evidence for a sexual selection mechanism for cuticular resistance, which could greatly affect the dynamics of spread of insecticide resistance by increasing the selective pressure on resistance traits.

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THE MALARIAGEN VECTOR OBSERVATORY: A NETWORK FOR THE GENOMIC SURVEILLANCE OF MALARIA VECTORS IN AFRICA AND SOUTHEAST ASIA

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We describe the launch of an open, collaborative network, leveraging genome sequencing technologies to undertake coordinated surveillance of malaria vector populations. In Africa the network has two overarching goals. The first goal is to realise the use of genome sequencing as an operational tool for insecticide resistance management (IRM), providing information and tools to aid the design of optimal IRM strategies, and providing feedback on the impact of IRM implementations on vector

populations and their adaptive responses. The second goal is to accelerate the development of new vector control tools, particularly new insecticides, synergists, and gene drive systems, by providing comprehensive data on natural genetic variation, and by using genetic data to estimate key parameters needed to model vector population dynamics, particularly aestivation and migration behaviours. In Southeast Asia the goal is to survey the full taxonomic diversity of malaria vector species, and to identify and characterise the species and populations that are primarily responsible for malaria transmission in different geographical locations, especially transmission of parasites resistant to antimalarial drugs. Building on insights, technologies and best practices developed in the *Anopheles gambiae* 1000 Genomes Project, the Vector Observatory has been operating in a pilot phase since 2017, and has been establishing lab, data processing and analytical pipelines to accommodate whole genome sequencing of 10,000 mosquito specimens per year, as well as developing new sequencing technologies that can scale to larger sample sizes and be deployed to regional facilities. We give an overview of current projects and partnerships within the network, the datasets generated so far, the model for participation in the network, and the roadmap for future data generation and technology development. We illustrate the network with three case studies: (1) longitudinal sampling of *An. gambiae* complex populations in West Africa; (2) A broad geographical survey of African *An. funestus* populations; (3) A taxonomic survey of *Anopheles* species from Cambodia.

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NOVEL SCREENING ASSAYS TO ACCELERATE THE PATH TO DELIVERY OF NEXT GENERATION VECTOR CONTROL TOOLS

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The development of new vector control tools, both insecticides and methods for their delivery, is essential if the challenges of resistance and other threats to malaria control are to be met. Widespread resistance across insecticide classes and the lengthy screening pipeline for new chemistries mean that rapid, accurate and informative assays to supplement or replace existing evaluation methods are urgently needed. We are developing a suite of bioassays incorporating human bait, ranging in scale from bench top (*e.g.* simple wind tunnel, modified cone tests, thumb tests) to room scale (video tracking at whole LLINs), to explore and quantify detailed behavioral responses to vector control products. Early results using pyrethroid susceptible and resistant *An. gambiae* in various bench top assays showed that behavior at untreated netting and LLINs (PermaNet® 2.0, Olyset) was not altered by insecticide susceptibility status. Without access to bloodfeed, mosquitoes mainly rested on nets, with some 'bouncing' (multiple rapid brief contacts) and probing. When allowed to bloodfeed through nets, feeding duration was reduced significantly on LLINs compared to untreated nets, in both susceptible and resistant strains. A modified WHO cone test that video records responses to a host demonstrated that mildly repellent effects seen with some net treatments, are overridden by the presence of the host, whether or not feeding is permitted. Since resistant populations of *An. gambiae s.l.* are now the norm across Africa, we have been exploring impacts of insecticides beyond 24-hrs post-exposure, by examining possible sub-lethal impacts on longevity, reproductive output and re-feeding rates in 'resistant' strains (as defined by WHO tests). Data and video from this suite of assays allows characterization of a wide and complex range of behavioral responses and consequences of mosquito exposure to, and interaction with, vector control products and illustrate their potential to provide meaningful data far beyond what is currently possible using existing standard assays.

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COPY NUMBER VARIATION AND INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE*

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Metabolic resistance in the form of increased expression of detoxification genes underlies much of the insecticide resistance observed in mosquito populations, yet the causal genetic variants for these differences remain poorly characterised. Using data from the *Anopheles gambiae* 1000 Genomes project, we have described 44 duplications found in four major detoxification gene clusters (GSTU4-GSTE3, CYP9K1, CYP6M2-CYP6Z1 and CYP6AA1-CYP6P2), with 10 of the 11 independent duplications in the GSTU-GSTE cluster encompassing GSTE2 and 13 out of the 15 independent duplication in the CYP6AA-CYP6P cluster encompassing CYP6AA1. We designed molecular assays, based on the duplication breakpoints to test for the presence of duplications in a wide range of recently collected samples. We found that some of these duplications have risen sharply in frequency, suggesting a response to selection by insecticide pressure.

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MUSCARINIC ACETYLCHOLINE RECEPTORS AS A TARGET FOR RESISTANCE-BREAKING INSECTICIDE DEVELOPMENT

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Widespread insecticide resistance amongst insect pests establishes a need for new insecticides with unique modes of action. Insects are known to possess muscarinic acetylcholine receptors (mAChRs), and muscarinic agonists are effective insecticides, but suffer from high mammalian toxicity. Of the three types of mAChRs in insects (A, B, and C), type-B activation is thought to decrease levels of cAMP and is pharmacologically distinct, having little sensitivity to the classical mAChR antagonist, atropine. Recently, we've identified a novel pyrazole oxime (747) that is toxic to *An. gambiae* (WHO paper assay LC_{50} = 0.122 mg/mL), has low toxicity to mice (oral LD_{50} > 2,000 mg/kg), and shares some structural similarity to pilocarpine, a non-selective mAChR agonist. Pilocarpine and 747 also shared similar signs of intoxication in mosquitoes, which include lethargic movement and loss of flight behavior. Pilocarpine toxicity was antagonized by atropine injection, whereas 747 toxicity was not. Consequently, we hypothesized that 747 affects the insect mAChR-B receptor. Exposure of a larval *Drosophila melanogaster* CNS preparation to 10 µM 747 resulted in a biphasic effect on the CNS firing rate; a slight increase followed by a nearly complete inhibition of nerve discharge. Neither effect was blocked by atropine. Whereas, pilocarpine's (10 µM) biphasic effect on CNS nerve firing, with strong initial neuroexcitation, was sensitive to atropine.. In addition to the electrophysiology preparations, biochemical assays using cockroach nerve cord showed that 747 (10 µM) resulted in a significant decrease in the production of forskolin-stimulated cAMP, and this effect was insensitive to atropine (100 µM). The insect mAChR-B is an attractive target for mosquitocide development that warrants further investigation.

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NOVEL MICROBIAL CANDIDATE MARKERS OF PYRETHROID RESISTANCE IN *ANOPHELES ALBIMANUS*, A MAJOR LATIN AMERICAN MALARIA VECTOR

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A deeper understanding of the biological mechanisms underlying insecticide resistance in malaria vectors is needed to mitigate its threat to vector control efforts. Our previous findings identified links between *Anopheles albimanus* microbiota and resistance to the organophosphate insecticide fenitrothion. We have now characterized the microbiota of *An. albimanus* with differing pyrethroid resistance profiles across four locations in Guatemala using 16S rRNA gene sequencing. We focused on identifying patterns of microbial composition across mosquitoes expressing susceptibility or resistance to alphacypermethrin, deltamethrin and permethrin, in both larvae and adults. F1 progeny of wild-caught mosquitoes from each location were reared separately under identical conditions, and characterized using the CDC bioassay as resistant (Res), susceptible (Sus) or unexposed to insecticide (Unexp). This resulted in 44 pools of late instar larvae and 45 pools of 2-5day old non-blood-fed virgin adult females - 3 mosquitoes/pool, 3 pools/category, with all insecticides tested in at least one location. Differential microbial compositions (p<0.05), mostly driven by type of insecticide (R2=0.51), were identified between Res, Sus and Unexp, for all three insecticides, in both larvae and adults, and across each location tested. With more than five-fold greater abundance in Res compared to any other category (p<0.05), *Klebsiella* (alphacypermethrin and deltamethrin), *Asaia* and *Pantoea* (permethrin) were identified as candidate markers associated with resistance in adults but not in larvae, suggesting the presence of transient microbiota in larvae. These results build upon our initial findings and identify candidate microbial markers of pyrethroid resistance in adult *An. albimanus* that are being functionally validated. The results also suggest a specificity of candidate microbial markers within the pyrethroid class of insecticides.

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SPATIOTEMPORAL MODELLING OF PREVALENCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MUTATIONS IN THE *DHPS* GENE ACROSS AFRICA, 1990 - 2015

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Despite global efforts to control and prevent malaria, it remains one of the most important global public health issues. Large-scale prevention programmes include intermittent preventive treatment for pregnant women (IPTp) using sulfadoxine-pyrimethamine (SP), and Seasonal Malaria Chemoprevention (SMC) with monthly courses of amodiaquine plus SP to all children under 5 years in the high transmission season in areas of the Sahel sub-region of Africa. Molecular surveillance of mutations in the *pfdhps* gene can guide the assessment of the effectiveness of IPTp. This study updated previously developed geospatial maps displaying the published evidence of the prevalence of *dhps*540E, a marker associated with resistance to SP. To update these maps a systematic literature search was conducted to identify publications with data on *dhps*K540E genotype and/or haplotype combined with other alleles from *P. f* pre-treatment isolates, study year and geo-position in publications up to 2017. Data were extracted from the publications and entered into a standardised relational database. In total, we used 366 data points (42514 samples) collected between1978-2015 across 41 countries and 253 sites. The data was used in a Bayesian model based geostatistics approach to create predictive distributions of the prevalence of *dhps*K540E in Africa from 1990 to 2015. These predictive maps provide predicted resistance levels in places where no data are available and give insight on the spatial and temporal spread of resistance in a way that the data alone do not allow. The maps present the changing spatio-temporal prevalence patterns across Africa. Interestingly, despite SP use in IPTp and SMC, the prevalence of dhps540E is remarkably stable from 2010-2016. In East Africa, although SP has not been widely used as a treatment regimen for many years now we do not see a return to the wildtype population. There is reduced drug pressure on the parasite population but the prevalence of this resistance marker remains high, confirming other studies that suggest the mutation does not confer a high fitness cost for the parasite.

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TRAVEL PATTERNS OF PATIENTS WITH MALARIA IN THE GREATER MEKONG SUBREGION AND NEIGHBORING COUNTRIES IN SOUTH ASIA

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Malaria elimination is an important public health goal for countries in the Greater Mekong Subregion (GMS), Bangladesh and India. In the GMS, the reported number of malaria cases has declined by 74% and deaths by 91%. However, malaria hotspots persist in remote rural and forested areas close to border regions, with disproportionate representation of migrant and marginalized communities. Dihydroartemisinin-piperaquine resistance first noted in Cambodia has also been reported in Southern Vietnam. We hypothesize that dissemination of malaria and anti-malarial drug resistance could take place through migration. From August 2015, we have interviewed 4469 patients with malaria from over 150 study sites and obtained information on demographics and travel patterns (including travel to forests). These were combined with census and surveillance data from the national malaria control programmes. Dried blood spots were also collected to examine malaria parasite DNA. Of the 4469 patients collected so far, 48% of cases are from Bangladesh primarily through the Bangladesh Genotype Mobility study, and the rest are from ongoing studies (Lao PDR: 21%, Cambodia: 13%, Vietnam: 10%, India: 6%, Myanmar: 2%, and Thailand: 1%). Of the study population, *Plasmodium falciparum, vivax* and mixed infections were at 74%, 20% and 5% respectively. 71% were males, median age was 29, with 1% of children under 5 and 14% aged 5 to 15 years. 85% of cases reported having traveled, and over 64% reported having lived in or visited the forest. Travel patterns were further analyzed to identify mobile population groups, nodes of high traffic and possible sources and routes of spread of malaria. Travel was heterogeneous across demographic groups and spatially from village up to inter-country level, determining risk groups at coarse and fine scales. We aim to collect and analyze data from at least 5000 cases by end of this malaria season. Through working in close partnership with national programs and health workers, this improved understanding of the impact of population movement aims to inform elimination efforts in the region.

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CLUSTERING OF SUBPATENT *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INFECTIONS AROUND PATIENTS IN A LOW-ENDEMIC SETTING AIMING FOR ELIMINATION: BATU DEGAGA KEBELLE, ADAMA WOREDA, OROMIA, ETHIOPIA

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The heterogeneous distribution of infections in settings aiming for malaria elimination challenges finding and treating all relevant infections. In this study we carried out a reactive active case detection: family members and immediate six neighbors of index cases (patients with RDT-confirmed malaria) and controls (who visited the health facility for non-malaria cases) for malaria using RDT and quantitative PCR between October and December 2016 in Batu Degaga kebelle, Adama Woreda, Oromia, Ethiopia. Overall parasite prevalence was 9.7%(90/931) by RDT and 24.0%(218/907) by qPCR. qPCR-detected *Plasmodium falciparum* (*Pf*) infection prevalence was higher in the community surrounding index cases (13.9%) compared to controls (9.5%; *P*=.038) while there was no difference for *P. vivax* (*Pv*) (*P*=.926). Four geographically non-overlapping hotspots of any malaria cases with relative risk of 2.11, 1.90, 1.89 and 1.86 were detected (within 280-590meters radius) using SaTScan. Self-reported previous malaria episodes were higher in individuals who lived within risk areas compared to outside risk areas (33.1%, 177/233 vs 1.5%, 3/203; odds ratio[OR], 32.9; 95% CI, 10.2–106.3). Family members who live in households with ≥1 RDT-confirmed *Pf* individual were considerably more likely to have qPCR-detected *Pf* infection (32.4%, 11/34) compared to individuals in households without RDT-detected infection (5.9%, 47/795; OR, 7.6; 95% CI, 3.5–16.5; *P*<.001). Similar findings were found for *Pv;* (25.0%, 17/68) vs (11.8%, 94/795; OR, 2.5; 95% CI, 1.4–4.5; *P*<.0045), respectively. Outdoor activities (staying outside late in the evening and leaving early morning) and presence of eave opening were found associated with malaria risk. Data on relatedness of infections within and between households is being assessed using microsatellite markers and will be completed in the coming months. The findings in the present study indicate that subpatent infections are clustered around patients that present to health facilities and detected by standard diagnostics. This suggests that interventions targeted to index case households may support malaria elimination initiatives.

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BIAS IN ROUTINELY COLLECTED MALARIA SURVEILLANCE DATA DUE TO ASYMPTOMATIC INFECTIONS ACCORDING TO TRANSMISSION INTENSITY (A PROXY FOR PROTECTIVE IMMUNITY): A POOLED ANALYSIS OF PAIRED HEALTH SYSTEM AND COMMUNITY CROSS-SECTIONAL SURVEY DATA

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Routinely collected health systems data is the foundation of most malaria programs, both to estimate burden and to inform control and elimination programs. However, in some communities, the prevalence of infected individuals that do not seek care due to being asymptomatic can be significant. Immunity acquired with repeated exposure modifies the probability that a person will become symptomatic. Therefore, the proportion of infected individuals at any given time that will become symptomatic is expected to be modified according to transmission intensity. The aim of this research was to determine the relationship between the proportion of all infections in the community that are identified within the health system and transmission intensity, as a proxy for levels of protective immunity present in the community. Paired community and health system data in both time and space were collected from 282 clusters in 9 countries. The proportion of infections detected in the health system was estimated by the quotient of the number of infections detected at the facility and the total number of PCR confirmed infections in the community and those detected in the facility. The proportion was modeled according to transmission intensity, measured by overall population prevalence by fitting a linear relationship on the log odds scale using Bayesian Markov Chain Monte Carlo methods to account for differences in health systems and other key factors across the clusters. Preliminary results indicate that the proportion of infections reported by the health system starts to increase when overall malaria prevalence in the community is 5.9% to a maximum of 100% of infections. These findings suggest that in elimination settings, health systems can be relied upon to detect most malaria infections in a community once overall prevalence is sufficiently low.

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ANTENATAL CLINIC SURVEILLANCE FOR MALARIA ACCURATELY REFLECTS COMMUNITY MALARIA INFECTION PREVALENCE IN A HIGH TRANSMISSION SETTING IN WESTERN KENYA

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Surveillance systems in malaria endemic countries rely on passive detection of laboratory confirmed cases presenting to outpatient health facilities, community health workers, or other monitored outlets. In areas where many infections do not result in clinical symptoms, such as western Kenya where >75% of infections are asymptomatic, this strategy likely does not reflect community transmission dynamics. Community-based cross-sectional studies may provide more accurate estimates of infection, but are not scalable due to cost. Where antenatal care (ANC) attendance is high (>90% in western Kenya), pregnant women may serve as an easy access group as they are routinely screened for malaria during their first ANC visit. We compared 11 months of malaria prevalence data collected through a continuous community-based cross-sectional survey to test positivity rates in women attending first ANC visits collected from routine ANC records, to assess this population as a proxy for community malaria prevalence. Between April 2015 and June 2016 there were 998 first ANC booking visits with RDT results in the 5 health facilities, representing 10 geographic sub-locations. Of these, 110 (11.0%; 95% Confidence Interval [CI]: 9.2–13.1%) were RDT positive, with a test positivity rate range by sub-location of 2.5–20.3%, and by month of 3.8–15.5%. In the same geographic sub-locations over this period, 2108 individuals of all ages were tested for malaria by RDT in cMIS, and 436 (20.7%; 95% CI: 19.0–22.5%) were positive. Prevalence in cMIS by sub-location ranged from 12.3–39.5%, and by month from 8.5–28.4%. The Pearson correlation coefficient between ANC surveillance and cMIS by sub-location was 0.84 (p= 0.0098), and by time was 0.69 (p=0.025). We found a strong correlation between malaria RDT test positivity rate amongst women attending their first ANC booking visit and community malaria infection prevalence estimated by cMIS when comparing proportions over time and by geographic location. This

suggests that in this area with a high first ANC coverage rate, pregnant women may present a sustainable proxy for trends in population malaria infection prevalence.

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WHAT PROPORTION OF MALARIA CASES IN AFRICAN CHILDREN RECEIVE EFFECTIVE TREATMENT?

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Understanding the proportion of malaria infections that receive and adhere to effective treatment and are subsequently cured is vital for measuring progress in lowering the burden of malaria. Using household survey datasets for over 170,000 children over the past decade, we extract information on two-week malaria positivity and fever and treatment history. By coupling this data with a predictive model to distinguish between fevers attributable to malaria and fevers caused by non-malarial febrile illness (NMFI), we estimate the proportion of malaria infections that are removed from the surveyed population by effective treatment. Further, we estimate the proportion of malaria-positive fevers that would not be cured by effective treatment due to co-infections with NMFI, and estimate the rate of inappropriate treatment of NMFI with antimalarial medication. We quantify the proportion of individuals within the household surveys whose infections are not removed as an individual passes through each stage between infection and cure: i) their infection is asymptomatic, ii) they do not seek treatment, iii) they do not receive an antimalarial at the health facility they visit (and the proportion of individuals who receive a parasite-based diagnosis in-clinic), iv) they are non-adherent to the prescribed antimalarials, and v) the treatment fails. By adding quantitative information to the reasons why infections remain untreated, we measure the achievements of malaria interventions through time and identify coverage gaps where further interventions should be targeted.

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ETHIOPIA: ASSESSMENT OF MALARIA TRANSMISSION DYNAMICS USING MULTIPLEX SEROLOGICAL ASSAY

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Serological methods are increasingly utilized to estimate malaria transmission in low transmission settings. Malaria burden measures using conventional diagnostic methods in cross-sectional household surveys may incompletely detect the intensity and pattern of malaria transmission over time. This study describes the seroprevalence and pattern of malaria transmission in Ethiopia. Dried blood spot (DBS) samples collected during the Malaria indicator Survey 2015 representing malarious areas of Ethiopia were analysed with bead-based multiplex assays for IgG antibodies to 12 *Plasmodium* spp. antigens. Species-specific responses to MSP1 antigens were used to estimate the seroprevalence for the four human malaria species (*Plasmodium falciparum, P. vivax, P. malariae and P. ovale*). Frequency of seropositivity and seroconversion rates were fitted to models stratified by altitude and administrative regions. Of 8045 DBS samples collected, 7961 serological results were available for analysis. The mean age of participants was 14.8 years (95% CI: 14.7-17.3); 4197 (52.7%) participants were female. National seroprevalence was 26.4 % (95% CI: 25.5-27.5) for *P. falciparum* and 20.6% (95% CI: 19.7-21.5) for *P. vivax*. Estimated seroprevalences for *P. malariae* and *P. ovale* were 3.7% (95% CI: 3.3-4.2) and 1.1% (95% CI: 0.9-1.4), respectively. Seroprevalences were higher at lower altitudes (<2000m) compared to higher altitudes (2000-2500m); OR 2.2 (95% CI: 1.8-2.6) and 1.3 (95% CI: 1.1-1.6) for *P. falciparum* and *P. vivax*, respectively. Among Regions, the *P. falciparum* seroprevalences ranged from 41.4% (95% CI: 36.3-46.8) in Benishangul-Gumuz to 3.6% (95% CI: 0.9-13.3) in Harari Region. For *P. vivax,* the seroprevalence ranged from 37.0% (95% CI: 33.7-40.3) in Amhara to 3.1% (95% CI: 2.0-4.6) in Somali Region. Models fitted to measure transmission patterns show variability by regions, antigen type and within species. Using serology, this study explored the cumulative malaria burden in Ethiopia and the use of multiplex assay on multiple antigens in a low transmission setting as a more sensitive biomarker using samples collected in a national survey.

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EVALUATING THE IMPACT OF ANTHELMINTIC-BASED INTERVENTION STRATEGIES FOR CONTROLLING *LOA LOA*: A MATHEMATICAL MODELLING STUDY USING EPILOA

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Although traditionally considered a benign condition, increasing evidence suggests that loiasis (caused by the filarial nematode *Loa loa*) poses a significant public health burden to the c.10 million infected individuals across Central Africa. Recent results have demonstrated a significant impact of high microfilaraemia on mortality, as well as an association between infection and severe cardiac, renal and neurological conditions, in addition to the more common "eyeworm passage" and "Calabar swellings". Mathematical models provide useful tools with which to understand and quantify the population biology and transmission dynamics of filariases, providing a rigorous framework to evaluate and compare different control strategies. We have previously presented a review of *Loa loa* biology and epidemiological dynamics, and here we build on this foundation to develop and parameterise a (deterministic) model of loiasis transmission and control (EPILOA). EPILOA was fitted to infection intensity (microfilariae/ml) data from Cameroon to estimate how exposure to infection varies with host age and sex. After capturing the demographic profiles of infection observed, EPILOA was used to explore the impact of strategies aimed at controlling *Loa loa*, centred around the delivery of anthelmintics. This model provides, for the first time, a means to inform the design and evaluation of interventions directly targeting this neglected but important disease (rather than as a collateral benefit of treating onchocerciasis and/or lymphatic filariasis). This work highlights the significant impact that programmes targeting loiasis directly could have on improving the health of millions across Central Africa, indicates operational research gaps (such as the potential impact of vector control), and identifies the pressing need for a better understanding of key aspects of *Loa loa* lifecycle and population biology.

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COMMUNITY TEST AND NOT TREAT (TANT), A COST-EFFECTIVE STRATEGY FOR THE ELIMINATION OF ONCHOCERCIASIS IN CENTRAL AFRICA

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Thirty-one years after the discovery of the effect of ivermectin on onchocerciasis, this drug has substantially reverted the severity of onchocerciasis in endemic countries. The success of onchocerciasis control, especially in African countries, is the result of the Community Directed Treatment with Ivermectin (CDTI). With this success, the paradigm has shifted from the elimination as a public health concern to complete elimination of this blinding disease. Therefore, mass treatment previously indicated for meso- and hyper-endemic communities should now be also distributed in hypo-endemic communities. Despite the success of CDTI, the extension of treatment in hypo-endemic areas remains a great challenge in Central Africa where loiasis is endemic and where mass ivermectin distributions were proscribed by fear of the occurrence of Severe Adverse Events (SAEs). Indeed, subjects heavily infected with *Loa loa* can develop severe encephalopathies after treatment with ivermectin. To overcome this difficulty, a new strategy (TaNT) was developed to help preventing SAEs. To reduce the costs of this intervention and to render this strategy operational, a community TaNT was developed, based on the CDTI model, with a duo of a blood drawer and a LoaScopist to perform the test prior treatment by Community Drug Distributors (CDDs) in each community. This strategy was implemented in the Soa Health District in Cameroon, known to be hypoendemic for onchocerciasis and endemic for loiasis. Adverse Events (AEs) surveillance was done by the research team. A total of 36,070 subjects were tested and 27,596 treated by the community team. Treatment coverage was 45.9%, ranging between 34.4% and 78.5%. A total of 226 (0.6%) were excluded from ivermectin treatment because of high Loa mf loads. During the campaign, 189 (1.3%) complained of AEs. This study reveals that after only two days training, the community team has been able to conduct the TaNT strategy, and importantly no SAE case was recorded. This strategy appears as a viable alternative strategy to accelerate onchocerciasis elimination wherever it is endemic.

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ASSESSING HYPOENDEMIC ONCHOCERCIASIS IN *LOA LOA* ENDEMIC AREAS OF SOUTHEAST NIGERIA

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Nigeria has a new national plan for onchocerciasis that moved its objective from control to transmission elimination. Under the control program, ivermectin mass drug administration (MDA) focused on hyper/meso-endemic local government areas (LGAs) designated by Rapid Epidemiological Mapping of Onchocerciasis (REMO) to have ≥20% nodule rates. The new elimination paradigm requires MDA be expanded into hypoendemic LGAs; WHO has suggested MDA where OV-16 antibody rates in adults are ≥2%. Southern Nigeria is coendemic for *Loa loa* and onchocerciasis; if a person has high density *L. loa* microfilaremia (HDLLMF), treatment with ivermectin can cause central nervous system adverse events (CNS-AEs). The country is faced with the challenge of determining 1) if there is onchocerciasis transmission in the untreated *Loa loa* endemic LGAs, and 2) if so, whether MDA can be given there with low risk due to absence of HDLLMF. Our study took place in 2016, in 19 LGAs likely to have onchocerciasis due to their proximity to hyper- or mesoendemic (MDA treated) LGAs. In 110 villages in these LGAs, 50 adults and 50 children (aged 5-10) were tested for HDLLMF by LoaScope and onchocerciasis antibody using an OV-16 rapid diagnostic test (RDT). In a subset of the sample, a blood spot was also taken for confirmatory OV-16 ELISA. The mean prevalence of RDT positives was 0.5% in the 5,401 children sampled (village range 0.0 - 6.0%) versus 3.2% in the 5,355 adults sampled (village range 0.0 - 58.0%). Five LGAs had no RDT positives. Preliminary blood spot results (600 analyzed so far out of 2,200) indicate 96.8% concordance between the OV-16 RDT and ELISA. Combining these results with a previously reported LoaScope assessment showing no HDLLMF, we analyzed our data by proposing MDA at three possible OV-16 thresholds: ≥1% in children, ≥1% of adults (as proposed by WHO), or ≥2% of adults. Each of these yields a different treatment subset of the LGAs tested: 4 (21%), 9 (47%) or 3 (16%) of the 19 LGAs, respectively. If children and adults (≥1%) are both used, 10 LGAs (53%) should be treated.

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ENVIRONMENTAL FACTORS ASSOCIATED WITH LOIASIS HOTSPOTS IN CAMEROON

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Loiasis is a vector-borne filarial nematode caused by *Loa loa* and transmitted by *Chrysops* vectors, which are confined to the tropical forests of Central and West Africa. Loiasis is a major impediment to lymphatic filariasis and onchocerciasis elimination programmes due to severe adverse events associated with ivermectin mass drug administration. There is an urgent need to better refine and map high-risk loiasis communities to enable the deployment of new diagnostics and alternative treatment strategies. Understanding the ecology of vectors and factors driving high transmission may help to identify loiasis hotspots. The availability of satellite and remote sensed data provided an opportunity to examine environmental factors associated with *L. loa* prevalence (%) and intensity (mf/ml) in Cameroon. Georeferenced data from 42 villages across five zones within two distinct bio-ecological regions were examined; Equatorial Rainforest in South-West region (deciduous equatorial rainforest; dense-humid equatorial rainforest) and the Savannah in North-West region (grassland savannah, mosaic forest savannah; forested savannah). Using qGIS software, annual precipitation, annual mean temperature, elevation, tree cover and canopy height were extracted from raster maps using a 3km village buffer. Overall temperature (r=0.51 %; 0.41 mf/ml) and tree cover (r=0.48 %; 0.41 mf/ml) were significantly positively correlated and elevation (r=-0.51 %; -0.41 mf/ml) negatively correlated with both *L. loa* prevalence and intensity. Within the Savannah region, significant differences between the three zones 20-50km apart were found, and a linear regression model indicated tree cover and elevation were important predictors explaining 75.9% of the variance in the model for *L. loa* prevalence, and tree cover explaining 57.1% for *L. loa* intensity in this region. Similar results were found between villages 2-5km apart within the high-risk forested zone in this Savannah region. The study highlights that

environmental analysis can help delineate risk at different geographical scales, which may be practical for developing larger scale operational plans.

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VALIDATION OF THE LOA ANTIBODY RAPID TEST (LART), A NOVEL RAPID DIAGNOSTIC TEST (RDT) TO REFINE LOIASIS MAPPING

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The elimination of onchocerciasis using ivermectin preventive chemotherapy is contraindicated in areas of Loa co-endemicity due to the risk of severe adverse events. In onchocerciasis hypo-endemic areas, the only alternative is a Test-and-Not-Treat (TaNT) strategy, where each individual is tested for *Loa* microfilaremia to assess their eligibility for ivermectin. This strategy, though successfully implemented in Cameroon with the LoaScope, is expensive and hardly scalable. High resolution maps of *Loa* are needed to minimize the areas where the TaNT approach must be deployed. The Loa Antibody Rapid Test (LART) is a novel lateral flow assay that detects a serological response to Ll-SXP-1, a validated marker of *L. loa*. The present study aimed at validating the performance of this new RDT for *Loa loa* infections using field data. A total of 924 dried blood spots (DBS) were collected in nine Cameroonian ivermectin naïve communities with different levels of *L. loa* endemicity. LoaScope data was available for all individuals and used as gold standard. The DBS were eluted and analyzed with the LART. The intensity of the test lines was visually quantified, by comparing the test line with a printed score card. Based on the Youden index, the optimal cut-off was set at 6, and the resulting sensitivity and specificity were 79.6% (72.2 - 87.1) and 76.3% (73.4 - 79.2), respectively. A strong correlation was observed between seroprevalence and mf prevalence (OR: 12.54; 95%CI: 7.62 - 21.40; $p<0.0001$), with the seroprevalence being approximately 4 times higher than the mf prevalence. Overall, 39% of the people who were amicrofilaremic responded to the LART. The data suggests that the Loa Antibody Rapid Test can be used to draw high resolution maps of *L. loa* infection and set a seroprevalence threshold above which the communities should be excluded from MDA and adopt the TaNT strategy.

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IMPACT OF REPEATED ANNUAL IVERMECTIN MASS DRUG ADMINISTRATION ON LOIASIS PARASITOLOGICAL INDICATORS IN CAMEROON: IMPLICATIONS FOR ONCHOCERCIASIS AND LYMPHATIC FILARIASIS ELIMINATION IN AREAS CO-ENDEMIC WITH *LOA LOA* IN AFRICA

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Loiasis is a filarial infection endemic in the rainforest zone of west and central Africa particularly in Cameroon, Gabon, Republic of Congo, and Democratic Republic of the Congo. Repeated treatments with ivermectin have been delivered using the annual community directed treatment with ivermectin (CDTI) approach for several years to control onchocerciasis in some *Loa loa-Onchocerca volvulus* co-endemic areas. The impact of CDTI on loiasis parasitological indicators is not known. We, therefore, designed this study to explore the effects of several rounds of CDTI on parasitological indicators of loiasis. The study was conducted in the East, Northwest and Southwest 2 CDTI projects of Cameroon. Individuals who consented to participate were interviewed for ivermectin treatment history and enrolled for parasitological screening using thick smears. Ivermectin treatment history was correlated with loiasis prevalence/intensity. A total of 3,684 individuals were recruited from 36 communities of the 3 CDTI projects and 900 individuals from 9 villages in a non-CDTI district. In the East, loiasis prevalence was 29.3% (range = 24.2%–34.6%) in the non-CDTI district but 16.0% (3.3%–26.6%) in the CDTI district with 10 ivermectin rounds (there were no baseline data for the latter). In the Northwest and Southwest 2 districts, reductions from 30.5% to 17.9% (after 9 ivermectin rounds) but from 8.1% to 7.8% (not significantly different after 14 rounds) were registered post CDTI, respectively. Similar trends in infection intensity were observed in all sites. There was a negative relationship between adherence to ivermectin treatment and prevalence/intensity of infection in all sites. None of the children (aged 10–14 years) examined in the East CDTI project harboured high (8,000–30,000 mf/ml) or very high (>30,000 mf/ml) microfilarial loads. In areas where onchocerciasis and loiasis are co-endemic, CDTI reduces the number of, and microfilaraemia in *L. loa*-infected individuals, and this, in turn, will help to prevent non-neurological and neurological complications post-ivermectin treatment among CDTI adherents.

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INVESTIGATION OF RISK FACTORS FOR TRANSMISSION OF GUINEA WORM DISEASE IN DOGS— CHAD, 2018

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Guinea worm (GW) disease, slated for global eradication, is historically a human disease spread by drinking stagnant water containing microscopic crustaceans infected with larvae of the roundworm *Dracunculus medinensis*, causing clusters of case-patients with worms emerging from the skin. However, dog infections, reported in Chad since 2012, have surpassed the few sporadic human cases reported annually, with 1011 infected dogs in 2016 and 817 in 2017. Chad dog GW infections peak each mass fishing season; lab and field studies have found *D. medinensis* larvae in frog tissues and fish intestines, suggesting a possible paratenic (transport) host. We conducted a case-control study to identify risk factors for GW transmission in dogs and recommend interventions to prevent infections. We used a standardized questionnaire to interview dog owners and their families about their dogs' behaviors, water and food consumption habits. Case-dogs had emerged worms in 2017 confirmed by Chad Guinea Worm Eradication Program (CGWEP) supervisors. Each case-dog was matched to 1–2 controls by age, sex, and residency; controls came from neighboring households without a history of GW disease in animals or humans. We performed univariable conditional logistic regression to calculate odds ratios (OR). We enrolled 73 case-dogs and 143 controls. Consumption of fish entrails was associated with GW disease (OR 2.4; 95% confidence interval [CI]: 1.1–5.2]. There was no association with frog consumption. Water accessible to dogs that was provided to other household animals was protective for high-worm burden dogs (>4 worms) compared to controls (OR 0.2; 95% CI: 0.04–0.9). This is the first epidemiologic study to link lab and field observations of a GW-infected paratenic/transport host with dog feeding habits. CGWEP should emphasize methods for safe disposal of fish entrails while research is ongoing to find ways to safely use this food source for dogs. Further

research is needed to understand the reason for the protective effect of water and capitalize on its underlying influence. However, CGWEP could consider adding potable water provision for dogs to its health messaging.

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POPULATION GENETICS OF SCHISTOSOME PARASITES IN SCHOOL AGED CHILDREN BEFORE AND AFTER PRAZIQUANTEL TREATMENT

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Despite over 14 years of mass drug administration (MDA), there are still schistosomiasis hotspots in Uganda. Here, we describe longitudinal surveys of school-aged children (SAC) to understand effects of praziquantel on parasite genetic diversity and population structure. We recruited SAC (6 – 14 yrs) across three schools on the shores of Lake Victoria in Mayuge District, Uganda from 2003 - 2018. We collected parasite infection intensity and genetic data up to 22 times during the study period. High prevalence (>70%) with mean moderate *S. mansoni* infection intensities were observed at all 'pre-praziquantel' treatment timepoints. We utilized up to 18 microsatellites to examine genetic diversity of parasites between individuals and schools before and after praziquantel treatment. There is a significant reduction in diversity with treatment within individual children but this is not reflected at the intra- and inter- school genetic diversity levels. Some of the parasites collected four weeks after praziquantel treatment cluster together despite being collected in different years, suggesting these parasites have survived repeated treatments. However, high rates of gene flow, coupled with high reinfection rates, rapidly dilutes these genotypes within a few months after treatment. Genetic diversity of parasites increased with host age and was higher in males, with a significant interaction between host age and sex. The majority of genetic diversity was observed within a host, with low genetic differentiation between schools despite large distances between villages. Genetic structure and estimates of the effective population size suggest that the thousands of treatments that have been administered in Mayuge District since 2003 have had minimal effects on populations of this diverse parasite. The findings emphasize a critical need for concerted drug treatment combined with wide uptake of other interventions along the shores of Lake Victoria to have long term impacts on transmission.

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OÙ SONT LES ESCARGOTS - WHERE ARE THE SNAILS? USING REMOTE SENSING METHODS TO COMBAT SCHISTOSOMIASIS IN NORTHERN SENEGAL

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Schistosomiasis, a neglected tropical diseases caused by the *Schistosoma* parasite, affects more than 240 million people a year. Ever since the construction of the Diama dam across the Senegal River in 1986, schistosomiasis has tormented villages along the Senegal River and its tributaries. The World Health Organization estimates that mass drug administration of praziquantel to treat schistosomiasis reaches less than 10% of the at-risk Senegalese population. Our objective was to assess a novel method of identifying high-density snail habitats in schistosomiasis endemic areas through remote sensing. Firstly, we identified environmental parameters significantly associated with the presence of the snail vectors. Data were collected from 36 water access points in 15 villages between June-August 2017. The number and species of snails and types of vegetation were recorded for each sweep of the net, with data analyzed through logistic regression models to determine whether the presence of certain vegetation was significantly associated with the presence of snails. *Ceratophyllum* was the most significant predictor of snail presence and abundance among lake and river water access sites. This held true across the two different species of snail vectors, *Bulinus* and *Biomphalaria spp*. Secondly, World View 2 images of northern Senegal were analyzed to identify areas containing signature characteristics of *Ceratophyllum* and other environmental parameters significantly associated with snail presence at a subset of sampled water access points. These spectral signatures were then used to predict the presence or absence of snail vectors among all sampled water access points. The third phase of this research is validating this model in previously unsampled sites in northern Senegal. Preliminary results suggest that a signature extracted from remote sensing images may be used to predict the location and abundance of snail vectors with accuracy. Once honed, this method should assist in predicting communities at risk for schistosomiasis in northern Senegal, and perhaps beyond, where ground-based epidemiological surveys are difficult to implement.

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ANTHELMITHIC TREATMENT UPTAKE AND PREDICTORS IN LAKE VICTORIA FISHING COMMUNITIES, UGANDA: INTERVENTION COVERAGE RESULTS FROM THE LAVIISWA CLUSTER RANDOMIZED TRIAL

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Mass drug administration (MDA) is a cornerstone of the strategy for control of helminths. In schistosomiasis-endemic areas with >50% of school-aged children infected, community-wide MDA with praziquantel is recommended by the World Health Organisation (WHO), with target coverage of >75%. In the Koome islands of Lake Victoria, Uganda, where baseline schistosomiasis prevalence (Kato-Katz) was 52% overall and 67% among school-aged children, we conducted a cluster-randomised trial of community-wide intensive MDA (quarterly single-dose praziquantel 40mg/kg; albendazole 400mg for three days) versus standard, Uganda government intervention (annual praziquantel 40mg/kg; 6-monthly single-dose albendazole). Twenty-six fishing villages were randomised, 13 per trial arm, for four years. At each treatment round, registers of village residents were updated and treatment uptake recorded. During the four year MDA period, 53,930 people were registered in the study area (25,055 and 28,875 in standard and intensive intervention villages, respectively). The age distribution of village residents was bimodal with 16% under-five and 44% 20-35 years; 53% were male. Praziquantel uptake was lower than albendazole uptake (60% versus 65%), more so in the standard arm (praziquantel 58%, albendazole 70% versus intensive arm: praziquantel 61%, albendazole 62%). In the standard arm, albendazole uptake was lower in the treatment rounds at which praziquantel treatment was given. Absence was by far the most common reason for non-treatment (81% albendazole, 78% praziquantel), with refusal the next most common (14% albendazole, 16% praziquantel). Uptake for both treatments was lowest among school-aged children (mainly due to absence at day school), but did not differ by sex. Refusal was more likely with the older age, more common among females. Persistent refusal (all treatment) was seen for 8% and 6% residents for praziquantel and albendazole, respectively. In

schistosomiasis transmission "hot spots", a combination of community-wide and school-based treatment strategies and educational interventions may be required to achieve WHO target coverage.

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RESEARCH CHALLENGES AND NEEDS FOR CONTROL AND ELIMINATION OF SCHISTOSOMIASIS: NEW DIAGNOSTICS

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Morbidity reduction and eventual elimination through integrated intervention approaches are the focal points of current schistosomiasis control. Precise diagnosis of schistosome infections will be critical in achieving these goals, particularly in areas such as China where extensive control has reduced schistosomiasis to very low levels to the point where elimination is on the horizon. Field surveillance and point of care testing will require sensitive, specific, inexpensive, field applicable diagnostic assays to determine worm burdens and to distinguish active infection and successful cure in high and low prevalence areas and for assessment of treatment failures. This paper will describe three new diagnostic tools for *Schistosoma japonicum* we developed and tested: • A real-time polymerase chain reaction (qPCR)-based (for humans and animals) assay which can be used as a future field diagnostic and surveillance tool in low-transmission zones where schistosomiasis elimination is targeted and for monitoring post-intervention areas to verify that elimination has been maintained. • A novel droplet digital PCR assay capable of identifying pre-patent and patent infections using *S. japonicum* DNA isolated from serum, urine, salivary glands, and feces in a murine model, and validated using clinical samples collected from subjects resident in an area moderately endemic for schistosomiasis in the Philippines. This verified method represents a valuable tool for the diagnosis and surveillance of schistosomiasis, particularly in low-prevalence and low-intensity areas approaching elimination and in monitoring where disease emergence or re-emergence is a concern. • An IgG-based ELISA using a combination of two recombinant *S. japonicum* antigens (SjSAP4 + Sj23-LHD (large hydrophilic domain)) which, on large scale testing with human serum samples from the Philippines provided a high diagnostic outcome with 87% sensitivity and 97% specificity. This combination could be used in future for serological diagnosis of schistosomiasis, thereby representing an important component for monitoring integrated control measures.

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PRECISION MAPPING IS THE WAY FORWARD TO SHRINK THE MAP AND ACCELERATE THE ELIMINATION OF SCHISTOSOMIASIS

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The major intervention for schistosomiasis control is mass administration of praziquantel to those shown to be, or presumed to be, at-risk of infection, on the basis of disease mapping. However, the current conventional mapping design for schistosomiasis has shown several limitations and may lead to several uncertainties and misclassification of some districts and their eligibility for preventive chemotherapy. Considering the high focality of schistosomiasis transmission, these mapping inaccuracies may result in over-treatment in some areas, and most importantly under-treatment or no treatment in areas that need it most. Thus preventing successful treatment coverage of all populations who need treatment, and jeopardizing the achievement of schistosomiasis elimination. To assess the role a more precise mapping could play in the stage moving towards elimination of schistosomiasis, we conducted studies in several health districts in Cameroon, using a new and innovative approach based on an exhaustive sampling of all schools within these districts. Over 300 schools and 15,000 school children were sampled. The results showed significant variations in schistosomiasis prevalence within health districts and sub-districts. Furthermore, a comparison of maps obtained using the conventional mapping method with those from precision mapping showed significant differences. The precision mapping of schistosomiasis gave high resolution information at the local level, and clearly provided detailed information on high risk zones and locations where intensified interventions should be focused primarily to obtain higher impact. By increasing the map granularity and spatial resolution, the precision mapping provides the best evidence based data to guide intensified interventions in targeted transmission zones, and allows a better and rational utilization of the donated praziquantel and available resources. We therefore believe that it is a promising and innovative tool to shrinking the map of schistosomiasis and accelerating the move towards the elimination of schistosomiasis in sub-Saharan Africa.

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SCHISTOSOMIASIS MONITORING AND EVALUATION PROGRAMS: THE IMPORTANCE OF COLLECTING ADULT DATA TO INFORM TREATMENT STRATEGIES FOR *SCHISTOSOMA MANSONI*

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Schistosomiasis remains an endemic parasitic disease affecting millions of people around the world. The World Health Organization (WHO) has set goals for controlling schistosomiasis morbidity by 2020, along with its elimination as a public health problem in certain regions by 2025 (defined by reaching ≤5% and ≤1% prevalence of heavy-intensity infections in school-aged children, respectively). Monitoring and evaluation (M&E) programmes are used to collect data which can inform treatment strategies required in a defined area and can also aid in assessing the progress of treatment strategies. Due to programmatic and financial constraints, data are typically collected from school-aged children (SAC; 5-14 years of age) as they are thought to be most likely to be infected. Using a mathematical model, we incorporate three different age-intensity profiles of infection for *Schistosoma mansoni* with low, moderate and high burdens of infection in adults to investigate how the age distribution of infection impacts the preventive chemotherapy coverage levels required to achieve the WHO goals. We find that for low to moderate prevalence regions, regardless of the burden of infection in adults, treating SAC only may achieve the WHO goals. However, for high prevalence regions with a high burden of infection in adults, adult treatment is required to meet the WHO goals with coverage levels varying with the burden of infection in adults. This highlights that prevalence and intensity of infection data in adults needs to be included within M&E programmes. Collecting data from a broader age-range, specifically the inclusion of adult data at baseline (prior to treatment) and throughout the treatment programme if possible, as well as SAC data will allow for more accurate determination of the optimal treatment strategy for a defined region.

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COUPLING DYNAMIC ENERGY BUDGET (DEB) THEORY WITH A MESOCOSM EXPERIMENT TO PREDICT AND VALIDATE THE EFFECTS OF TEMPERATURE ON A HOST-PARASITE SYSTEM

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Schistosomiasis, caused by trematodes of the family *Schistosoma*, is a widespread human parasitic disease that might increase in prevalence with global climate change. However, the net effects of temperature on host-parasite systems have been historically difficult to model because parasites and their hosts often have different optimal temperatures across life history traits (e.g. hatching, infectivity, growth, reproduction). Dynamic energy budget (DEB) theory addresses this hurdle by utilizing metabolic principles, which are temperature-dependent, to predict the trajectory of individual life history traits. A DEB-based model for *Schistosoma mansoni* and its intermediate snail host, *Biomphalaria glabrata*, was used in this study to predict parasite cercarial shedding rates and snail growth and reproduction across a temperature gradient (18, 21, 25, 29, 33, and 37°C). Additionally, 16-L mesocosms (n = 4 per treatment) were inoculated with 1-L of local pond water containing zooplankton and algae to mimic a freshwater environment. After algae established, five snails between 3-12 mm were added to each mesocosm. *S. mansoni* miracidia were added in four doses during the first 10 weeks of the experiment. Mortality was monitored daily and DEB predictions were compared to measurements of snail life history traits, sampled weekly for 4 months. Optimal snail growth occurred between 25-33°C, reproduction between 21-29°C, and cercarial shedding between 25-29°C, which aligned with DEB predictions and temperatures where schistosomiasis is endemic. These results validate DEB theory as a tool for predicting the effects of global climate change on the disease dynamics of schistosomiasis and perhaps similar host-parasite systems.

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EVIDENCE OF PERENNIAL MALARIA TRANSMISSION UNDER ARID CONDITIONS AND DRY SEASON REFUGIA FOR ANOPHELINE LARVAE: A CASE STUDY AT KANDI IN NORTHEASTERN BENIN, WEST AFRICA

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In arid settings, droughts usually lead to periods of very low or no malaria transmission. However, in rural Kandi (Sonsoro) in northeastern Benin, several malaria cases are often diagnosed during dry seasons. The underlying factors accounting for this phenomenon remain unknown. For our investigations, the entomo-parasitological profile of the location (Sonsoro) was compared to a site in urban Kandi (Gansosso), meticulously focusing on what happens in the dry season. A GIS approach was used to access in 1-year period the spatial and seasonal distribution of mosquito larval habitats and identify their drought-refugia. Conjointly, adult *Anopheles* vector collections were monthly performed using Human Landing Catches (HLC). Elisa assays for *P. falciparum* circumsporozoite protein were conducted on vector specimens and the entomological inoculation rates (EIR) were determined per season. To provide a global view of drought-malaria prevalence, Rapid Diagnostic Tests (RDTs) were conducted in children < 10 years in both sites. Overall 187 mosquito larval habitats were identified of which 56 were recorded during the dry season (73% in rural site against 27% in the urban). The drought-refugia for mosquito breeding were all of domestic nature mainly canaries, jars, and flower pots. 100% of drought larval habitats identified in rural Kandi contained anopheline larvae against only 20% in urban Kandi. HLC provided 966 mosquitoes belonging to 12 species and *Anopheles gambiae* is the main species sampled (69 %). It represented respectively 94% (628/668) and 13 % (39/298) of the collections in rural and urban Kandi. From wet to dry season, we observed a drastic 96% reduction of EIR in rural Kandi (23 infected bites/man/month to 1 infected/man/month). Same trend was observed in urban Kandi (1 infected bite/man/month to 0 with the drought). RTDs data on 400 children showed that *P. falciparum* infections were significantly higher in rural Kandi (41%) than in urban (7.5%). Our results suggest that a suitable domestic management of anopheline larval habitats in droughts would provide a huge impact on the dynamics of mosquito populations and malaria transmission.

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FROM INSIGHT TO INNOVATION: HOW VIDEO-TRACKED *ANOPHELES GAMBIAE* BEHAVIOR LED TO THE 'BARRIER BEDNET' TARGETING INSECTICIDE RESISTANT VECTORS

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Designing the next generation of long-lasting insecticidal bed nets (LLINs) is a primary goal in malaria vector control today, as the emergence of pyrethroid-resistant vector populations across Africa threatens their future. We approached this challenge by initiating a series of studies on the basic behavior of *Anopheles gambiae s.l.* at human-occupied LLINs, using a state-of-the-art video-tracking system. Initial results provided extraordinary insight into the behavior of *An. gambiae s.l.* at a human-occupied LLIN. Video analyses revealed previously unseen spatial characteristics of mosquito activity that had potential as novel targets. A key feature of host-seeking behavior was that the vast majority of activity occurred above the roof of the human-occupied bednet (*i.e.* outside the net, where contact with the human sleeper's skin would not occur and above the reach of children at all times). Conceivably, a range of insecticides might be delivered via an added 'net barrier' fitted above the net roof and on which mosquitoes might land or collide. The results of a series of completed studies investigating the barrier bednet performance will be presented. Results of video-track recordings of pyrethroid-resistant *An. gambiae* at barrier bednets treated with combinations of pyrethroid, organophosphate and other treatments will be presented and the nature of mosquito-net interaction interpreted. We also investigated the effectiveness of barrier designs and treatments using a novel *in silico* computer model (InVeCTS). Finally, the results of a hut trial carried out in Burkina Faso, targeting a wild pyrethroid-resistant population of *An. gambiae s.l.* using a number of barrier bednet treatments will be presented, during which one barrier design significantly outperformed other LLINs as measured by personal protection (P=<0.01), immediate (P=<0.001) and delayed (P=<0.001) vector mortalities. We present the barrier bednet as a simple affordable method of restoring LLIN effectiveness in areas with high levels of insecticide resistance and consider its potential for malaria prevention in an insecticide resistant Africa.

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IDENTIFICATION OF CANDIDATE GENES UNDERLYING HUMAN HOST PREFERENCE IN THE MALARIA MOSQUITO *ANOPHELES COLUZZII*

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The malaria mosquito *Anopheles coluzzii* has a strong preference for human hosts, which contributes greatly to its vectorial capacity for malaria. We combined several approaches to study the genetic basis of this important trait. Previously we made use of the fact that *An. coluzzii's* sibling species. *An. quadriannulatus* is largely zoophilic,

by conducting a QTL mapping analysis on backcross between the two species. This identified six autosomal QTL containing numerous olfactory genes. Additionally, we compared gene expression between the two species in the two major olfactory organs, the antennae and maxillary palps, to identify olfactory genes with species- and sex-biased expression. Furthermore, we analyzed sequence data for olfactory receptors, ionotropic receptors and odorant binding proteins obtained from field collected specimens representing six of the species in the *An. gambiae* complex, and identified genes under positive selection in the anthropophilic *An. coluzzii* and *An. gambiae ss.* Taken together, these genetic mapping, expression and selection analyses identify a set of candidate olfactory genes for human host preference in this important malaria vector.

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NEW INSIGHTS INTO *ANOPHELES* MATING BEHAVIOR: BOTH MALES AND FEMALES OF *ANOPHELES COLUZZII* AND *ANOPHELES GAMBIAE* USE VISUAL MARKERS TO SWARM … BUT EACH IN ITS OWN WAY

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Effective malaria vector control strategies require a good understanding of mosquito behaviors. However, in *Anopheles* species, mating behavior has been deeply overlooked. Nowadays, it is known that males of *Anopheles gambiae s.l.* form swarms at dusk in which virgin females come to mate. While *An. gambiae* has been described to swarm over bare ground, *An. coluzzii* swarms over dark markers. However, both the exact way males use these visual cues and whether females also use these cues to join species specific swarms are unknown. In laboratory, we first, tested whether males of the two species use a marker to locate the swarm. The marker was moved during swarming and the relative position of the swarm was recorded. Second, we investigated the male swarm characteristics (height, width and number of mosquitoes) as a function of the presence/absence of a marker and the marker size (20x20cm *vs*. 60x60cm). Finally, we repeated these experiments with virgin females to observe their behavior towards ground visual markers. We observed that *An. coluzzii* males swarmed only in presence of a marker and always right above it. With the large marker, swarms were higher, wider and had more mosquitoes (*ca.* x2) than with the small one. In *An. gambiae*, males swarmed next to the marker and were also able to form a swarm without a marker. However, only the number of swarming mosquitoes and swarm width increased in presence and with larger markers (*ca.* x1.3). The two species instantly followed the marker movements with *An. coluzzii* being over the marker and *An. gambiae* keeping a constant distance of ≈75cm to the marker. The females of the two species exhibited a swarm-like behavior very similar to those of the males. Our findings show that both *An. gambiae* and *An. coluzzii* males use a ground marker to swarm but in a different way and their females also use this visual cue like their specific-specie males to exhibit swarm-like behaviors. It suggests that ground visual markers could be a key cue in specific swarm recognition and location which is highly important for assortative mating in these sibling species. These results raise new avenues to explain diversification within the *An. gambiae* complex.

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TIMING IS EVERYTHING: *AEDES AEGYPTI* REPRODUCTIVE PHYSIOLOGY EXPLAINS BEHAVIOR

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Before a female *Aedes aegypti* mosquito reproduces, several events must occur. She must mate, store sperm, blood feed, produce eggs, and finally fertilize eggs as she lays them. Such processes independently are well known to mosquito biologists, but confusion remains about the order of these events and their physiological pre-requisites. We sought to identify the timing and order in which important reproductive events occur. Laboratory-adapted *Aedes aegypti* females blood feed and produce some eggs even when their virginity is enforced, and we used these gravid virgins to test how soon after mating females were able to fertilize their eggs. We demonstrate that gravid females, when forced to lay eggs via a death stressor, gradually gain the capacity to fertilize eggs over 16 h after mating. Readiness to fertilize eggs coincides with drastic changes in sperm activity and morphology, suggesting that sperm processing may be necessary for fertilization. When oviposition was not forced, females naturally began laying eggs 16 h after mating, suggesting that oviposition behavior and fertilization competence are tightly coordinated. Whether wild females produce eggs before mating remains unclear; if this were the case, females would experience a three day delay between mating and fertilization. Given that sperm and female behavior are prepared for fertilization in just 16 h, we tested whether wild virgins sometimes produce eggs. Field-collected females from Medellin, Colombia, included a small proportion of gravid virgins, suggesting that mating may not always be necessary for oogenesis. Thus, females are not physiologically prepared to fertilize eggs until 16 h after mating, and this delay may be mediated by sperm. Oviposition behavior begins at the same time that females become fertilization competent. Finally, preparing to fertilize eggs in 16 h may benefit females that blood feed and produce eggs prior to mating. Understanding reproductive events and their timing in this important disease vector will contribute to a deeper understanding of mosquito behavior, ecology, and the cellular and molecular mechanisms that mediate reproduction.

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TEMPERATURE DRIVES ZIKA VIRUS TRANSMISSION: EVIDENCE FROM EMPIRICAL AND MATHEMATICAL MODELS

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Temperature is a strong driver of vector-borne disease transmission. Yet, for emerging arboviruses we lack fundamental knowledge on the relationship between transmission and temperature. Current models rely on the untested assumption that Zika virus responds similarly to dengue virus, potentially limiting our ability to accurately predict the spread of Zika. We conducted experiments to estimate the thermal performance of Zika virus (ZIKV) in field-derived *Aedes aegypti* across eight constant temperatures. We observed strong, unimodal effects of temperature on vector competence, extrinsic incubation period, and mosquito survival. We used thermal responses of these traits to update an existing temperature-dependent model to infer temperature effects on ZIKV transmission. ZIKV transmission was optimized at 29°C, and had a thermal range of 22.7°C-34.7°C. Thus, as temperatures move toward the predicted thermal optimum (29°C) due to climate change, urbanization, or seasonally, Zika could expand north and into longer seasons. In contrast, areas that are near the thermal optimum were predicted to experience a decrease in overall environmental suitability. We also demonstrate that the predicted thermal minimum for Zika transmission is 5°C warmer than that of dengue, and current global estimates on the environmental suitability for Zika are greatly over-predicting its possible range.

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HIGH-ACCURACY DETECTION OF MALARIA VECTOR HABITATS USING DRONE-BASED MULTISPECTRAL IMAGERY

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Interest in larval source management (LSM) as an adjunct intervention has recently increased mainly because LLINs and IRS are ineffective against malaria transmission by exophagic and exophilic mosquitoes. Knowledge of the characterization of the most productive, positive water bodies would provide a more cost-effective tool and increase the impact of targeted mosquito control on aquatic life stages. The present study explores the use of unmanned aerial vehicles (UAV) for identifying potential *Nyssorhynchus darlingi* (former *Anopheles darlingi*) breeding sites by mapping characteristics of water bodies in four localities in Mazan District, Amazonian Peru. There were two primary goals: 1) to evaluate the feasibility of high-resolution mapping (~0.02m/pixel) in Amazonian areas to detect inaccessible malaria vector breeding sites; and 2) to investigate the relationship between the multispectral profile and *Ny. darlingi* breeding sites. Imagery was acquired during April 2017 in four localities in the Mazan river basin. *Ny. darlingi* larvae collections were performed simultaneously and information about positive and negative breeding sites from two additional surveys was incorporated into the calculations. A supervised Random Forest classification was performed to detect whether or not water bodies harbor *Ny. darlingi* immature stages. Our results showed that multispectral imagery collected from a UAV could discriminate a profile of water bodies where *Ny. darlingi* was most likely to breed. We performed the analysis in Google Earth Engine (GEE) using three classification approaches. The overall accuracy ranged between 89.79% and 97.46% and statistical analysis showed clear differentiation of spectral bands of water bodies positive *vs* negative for *Ny. darlingi*. This work provides proof-of-concept of the use of high-resolution images to detect malaria vector breeding sites in the Peruvian Amazon, where the use of such an innovative methodology could be crucial to maximize resources for the incorporation of LSM into integrated interventions and a powerful additional tool for malaria elimination in the Amazon and elsewhere.

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ARTEMISININ RESISTANCE AND THE *PFK13* C580Y MUTATION IN GUYANA: A CONFIRMED LINK AND EMERGENCE

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The emergence and spread of artemisinin resistance in Southeast Asia has raised concerns about malaria control worldwide. Non-synonymous mutations in the propeller domain of the *pfK13* gene have been associated with artemisinin resistance in this Asian region. Recent evidence indicates suspected partial artemisinin resistance in the Guiana shield region of South America, a locality where historically, resistance emerge *de novo*. As a previous retrospective study indicated an independent emergence of *pfk13* C580Y mutation in Guyana, a follow up molecular surveillance was conducted to evaluate the presence and/or extent of artemisinin partial resistance in part of this the Guiana shield region. The *pfk13* gene was genotyped through PCR-based sequencing in 683 blood spots collected in Guyana in 2016-2017. Thirteen samples were found to contain the *pfK13* C580Y mutation, indicating a population frequency of 1.9% [0.8-2.8]. No other mutation was found in the propeller domain of this gene. The impact of this C580Y *pfK13* mutation on the phenotype to artemisinin derivatives in parasites exhibiting a South American genetic background was also evaluated by genome editing. The analysis confirmed that C580Y mutation confers a high level of artemisinin resistance *in vitro*. Therefore, this finding confirms circulating C580Y alleles in Guyana can lead to partial artemisinin resistance. The impact on therapeutic efficacy is being evaluated. During this presentation, the geographic extent of this

new focus of artemisinin partial resistance will be presented as well as the origins and spread of these C580Y mutants under drug pressure, based on whole genome sequencing data.

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MUTATIONS IN PFCORONIN CONFER RESISTANCE TO ARTEMISININ IN WEST AFRICAN *PLASMODIUM FALCIPARUM* ISOLATES

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Global public health efforts for control and elimination of malaria have made considerable progress since the success of the Artemisinin-based combination therapies, which are highly effective against *Plasmodium falciparum*. However, an emerging drug resistance to Artemisinin (ART) in Southeast Asia poses one of the greatest threats to eradicate malaria. If this resistance spreads to the African continent, which bears the highest global malaria burden, efforts to control malaria will be in serious jeopardy. With this in mind we chose two Senegalese field isolates to investigate ART resistance. These field isolates are susceptible to ART treatment and do not have mutations in the propeller domain of PfKelch13, a known marker of ART resistance. After intermittent and step-wise dihydroartemisinin (DHA) pulses started in 2011 and continued over four years, we obtained three independent ART resistant lines. All three parasite lines had a significant increase in their $^{0\text{-}3hr}$Ring Stage Survival Assay (RSA) survival percentage (6%, 7.9% and 9.6% respectively) compared to their parents ($^{0\text{-}3hr}$RSA<1%). They had no change in EC_{50} response to ART derivatives in a 72-hour dose-response assay. Whole genome sequencing of ART resistant lines identified thirteen different single nucleotide polymorphisms (SNPs) in ten genes. There were no mutations in the PfKelch13 gene. One gene, PfCoronin (PF3D7_1251200), which codes for a protein similar in structure to PfKelch13, had new SNPS in its WD-40 propeller domain of all three resistant lines. To test whether the PfCoronin SNPs (G50E, R100K, E107V) were sufficient to confer ART resistance, we generated CRISPR-Cas9 edited parasite lines containing PfCoronin SNPs in both 3D7 and the Senegalese parental backgrounds. Our preliminary results in one of the Senegalese parental backgrounds indicate PfCoronin SNPs are sufficient to confer ART resistance. This suggests that PfCoronin is a determinant of ART resistance in West African parasites. Further investigation of biological mechanism of ART resistance conferred by PfCoronin will make significant contributions to the current understanding of ART resistance.

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THE EMERGENCE OF MULTIDRUG RESISTANT MALARIA PARASITES IN SOUTHEAST ASIA AND IMPLICATIONS ON FUTURE MALARIA TREATMENT

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Thailand and Cambodia have utilized dihydroartemisinin-piperaquine and artesunate-mefloquine as first line treatment for *Plasmodium falciparum*, alternating between these ACTs due to PIP and MQ counteracting resistance mechanisms. The recent identification of 'triple mutant' malaria strain exhibiting molecular markers of resistance to all three components—the artemisinin, mefloquine and piperaquine—threatens the long-term sustainability of this approach and underscores the need for timely surveillance to characterize the rate and geographic extent of such "triple mutants". A total of 528 samples were collected from 2015 to 2017 from Cambodia patients infected with *P.falciparum* to assess temporal increase and geographical differences in parasites bearing copy number amplification in both *pfmdr1* (conferring MQ resistance) and *plasmepsin 2* (conferring PIP resistance) genes and *pf*Kelch13 mutations. We collected samples in Cambodia along the Thai border (military population) and northern and eastern Cambodia (civilians). Out of 218 samples collected in 2015 in civilians, 48 (22%) carried increased copy numbers of MQ and PIP markers of resistance, compared to only 1 (1%) collected in 2016 along Cambodia-Thai border in military. Eastern Cambodia had highest prevalence of doubly amplified parasites (52%). Amplification of *pfmdr1* and *plasmepsin2* was exclusively observed among *pf*Kelch13-580Y mutants suggesting amplification is occurring on a highly artemisinin-resistant genetic background. Analysis of *P.falciparum* malaria parasites for the prevalence of 'triple mutant' phenotype is ongoing for the remaining 316 samples collected in 2017. Origins of the identified triple mutant parasites based on loci flanking and in linkage disequilibrium with resistance genes will be determined. Based on these observations, new drug regimens will be required for treatment of multidrug resistant malaria in Southeast Asia. Triple drug combinations of already marketed drugs may be a solution for possible imminent malaria outbreaks with these multidrug resistant parasite subpopulations that may soon become untreatable by the first-line ACTs.

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IMPACT OF *CYP2C8*2* ON ARTESUNATE-AMODIAQUINE METABOLISM IN MALI

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The clinical role of genetic polymorphism in drug metabolizing enzyme such as the cytochrome P450 (CYP450) protein has been reported for many diseases including malaria. *CYP2C8*2*, a variant shown to reduce CYP2C8 enzymatic activity, was associated with severe side effects following amodiaquine (AQ) administration. *CYP2C8*2* is the most common mutation in Africa and the use of AQ in several antimalarial treatments (ACTs) and preventive strategies (Seasonal Malaria Chemoprevention strategies SMC) in many sub-Saharan Africa countries raise concern on the sustainable efficacy and safety of AQ. This study aimed to assess the impact of the *CYP2C8*2* allele on the Artesunate-Amodiaquine (ASAQ) metabolism and efficacy following uncomplicated malaria treatment with ASAQ. A retrospective pilot study was conducted on archived dried blood spot samples and sera from a malaria clinical trial study (WANECAM) conducted in Mali between October 2011 and December 2015. Samples from Bougoula-Hameau patients treated with ASAQ were collected. All patients were followed for two years. Human DNA was extracted using Qiagen kit and PCR-RFLP was used to identify the *CYP2C8*2* allele. Desethylamodiquine level was measured on day-7 sera by HPLC. We analyzed 159 samples out of 213. Of the

159 samples selected for this study, 76.1 %, 7.5 % and 16.4 % of the patients were carrying the wild type (*Wt/Wt*), mutants (**2/*2*), and both wild and mutant (*Wt/*2*) alleles, respectively. Desethylamodiaquine day-7 median concentration was 75.2, 83.2 and 91.3 ng/mL for the *Wt/Wt*, *Wt/*2* and **2/*2* respectively ($p = 0.01$). We provide for the first time the prevalence of the *CYP2C8*2* variants in Mali. We also show that day-7 desethylamodiaquine concentration was significantly higher in mutants than wild-type *CYP2C8*. These preliminary results indicate that additional studies on AQ and desethylamodiaquine metabolism are needed to improve our understanding of antimalarial treatment failure.

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PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES OF DIHYDROARTEMISININ-PIPERAQUINE IN SEASONAL MALARIA CHEMOPREVENTION IN YOUNG CHILDREN

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Young children are the population most severely affected by *Plasmodium falciparum* malaria. Seasonal malaria chemoprevention (SMC) with amodiaquine and sulfadoxine-pyrimethamine is recommended and provides substantial benefit to this vulnerable population in high transmission areas. Resistance to this regimen limits preventive efficacy, and the long-acting artemisinin-based combination, dihydroartemisinin-piperaquine is under study as an alternative regimen. However, the pharmacokinetic and pharmacodynamic properties of artemisinin-based combination SMC therapy are not well characterised. Sparse piperaquine plasma concentration measurements were available for 179 children (aged 2.33-58.1 months) after monthly SMC using dihydroartemisinin-piperaquine for three consecutive months in Burkina Faso. Piperaquine pharmacokinetics were characterised by a flexible transit-compartment absorption model followed by a three-compartment disposition model. Body weight was a statistically significant covariate, resulting in lower drug exposures to piperaquine in smaller children after a standard mg/kg dosage. A covariate-free sigmoidal E_{MAX}-model described the time to malaria re-infections satisfactorily. The model-predicted the minimum inhibitory plasma concentration of piperaquine to range between 33.9 and 45.5 ng/mL. Population-based simulations using the final pharmacokinetic-pharmacodynamic model suggested that small children would benefit from a higher dosage. Increasing the dosage and extending the dose schedule to four monthly doses in 4-20 kg children resulted in a predicted relative reduction in malaria incidence of up to 58% during the high transmission season. Use of a higher dose of dihydroartemisinin-piperaquine and extending the dosing schedule to cover the high transmission period for SMC could improve the preventive efficacy of this regimen substantially.

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SAFETY, TOLERABILITY, EFFICACY AND PHARMACOKINETICS OF HIGH DOSE, SHORT COURSE PRIMAQUINE REGIMENS IN PAPUA NEW GUINEAN CHILDREN

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Plasmodium vivax (Pv) is geographically the most widely distributed human malaria parasite with more than 2.2 billion people in the Asia Pacific region at risk. In countries such as Papua New Guinea (PNG) where both *P. falciparum* and Pv are transmitted, the complex biology of Pv represents a challenge to malaria control and chemotherapy. Primaquine (PMQ) remains the only FDA approved drug for elimination of Pv liver stage infection. However, the conventional 14-day treatment regimen is limited by poor adherence and there is an urgent need for shorter regimens. This study evaluated the safety, tolerability, pharmacokinetics and pilot efficacy of short course, high dose PMQ in PNG children. Treatment allocation was conducted in a stepwise design, with three regimens investigated; (i) 0.5 mg/kg daily for 14 days (Group 1), (ii) 1.0 mg/kg daily for 7 days (Group 2), and (iii) 1.0 mg/kg twice daily for 3.5 days (Group 3). Children (5-10 years) with normal G6PD activity and confirmed Pv infection were treated with a course of artemether-lumefantrine (AL) and recruited into the study. On completion of AL, all participants underwent a full clinical examination and received their first nominated dose of PMQ. Safety assessments including monitoring of haemoglobin, methaemoglobin, urinalysis and hepatorenal function were performed after doses 1, 3, and 7 in all patients, and dose 14 for Group 1 participants. Adverse effect questionnaires were administered after each dose. Each child was randomly assigned to 3 paired sparse blood sampling time-points (immediately prior, and 2 hours after PMQ) for drug assay during the treatment period. To evaluate treatment efficacy, participants were followed for 2 months after treatment, including active fortnightly surveillance for malarial illness not reported at a health facility (including finger prick blood sample for PCR) and passive detection of participants presenting at the clinic with malaria confirmed by RDT and PCR outside scheduled study-related assessments. This study will be the first to report the safety, tolerability and efficacy of high dose, short course, PMQ treatment regimens in paediatric patients.

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RISK OF ANEMIA AND TIME TO HEMATOLOGICAL RECOVERY FOLLOWING ARTEMISININ-BASED COMBINATION THERAPIES AMONG HIV INFECTED INDIVIDUALS STABILIZED ON ANTIRETROVIRAL THERAPY WITH OR WITHOUT MALARIA CO-INFECTION IN SUB-SAHARAN AFRICA: POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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In sub-Saharan Africa (SSA) where malaria is highly prevalent, HIV infection is also endemic. Co-infection and co-treatment of malaria and HIV is therefore frequent, and their occurrence is associated with increased risk of anaemia as a result of haemolysis and decreased red blood cell production. Cases of transient anaemia, observed after artemisinin-based combination treatment (ACT) for uncomplicated malaria, have resulted in a debate on whether the changes in haemoglobin are due to an adverse effect of the ACT or a result of the malaria infection. Recent evidence has shown that the extent of the drop in haemoglobin following treatment offuncomplicated malaria is determined by pre-treatment parasitaemia and parasite clearance rates, but is independent of the antimalarial(s) used. However, it is unclear if this finding is equally true for the HIV-malaria co-infected sub-population on different antiretroviral regimens. To explore the potential impact of HIV-malaria co-infection and antiretrovirals on the risk of anaemia, we pooled longitudinal data of haemoglobin measurements from 39 uncomplicated malaria clinical trials in SSA and used logistic regression, accounting for between study variations, to investigate factors associated with anaemia among HIV infected individuals (n=630) compared with HIV uninfected individuals (n=2,568). Furthermore, we will present comparisons of changes in haemoglobin following intake of different antiretroviral regimens (nevirapine-, efavirenz- and lopinavir/ritonavir-based) and ACTs (artemether-lumefantrine, artesunate-

amodiaquine and dihydroartemisinin-piperaquine) in these sub-groups. Understanding any potential impact that commonly used antiretroviral drugs and ACTs may have on haemoglobin concentrations in high risk sub-groups, such as HIV infected individuals, is an important step in optimising antimalarial treatment in this key vulnerable sub-population.

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ETHNICITY-RELATED MALARIAL SPLENOMEGALY: THE HYPER-REACTIVE RED CELL FILTRATION TRAIL

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Fulani subjects are partially protected against clinical malaria, have lower parasitemia but more frequent splenomegaly and anemia, as well as elevated levels of both total IgM and anti-*Plasmodium* antibodies. The triggering mechanisms of this phenotype are poorly understood. This collaborative study explored the genetic, parasitological and behavioral determinants of ethnicity-related malarial splenomegaly. In December, 2017, 482 subjects, living in Northwestern Benin, and belonging to 4 ethnic groups (Fulani, Gando, Bariba, and Otamari) were included. We captured age, gender, ethnicity, temperature, spleen size, malaria rapid diagnostic test (RDT), and rapid haemoglobin determination (Hemocue©). Red blood cells (RBC) from venous blood were analyzed using microsphiltration, a method assessing the ability of RBC to squeeze through narrow spaces between microspheres, as a proxy for their splenic filtration. RBC deformability was also assessed with ektacytometry. In a subgroup, (n=120) the deformability of RBC infected *in vitro* with *Plasmodium falciparum* was also assessed by microsphiltration, as well as parasite growth over 72 hours. As expected, Fulani were more often splenomegalic than subjects from the other groups (41% v 23%, p=0.0003) with a trend towards more frequent anemia. Microsphiltration showed that, compared to other groups, Fulani had more deformable RBC in circulation (p<0.0001). This difference was greater in subjects with a positive RDT. Ektacytometry at high shear stress (30 pascals) also showed an increased deformability of RBC in Fulani (p=0.0057). In vitro, the deformability of RBC after infection with *P. falciparum* and parasite growth were similar across the different ethnic groups. We establish a new link between ethnicity and the deformability of circulating RBC. Our results suggest that, in Fulani, splenic filtration of RBC becomes more stringent upon infection with *P. falciparum*, which may explain anemia, splenomegaly and the partial control of parasitemia. Upcoming work will search for determinants of this peculiar, apparently innate splenic reactivity to plasmodial infection in Fulani.

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IMPACT OF MALARIA-PROTECTIVE GLYCOPHORIN POLYMORPHISM ON *PLASMODIUM FALCIPARUM* INVASION

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Malaria has had a major selective effect on the human genome. Mutations that provide partial protection against severe malaria can be found at high frequency in malaria endemic regions, even when they can have major deleterious effects, such as sickle cell hemoglobin and resultant sickle cell anemia in homozygotes. Large genome-wide association studies have identified a number of new human genetic polymorphisms that are associated with protection against severe malaria, most of which are found in or near genes encoding proteins important for the structure and function of red blood cells (RBCs), the primary host cell for *P. falciparum* parasites. These include a novel complex structural variant in the glycophorin gene cluster that encodes the Dantu blood group antigen which confers a similar level of protection against severe malaria as HbS, but for which the mechanisms of protection remain unknown. We used flow cytometry-based *in vitro* assays to investigate the impact of the Dantu polymorphism on *P. falciparum* RBC invasion and RBC membrane protein expression for the first time, using RBC samples from a genotyped cohort of Kenyan children. We observed a strong and linear reduction in the ability of multiple *P. falciparum* strains to invade RBCs from Dantu heterozygote and homozygotes respectively. We also observed a significant reduction in the surface expression of glycophorin A (GYPA) and glycophorin B (GYPB), and a significant increase in glycophorin C (GYPC) expression on the surface of RBCs in these same cells. The reduction in invasion observed in carriers of the Dantu variant allele indicates that this polymorphism could confer protection against malaria infection by significantly reducing parasite invasion into the RBC, perhaps mediated by altered expression of the glycophorin receptors on the RBC membrane.

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GENERATION OF AN IMMORTALIZED ERYTHROID CELL LINE FROM HUMAN PERIPHERAL BLOOD FOR THE FUNCTIONAL ANALYSIS OF INVASION IN *PLASMODIUM* SPECIES

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Plasmodium invasion and proliferation within host erythrocytes is responsible for the clinical manifestations of malaria. The identification of strain-transcendent host molecules for *P. falciparum* invasion (basigin and CD55) has highlighted the importance of identifying critical host determinants for *Plasmodium* spp. invasion to inform the design of vaccines and antimalarials. Recently, we have used forward genetics in erythroid progenitors derived from primary CD34+ hematopoietic stem cells (HSCs) to identify CD55 as an essential host receptor for *P. falciparum* invasion. However, the use of primary cells is time-consuming, subject to donor variability, and these cells are harder to manipulate genetically. Additionally, the few available immortalized erythroid cell lines do not fully differentiate into enucleated normocytes. In this study, we immortalized erythroid progenitors from peripheral blood using the Tet-inducible HPV16-E6/E7 expression system. This cell line (ejRBC) resembles HSC-derived basophilic erythroblasts based on morphology and surface erythopoietic markers, and can be induced to produce

~95% orthochromatic cells and ~5% reticulocytes in eight days, a period considerably shorter than that reported for other cell lines. ejRBCs are genetically tractable *via* shRNA and CRISPR-Cas9 with high efficiency knockdown and knockout evident at 7-10 days post-transduction. Additionally, the levels of the previously identified *P. falciparum* and *P. vivax* receptors are comparable between ejRBCs and reticulocytes. Terminally differentiated ejRBCs support invasion of sialic acid-dependent and independent *P. falciparum* strains, as well as *P. vivax*. CRISPR-Cas9 knockout of DARC in ejRBCs ablates *P. vivax* invasion without affecting *P. falciparum* invasion, emphasizing the robustness of this model for the functional study of *Plasmodium* invasion. Importantly, this method of immortalizing erythroid progenitors from peripheral blood makes practical the generation of cell lines from individuals from diverse genetic backgrounds and with unique polymorphisms to interrogate the mechanisms of malaria susceptibility.

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COMBINING RNA-SEQUENCING AND MATHEMATICAL MODELLING TO IDENTIFY MECHANISTIC CORRELATES OF PROTECTION IN MALARIA

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Parasite load is a key determinant of severe malaria and is a consequence of pathogen multiplication rate, time and the ability of the host to constraint parasite growth. We aimed to develop a new method to identify the host mechanisms which constrain parasite growth. Using a mathematical model of longitudinal infection dynamics for orientation, we made individualized estimates of parasite multiplication and growth inhibition in Gambian children at presentation with acute malaria and used whole blood RNA-sequencing to identify their correlates. We identified 26 human genes positively correlated with parasite growth inhibition, largely linked together in a network focused around extracellular signal-regulated kinases, which integrate cellular inflammatory and metabolic responses in innate defence. Focusing on two genes which encode secreted products, we identified novel roles for cathepsin G and matrix metallopeptidase 9 (MMP9) as direct effector molecules which inhibit *P. falciparum* growth. Cathepsin G acts on the erythrocyte membrane, cleaving surface receptors required for parasite invasion, whilst MMP9 acts on the parasite. Our findings underline the importance of accounting for the interaction between host and pathogen when seeking to identify correlates of protection, and reveal novel mechanisms controlling parasite growth in humans.

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TIMING OF HOST FEEDING DRIVES RHYTHMS IN PARASITE REPLICATION

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The synchronous manner with which malaria parasites replicate asexually in the blood of the host is responsible for many of the clinical symptoms of malaria, with fevers arising from parasites undergoing synchronous schizogony. How malaria parasites maintain synchrony and coordinate the timing of transitions between their developmental stages (e.g. ring, trophozoite, schizont stages) during asexual cycles is unknown. We reveal that the timing of asexual cycles is driven by the host's circadian rhythms, specifically rhythms in food intake. By feeding mice either in the day-time of night-time we show that the timing of asexual parasite development in *Plasmodium chabaudi* becomes inverted in day-fed hosts relative to the rhythms of parasites in night-fed hosts, with schizogony occurring in the middle of the day or night respectively. Our data suggest rhythms in glucose availability are responsible for rhythms in asexual development, not rhythms in host immunity. Furthermore, the host's circadian clock is not required for rhythms in asexual development. Specifically, parasite rhythms are maintained in clock mutant mice but only when rhythmic food intake is enforced; when hosts eat all day long, parasites lose synchrony. We propose that daily fluctuations in resource availability both directly regulate asexual development and are used by parasites as a time-of-day cue to synchronise their rhythm to those of the host. Because parasite rhythms matter for their fitness, identifying the molecular mechanism underpinning parasite rhythms may expose ways to disrupt parasite development and ultimately lead to a reduction in disease and transmission.

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SURVIVAL RIGHTS SURVEILLANCE OF CHILDREN PRESENTING TO A LARGE REFERRAL HOSPITAL IN MALAWI

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While medical interventions may prevent children from dying after they fall sick, to eradicate avoidable child mortality, there is a need to ensure that children have access to the most important determinants of health, which are basic rights or survival rights. Our aim was to quantify access to survival rights (adequate water and sanitation, shelter, and an educated mother) of children presenting to a major referral hospital in Southern Malawi. A quantitative observational cross sectional approach was used. Routinely collected socioeconomic data for children attending the hospital's services during three calendar years from January 2013 were analysed. As maternal education is associated with child survival, we included this as a survival right. Data from 98,284 children were included in the study. 35.9% and 3.6% of the children did not have access to an improved sanitation and improved drinking water source respectively. There were not significant differences between Blantyre urban and rural residents. 7.2% of children's guardians (90% mothers) did not have access to any education, with 65% having access to 'some primary' education. We also found that in 90.3% of the households, more than 3 people were living in one room. In conclusion many children did not have access to all their survival rights. Deprivation of the right to shelter was the most pronounced followed by the right to sanitation, then maternal education with the right to access to an improved water source being least deprived. A human rights approach to improving child health with routine surveillance of survival rights or data on the social determinants of health at every health facility could be a useful adjunct to national surveys. The collection of data and advocacy at a local level has the potential to increase access to survival rights.

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MORTALITY TRENDS FOR THE POLITICALLY VOICELESS AND THE OUTBREAK OF "PEACE"- POST-CIVIL WAR MATERNAL AND CHILD HEALTH BY PEACE TYPE, 1990-2015

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The outbreak of "peace" after civil war is characterized by deeply entrenched grievances and insecurity with violence often persisting at high levels with disproportionate impacts on different segments of society, particularly for the politically voiceless. This study updates our previous work with an expanded dataset, new health indicators, and more robust statistical analysis to test the assumption that diplomatic negotiations at the end of war protect the health and interests of vulnerable, non-combatant populations such as women and children. Specifically, we examined mortality trends after civil war whereby under-five child mortality rates and maternal mortality ratios are compared between two peace types, a peace imposed by the victor and a negotiated peace between capable warring parties, following five years of peace. The Uppsala Conflict Data Program Armed Conflict Dataset was queried for global civil wars ending between 1990 and 2015 and nominally categorized by peace type. UNICEF under-five mortality rates (U5MR) and UNMMEIG maternal mortality ratios (MMR) at five years after conflict termination were then compared by net change relative to health at the point of peace declaration. Health differences by peace type were compared by one-way analysis of variance at α=0.05 with 95% confidence intervals obtained by Welch-Satterthwaite two-sample t-testing. We found that U5MR fell by 13.0 and 19.2 deaths per 1,000 births in VP and NP, respectively, with no statistical differences seen; we found that MMR fell by 73.7 and 110.1 deaths per 100,000 births in VP and NP, respectively, with no statistical difference seen. Further analysis compared peace time mortality trends to those in continued active war zones. Therein, statistically significant health dividends of peace relative to their continued war-affected peers could not be appreciated until three years after conflict resolution. This study concludes that the maternal and child mortality after civil war does not differ between peace types and quantifies the time delay in which populations exiting civil war experience mortality improvements as the beneficiaries of peace.

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PATTERN OF MEDICATION PRESCRIPTION IN PRIMARY HEALTH CARE CENTERS IN NIGERIA: IMPLICATIONS FOR SDG3 AND IN ANTI-MICROBIAL RESISTANCE

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Our aim was to investigate the pattern of medicines prescription in selected peri-urban PHC centres in a state of Nigeria, and also attempt to estimate the cost implications of that prescription pattern. Multiple medication and medicines over-prescription were regular features in the PHC centres, with prescription of over nine ingredients in extreme cases. In some instances, the cost of prescribed medications was over 3/15/2018 ASTMH - Nomination Submissions https://www.astmh.org/awards-fellowships-medals/awards-and-honors/nomination-submissions?aid=305&sid=15354 5/20 500% higher than what would be determined for that of a rational or justified medication for the same presenting complaint. Uptake of nonrational or excessive prescriptions in PHC settings could cause family health expenses that exacerbate financial distress. Also, based on their past experience on the prescription profile, community members may delay visit to health centres in anticipation of the level of prescription charges. Forty-two to 50% of the prescriptions featured anti-biotics, in most cases incomplete dosage which at the cellular level may favour development of anti-microbial resistance. In view of the increasing incidence of antimicrobial resistance as a public health threat, there would be justification to carry out a prospective and wider population study of this subject to better identify and characterize and also quantity incidence of the health system governance and health care provider self-interest attributable factors that underpin this pattern, such that more effective measures could be entrenched to curtail the pattern.

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THE NEXUS OF CONFLICT, MIGRATION, AND DISEASE: A CASE STUDY OF THE CURRENT AND IMPENDING HEALTH CRISIS OF THE ROHINGYA REFUGEES IN BANGLADESH

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The majority of active and protracted conflicts are located in low or middle income countries - 10 of which have been on-going since 1991 and, per the World Bank, have caused the majority of forcible displacements worldwide. UNHCR reported that there were 65.6 million forcibly displaced persons worldwide by the end of 2016 and this number continues to increase. Predictably, conflict causes a crisis of migration that inevitably leads to a public health crisis. Evidence of this lies in the thousands of those directly injured through warfare in conflicts such as Syria and Iraq, as well as epidemic outbreaks of Cholera in Yemen and Measles seeping into surrounding countries from Venezuela. Taking the most recent conflict within Myanmar as an example, a genocide of the Muslim Rohingya people, what started as an internal conflict, lead to almost 700,000 Rohingya fleeing to Bangladesh from August to December of 2017. As a result of joining an existing Rohingya population and host community, the current appeal for support is for 1,300,000 persons and requested funding totals 950.8 million USD. Nearly 10% of this appeal is slotted for healthcare. In this sector, Samaritan's Purse is actively responding to the current Diphtheria outbreak and planning for the impending Cholera outbreak once the rainy season begins. Diphtheria, an antiquated disease and inactive for decades in the western hemisphere, has ravaged the Rohingya community who were not provided vaccinations by their home country. The majority of patients being children, this bacterial infection leads to a buildup of toxins requiring administration of Diphtheria Antitoxin that is in short supply worldwide and often causes severe adverse reactions. After treating over 1,000 individuals with Diphtheria, Samaritan's Purse presents clinical findings and operational lessons learned, as well as the necessary transition within an active humanitarian and health crisis to also caring for Cholera among the forcibly displaced Rohingya, who are an unfortunate case study of the intersection of conflict, migration, and disease.

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LESSONS LEARNED FROM COMMUNITY ENGAGEMENT AND OUTREACH PRIOR TO INITIATION OF COMMUNITY-WIDE AUTOPSY PROGRAMS TO IMPROVE MORTALITY SURVEILLANCE, KISUMU, WESTERN KENYA

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Several new international initiatives are underway to help address limited cause-specific mortality data in low-resource settings. KEMRI-CGHR/CDC in Kisumu, Kenya, was selected as a site for two of these new initiatives that use autopsies: Child Health and Mortality Prevention Surveillance program and the Kenya Mortality Study. Extensive community engagement and outreach were required prior to starting enrollment due to the sensitivities of this type of work. Community engagement was done through national administration, Ministry of Health ,and Community health strategy. Engagement involved meetings with local leaders including

MoH department heads, chiefs, and assistant chiefs. Trainings were held for all local partners, including community health volunteers, pathologists, laboratory technologists, hearse drivers, and medical staff at health facilities. Community members were engaged through presentations at community meetings. Site preparation and community engagement took place October 2016 through May 2017. We held 20 community meetings, 7 continuing medical education lectures, and sponsored weekly meetings and monthly health dialogue days with CHVs. Topics covered during meetings included background on the studies, the benefits of knowing mortality rates and causes of death, and autopsy procedure details. We trained 3 pathologists on minimally invasive tissue sampling, 2 lab technologists on tissue histopathology preparation, and 8 hearse drivers on safe body handling. We also provided prophylactic vaccinations to 3 pathologists, 4 pathology technicians, 8 hearse drivers, and 8 mortality surveillance staff. To date, we received 83 death notifications and have enrolled 12 pilot deaths. In conclusion, our community engagement strategy has engaged members of the community and has contributed to a quick rollout with receipt of many death notifications. Trainings have equipped partners to understand the objective and effectively handle study procedures. We need to enhance community engagement by continuing to share information to assist with death notifications.

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STRENGTHENING COMMUNITY HEALTH SERVICE DELIVERY THROUGH COMPLETE HOUSEHOLD MAPPING AND CENSUS DATA, IN A CASE OF MANYATTA URBAN HDSS

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Lack of accurate population-level denominators for underserved urban populations hinders the assessment of disease burden, and affects proper development and evaluation of health care interventions. Through the support of the Child Health and Mortality Prevention Surveillance (CHAMPS) network, the Kenya Medical Research Institute (KEMRI) in collaboration with the Kisumu county department of health, Emory Global Health Institute and Centers for Disease Control and Prevention (CDC) are establishing an urban Health and Demographic Surveillance System (HDSS) in Manyatta informal settlement area of Kisumu town, western Kenya. This study was carried out with an aim of generating surveillance data to advice stakeholders on policy and implementation towards reducing child mortality. After verbal consent was obtained from household heads physical mapping geo-location and unique numbering of all houses was conducted in Manyatta between November 2016 and February 2017. Subsequently, all members of the mapped households were enumerated and their socio-demographic data collected. These Data were collected electronically using smart phones by trained Community Interviewers. We compared the data obtained versus the department of health data used in service provision. The total number of enumerated households was 31,266, which was two and a half times the number of households reported and served by the county health department at 11,868. We noted that although each community health volunteers (CHV) is allocated 100-120 households by the health department, our data shows that they should actually cover 650-800 households. A comparison of the workload also showed that only 11 out of 110 villages met the set criteria of assigning 100-120 households per CHV. As observed, emphasizing the importance of re-assessing census figures for urban area catchments is of paramount importance since CHVs are recruited and allocated duties on the basis of number of households, this finding has important implications for service delivery and health outcomes in Manyatta.

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DEVELOPMENT OF RUMOR SURVEILLANCE IN SUPPORT OF MINIMALLY INVASIVE TISSUE SAMPLING FOR DIAGNOSING CAUSE OF CHILD DEATH IN BANGLADESH

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In low and middle-income countries, determining cause of death (CoD) is impaired by limited diagnostic facilities, poor access to health care systems, and limited acceptance of autopsies. Minimally invasive tissue sampling (MITS), a post-mortem procedure to extract small tissue specimens for laboratory analysis could reduce uncertainty of CoD. A study to identify the CoD in Bangladeshi children using MITS could lead to rumors, given the cultural sensitivities around any type of postmortem exam. Our objective was to implement a system to identify and track rumors and misinformation about the MITS procedure to enable management and response to rumors through community engagement. We recruited 1,275 volunteers in 261 villages to notify any rumors being spread in the community, defined as attitudes, expectations, interests, concerns and anxiety towards MITS. Once a rumor was reported, the field team selected respondents for in-depth interviews and group discussions to track, verify and understand rumors. From September 2017 through February 2018, the team identified three rumors from communities where deceased children underwent the MITS procedure. Two rumors indicated that the study was harvesting organs for sale or transplantation. Another rumor was that the MITS procedure had been conducted on a living child, and because of this, there was excessive seepage of fluids from the needle wound sites. Rumors reportedly originated from some villagers who were unfamiliar with the MITS procedure. The team visited the rumor affected villages, talked to residents who started or perpetuated the rumors to understand their concerns and queries, and then convened group meetings to correct misinformation. The rumor surveillance was critical for successfully identifying and responding to tensions between community's perceptions and the study´s objective, improving the acceptability of the procedure and ensuring that families who agreed to MITS did not suffer stigmatization. Rumor surveillance could be used as a part of any public health program where there is a need to identify and act upon public misinformation or other community events in real-time.

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"BLEEDING BODIES", "UNTRUSTWORTHY BODIES"? A SOCIAL CONSTRUCTIONISTS APPROACH TO THE HEALTH AND WELL-BEING METRICS FOR FEMALE YOUTHS IN KENYA

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The diverse changes - biological, social, cultural and economic - experienced by female youth and that affect their health and well-being is well researched. What is yet to be understood is how female youths in LMICs socially construct their health and well-being in the time of transition to adulthood in a resource scarce environment and how this impacts on their perception and meanings of health and well-being. Empirical evidence has shown that human experiences and perceptions of space and time are remarkably specific to certain groups and cultures in particular places and times. Using this premise, we employed a social constructionist perspective to explore health and well-being of female youth in Kenya with an objective of highlighting the health indicators that matter to the lay female which are youths essential in well-being assessment. Using an ecological model, we conducted six in-depth interviews (n=6) and four focus group discussions (n=4) with female

youth 15-24 years in different regions in Kenya. The regions were selected based on socio-economic status, urban, arid and semi-arid areas. Phenomenological analysis of the data gave rise to the conceptual categories of the female youth being "bleeding bodies", "unemployed and over dependent bodies", and "untrustworthy bodies", "culturally disadvantaged bodies" and "bodies prone to diverse health risks". We found out that poverty and lack of basic needs contributed to feelings of powerlessness and inability to take charge over one's life in the phase of transition. Strong social support was demonstrated as the only hope and fallback for the youth in these of contexts. These insights are important in formulation of context specific policies that target neglected groups.

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INTEGRATING ACTIVE AND PASSIVE CASE SURVEILLANCE IN PREDICTIVE DISEASE MAPPING

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Statistical relationships between environmental predictors and disease incidence can be observed either via localised active surveys or via passive case surveillance data collated at health centres. Active surveys can achieve monitoring with high explanatory power, but they are expensive and highly localised. Passive detection allows data collection across a broader scale, promising wider spatial predictions, but with limited explanatory power, due to the lack of detailed aspects of the disease and its drivers. Here, we aimed at achieving the best of both worlds via an inferential framework capable of simultaneously drawing insights from both these types of data.We used a Bayesian latent point approach, incorporating a combination of active data collection in some points, where a full set of covariates were measured, and passive cases detection, where an important covariate (insecticide resistance) was unavailable. We validated our approach on some hypothetical scenarios of reported malaria incidence as a combination of both an ecological process (incidence as a function of some environmental covariates) and an observation process (probability of reporting malaria cases as a function of distance from clinics). Models considering active sampling sites only failed in describing the relationships between the environmental predictors and malaria incidence. Models using passive detection data only gave better results, allowed to estimate the probability of case reporting at health centres and to generate full predictions of incidence in space, but not to infer the latent variable. When jointly considering active and passive cases, we obtained the best estimate of coefficients describing the biological and the observation processes, together with full spatial prediction of both incidence and the latent variable of insecticide resistance.By integrating active sampling and passive case detections in a complementary way, we provide a simple solution to a widespread problem in spatial epidemiology, combining latent process modelling, spatially autoregressive modelling and point sampling distance sampling.

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HEALTHCARE WORKERS' PERSPECTIVES ON PREPAREDNESS OF HEALTHCARE FACILITIES FOR OUTBREAK OF COMMUNICABLE DISEASES IN NIGERIA: A QUALITATIVE STUDY

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The probability of a future outbreak of communicable disease after the recent Ebola virus outbreak is high. The healthcare facilities may not be prepared to respond in case of such eventualities. The Nigerian healthcare workers' (HCWs) knowledge for preparedness, perception of level of preparedness existing in their facilities, militating factors and possible ways to improve were evaluated through a qualitative data collective: Focus Group Discussion and In-depth interview. Among the 193 HCWs that participated in the study, 98.4% (190/193) considered their health facilities to be insufficiently equipped for disease outbreaks. None of the facilities has an emergency operation unit. The HCWs' idea about preparedness centered essentially on universal precaution. Training and routine emergency drill, disease surveillance, and adequate waste management are lacking. None 0% (0/193) of the participants had undergone any form of emergencies drill. Among the suggestions on how to improve on preparedness were: immunization of staff, better communication among different departments in the health sector, routine training, fund and relocating some of the hospital services to an annex. In conclusion, the overall poor level of preparedness which exist in most health facilities cannot contain any outbreak. There is need to look beyond universal precaution to areas like improved communication and information sharing, and routine interpretation of surveillance data by epidemiologist. Institution of emergency operation units in every health institute capable of functioning during non-outbreak periods.

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KNOWLEDGE OF NEURAL TUBE DEFECTS, ATTITUDES AND PREVENTION PRACTICES AMONG WOMEN SEEN IN PRENATAL CONSULTATION-NIAMEY-NIGER, 2017

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Congenital abnormalities are functional, structural, or metabolic abnormalities that occur in the uterus and are identified before or after birth. In Niger, the field of congenital abnormalities has not been sufficiently explored. Our goal was to describe the knowledge of neural tube defects (NTD), attitudes and preventive practices among women seen in prenatal consultation (PNC). We carried out a cross-sectional survey from February to March 2017 among pregnant women seen in PNC (1) in maternity hospitals in Niamey. The sample, calculated with Open Epi was 421.These women were interviewed and the data were collected with a questionnaire. Univariate, bivariate and multivariate analyzes were performed. The prevalence ratio (PR) and the prevalence of 95% OR (POR) were calculated. Variables with $p < 0.25$ in bivariate analysis were integrated in the logistic model. The median age of the respondents: 25 years. There were 60.81% schooled; 24.33% had their first pregnancy. Among the respondents 30.65% knew the NTD; 15.92% had good attitudes; 41.81% had used folic acid (FA). Age (25-49 years) was independent factor of NTD knowledge ($p = 0.0008$); for attitude: age (25-49 years) ($p = 0.03$), FA intake ($p = 0.0002$) and for practice, being literate ($P = 0.01$), information by health workers ($p < 0.0001$), knowledge of other sources of FA ($p = 0.04$). There is a lack of knowledge, attitudes and practices regarding NTDs among women in Niamey. Continuing education for women and the implementation of a mandatory policy for the fortification of certain foods with FA are necessary.

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ACCESS TO VISCERAL LEISHMANIASIS DRUGS IN EASTERN AFRICA: WHY EFFECTIVE SUPPLY IS SO ELUSIVE?

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Visceral leishmaniasis (VL) is fatal without timely treatment. As a neglected tropical disease, the control program in eastern Africa region is hindered by vast challenges. Conflict, population displacements, malnutrition and poverty influence the supply of life-saving drugs to treat VL. The treatment regimens are limited: toxic, complex administration, too expensive, or produced by a single source. Drug shortages are common, leading to

the severe socio-economic burden of the people, often the poor and most marginalized. Despite the external support, effective supply of VL commodities remains uncharted territory. There is no data on the magnitude of drugs shortages and the bottlenecks in procurement. We aim to evaluate access barriers to supply of quality VL drugs and diagnostics, as part of a larger study on access to care. We conduct landscape on policy analysis at the global level, which then narrows down towards mapping of barriers at the regional level. We have launched an online survey for participants working in neglected disease in the region through an e-drugs mailing list. A series of in-depth interviews with key stakeholders at different levels are conducted, including from multilateral and international organizations using a piloted topic guides and conducted by an experienced qualitative researcher(s). A focus will be dedicated to Sudan, the country bearing the highest burden of VL (30,000 cases in 2010-2014). We perform analysis on stock-outs between 2007-2017 will be performed, ranging from the central medical stores to the health facility level. Perspectives from the national, state and health workers will be solicited as well. The data collection is planned to start in May 2018. With the combination of quantitative and qualitative data, we expect to have -for the first time- a clear map of barriers to effective supply of VL drugs and strategies moving forward for this region. Access to available drugs is often overlooked. Thus ensuring supply to the drugs that save lives, should not be neglected further.

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PILOTING NURSE-LED QUALITY IMPROVEMENT PROJECTS IN NENO DISTRICT, MALAWI

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This abstract describes a component of the authors' ongoing study "Improving Health Outcomes in Mothers and Neonates using the WHO Safe Childbirth Checklist in Neno District, Malawi". There is a persistent gap between the intended quality of maternity care and that experienced by Malawian women, as evidenced by country's high maternal and neonatal mortality rates. In response, the Malawian Ministry of Health (MoH) has decided to focus on quality improvement (QI), through the formation of a national Quality Management Directorate and facility-level QI activities. Guided by the Reproductive Health Directorate, these initiatives have focused on systems-level improvements to maternity care. In Malawi, most maternity care is provided by nurses and midwives at the community and district level. This project uses nurse-midwives as the drivers of QI to conduct projects targeting the most significant challenges or deficits in their workplaces. As part of the Global Action to Improve Nursing and Midwifery care (GAIN) initiative in Neno, a district in southern Malawi bordering Mozambique, 30 local nurse midwives were trained in leadership and QI in two cohorts. GAIN is a collaboration among the University of California San Francisco (UCSF) School of Nursing, Partners in Health/Abwenzi Pa Za Umoyo, and the Malawian MoH. Working on interdisciplinary teams and with guidance from two expert nurse midwife mentors, nurse midwives designed and implemented QI projects in their workplaces in March, 2018. Topics included improving management and documentation of the immediate postpartum period; improving routine neonatal care through checklist use; decreasing rates of puerperal infection; and improving blood pressure monitoring for antenatal mothers. Project targets include complete charting of all postpartum assessment and care, consistent use of a checklist for neonatal care, and documentation of blood pressure for all mothers at every antenatal visit. Given the vital role of nurses in this setting, QI initiatives designed and led by nurses have the potential to eliminate systems-level barriers to comprehensive and safe care for mothers and newborns.

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ASSESSMENT OF THE DETERMINANTS OF NON-ADHERENCE TO ANTIRETROVIRAL THERAPY DURING PREGNANCY IN THE DISTRICT OF MANHIÇA, MOZAMBIQUE: A PRELIMINARY ANALYSIS

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Inadequate adherence to antiretroviral therapy (ART) and retention to care among pregnant women and breastfeeding mothers has been an important barrier to effective prevention of mother to child transmission (PMTCT) of HIV infection in sub-Saharan Africa. Intending to address the many challenges of PMTCT implementation, WHO revised international guidelines to recommend lifelong ART treatment for all HIV infected pregnant women at the time of diagnosis in antenatal care and during breastfeeding, regardless of their clinical condition. Mozambique, adopted this strategy in 2013. However, despite commitments, this strategy has been severely stressed by continued high rates of non-adherence to ART and lost to follow up. The aim of this study was to identify the determinants of non-adherence to ART among pregnant women in the district of Manhiça, Mozambique. In this preliminary analysis we used de-identified, aggregate data collected within a large parent study. Data included age groups, marital status, education, occupation, religion, and residence. For select pregnant women, a more detailed in-depth interview was conducted to explore the association of the socio-demographic factors on consumption of ART medications. Non-adherence was determined through pill counts and self-reports, and defined as taking less than 95% of the prescribed ART doses for each of the first two months since initiating prenatal care visits. Preliminary analysis of the 127 participants recruited into this study, based on pill count, only 33% of our population was adherent to ART during each of the two months period following initiation of their prenatal care. In multivariable models of analysis, the characteristics most significantly associated with being non-adherent to one´s ART were decreased age, being a widow, having lower education, and initiating ART during the current pregnancy. It is very likely that greater attention needs to be given to actual pill consumption and clinical measurements such as viral load to better monitor the adherence to ART and retention in care rather than just simply maintaining clinic visits and medication pick-up.

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MOTIVATION FOR FRONTLINE HEALTH IMPLEMENTERS IN NEGLECTED TROPICAL DISEASE PROGRAM: THE KEY TO SUSTAINABILITY AND ACHIEVING ELIMINATION GOAL FOR NEGLECTED TROPICAL DISEASES IN NIGERIA

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Neglected Tropical Diseases (NTDs) affect the poorest and most marginalized people in low resource countries. Despite global efforts to combat these diseases, equitable access to freely donated drugs is still a problem. Frontline implementers (Health Facility staff, Community Drug Distributors and Teachers) are the workforce of the NTD control programme and serve as essential link between the health system and endemic populations. There is an evident gap in understanding best practices for capacity strengthening of health workforce and performance management of NTD programme in Nigeria. This study sought to explore current strengths and weaknesses in Human Resource management for NTD programme delivery so as to find solutions to current programmatic

challenges informed by the perspectives of these implementers. The study was conducted in two purposively selected states, Kaduna and Ogun State, to allow for maximum contextual variation. We conducted participatory stakeholder workshops with frontline implementers in 6 LGAs (3 per state). Experiences, challenges and enablers faced by each group were explored, synthesised and analysed thematically according to the WHO Health Systems building blocks. Motivation of implementers to work is affected by challenges they experience, which include lack of supportive supervision by the leadership and governance of NTD programmes, overburdened health workforce due to staff shortages and inadequate health financing resulting to out-of-pocket expenditure. Poor health management information systems for rapid response and management of adverse reactions leading to drug refusals in subsequent treatment rounds also discourages the implementers. Lastly, there is inadequate training of implementers resulting in lack of confidence and poor **service delivery**. Implementation research is required to understand how the health system can be strengthened to produce well motivated workforce for NTD programmes. Since the frontline implementers connects the health system and endemic populations, their motivation is key to sustainability and achieving elimination goal for NTD programme in Nigeria.

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PERFORMANCE OF COMMUNITY HEALTH WORKERS IN PROVIDING INTEGRATED COMMUNITY CASE MANAGEMENT SERVICES (ICCM) IN EIGHT DISTRICTS OF RWANDA

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Integrated Community Case Management (iCCM) is a proven evidence-based strategy that trains, equips and supports various cadres of community health providers to deliver high-impact treatment interventions in the community. It is an important component of Integrated Management of Childhood Illness (IMCI), which was developed by WHO in the 1990s. Community health workers (CHWs) in each of Rwanda's nearly 15,000 villages were trained in iCCM and equipped for empirical diagnosis and treatment of pneumonia, diarrhea, and malaria; for malnutrition surveillance; and for comprehensive reporting and referral services. This study was designed to evaluate the performance of CHWs in managing malaria, pneumonia and diarrhea in 8 districts of Rwanda. In August 2017, a cross sectional study was conducted among 64 CHWs in 8 districts. Data was collected using interviews and record reviews. Of the 64 CHWs, 35 (54%) were males, and 94.6% of CHWs reported being satisfied with their work. In the previous 3 months, the most common illness seen by CHWs was malaria (38%), followed by pneumonia (33%). Overall, 70% of cases were correctly treated. Malaria was correctly treated at 85%, while pneumonia was correctly treated at 60%. Artemether lumefantrine was found among 67% of CHWs' supplies, while malaria RDTs were the most available commodity at 80% of all CHWs. The results showed that CHWs are providing Integrated Community Case Management services but their performance is affected by drug availability. Strategies to improve drug supply, and supportive supervision from the formal health system are necessary to improve the performance of CHWs.

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ENGAGING COMMUNITY RESIDENTS IN INTRODUCING MINIMALLY INVASIVE TISSUE SAMPLING (MITS) PROCEDURE FOR CHILDREN IN BALIAKANDI, BANGLADESH

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Minimally invasive tissue sampling (MITS), a post-mortem procedure that uses needle to collect specimens has emerged as an alternative to autopsies, the gold standard to diagnose cause of death. In Bangladesh, diagnosing cause of child death is difficult due to poor diagnostic infrastructure, limited access to qualified doctors in rural settings and lack of documented medical histories. Considering the context, we aimed to use MITS to identify the aetiology of death among children less than five years of age in Baliakandi, Bangladesh. A previous small-scale MITS pilot in Bangladesh showed that rural people often confuse MITS with full autopsies, sometimes leading to mistrust between the study team and participants. Therefore, our objective was to engage and inform rural residents about MITS objectives and procedure, and enquire whether the MITS procedure is aligned with their social, cultural and religious practices and their motivations to know the aetiology of child death. Between January - March in 2017, we conducted 14 workshops with community leaders (7) and community members (7) using participatory approach. The majority of the participants reportedly accepted the MITS as it would not require opening the deceased body and believed MITS could be an innovative way to know the aetiology. Some participants provided examples of multiple child loss within families and believed MITS could help knowing the causes of such tragedies in the future. Participants stated that it would be socially and religiously permissible to perform MITS. Collecting samples and soliciting consent might be difficult from distressed and grieving families and due to community perceptions that MITS is not necessary for already known causes of deaths by severe diseases, accidents or drowning. Participants suggested engaging elder community members including religious leaders to address queries and concerns. Participatory community engagement approaches can be useful for population based public health programs through gaining community input and further insights into how to build trust and relations with rural residents to engage them in the program.

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EMERGENCIES, ETHICS AND COMMUNITY ENGAGEMENT

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December 2013, the first case of Ebola occurs in southern Guinea. Due to emergency, to the rapidity of its spread and its virulence, experts announce the risk of an international health disaster. Stakeholders in charge of the response against EVD are implementing a series of initiatives on the ground to limit the spread of the epidemic (sorting of patients, quarantine, rapid clinical trials, etc.). These initiatives lead to community resistance that encourages efforts at the local, national and international levels to improve their ethical dimensions (commitment of religious and community leaders, mobilization of AVAREF, development of guidelines at international level to support ethics committees This presentation is based on (1) documentary review, (2) then the exploitation of the data coming from ethical committees and (3) the analysis of the interviews and observations made in the field with communities and stakeholders in charge of the response. The analysis of the data set has shown: (1) an explosion of research and

intervention activities against EVD in West Africa, (2) that raised important ethical concerns because of emergency and lack of knowledge on EVD in West Africa, (3) and mobilization of all actors at different levels of the response to tackle these ethical concerns. In conclusion. the Ebola outbreak has highlighted the fragility of health systems in West Africa and the need for these countries to build the capacity of their ethics committees in developing tools and procedures against the emergence of emerging diseases and re-emergences.

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SYSTEMATIC REVIEW OF COMMUNICATION STRATEGIES IN NEGLECTED TROPICAL DISEASES ERADICATION, ELIMINATION AND CONTROL PROGRAMS: A CALL FOR ACTION

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Many NTD interventions call for community engagement, health promotion, or behavior change strategies to enhance coverage and adherence. Although communication theories and practitioners have conceptualized and developed strategies, it is unclear whether NTD interventions use these perspectives. To understand how communication practice is operationalized in NTD interventions, this systematic review identified communication actions implemented to address Guinea worm (GW), lymphatic filariasis (LF), schistosomiasis (SC) and Chagas disease (CD). We examined studies published between 2012 and 2017 in five electronic databases. Articles extracted met three criteria: references to communication, guiding theoretical framework (if available) and communication domains, including community participation. Information about media and messages was also collected when available. A total of 114 articles were extracted (GW=11; LF=29; SC=29; CD=45). Behavior change is the most common goal pursued and expected from communication strategies. Risk behaviors are usually addressed through health education and awareness campaigns. Media-based strategies are used for dissemination and persuasion. Community participation is frequently associated with adherence, although more complex definitions have been tested. References to communication theory are scarce. These results illustrate efforts in NTD programing to reach populations at risk; however, they do not account for the complexity of communication dynamics associated with prevention and treatment of this group of diseases. For instance, MDA strategies consistently showed challenges derived from the proliferation of rumors that can be effectively reduced through communication action. Similarly elimination, eradication and control programs did not meaningfully differ in their communication approaches despite the specific demands of each stage. More systematic and strategic use of communication frameworks adjusted to the specific public health goals of NTD interventions and context of affected populations is recommended.

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VICTOR CHARLIE AND DISEASE PREVENTION: *USARV MEDICAL NEWSLETTER* CARTOONS, INFECTIOUS DISEASE AND THE VIETNAM WAR, 1965-1972

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The US lost more than 58,230 troops during the Vietnam War. Although most died due to combat-related circumstances, thousands also died due to non-combat causes. Deaths in the latter group were the result of causes that ranged from suicide to heart attack, malaria to hepatitis. While mortality from infectious diseases was relatively rare, morbidity from such maladies proved an ongoing problem for medical officers and field commanders alike. As General Slim quickly learned in the India-Burma Campaign during WWII, troops who were too sick to fight were of little use against the enemy. The medicos of the USARV (United States Army Republic of Vietnam) focused much of their prevention efforts on infectious diseases. As such, they published a type-written, semi-monthly tract, the *USARV Medical Newsletter*. Its purpose? "To provide information of interest and assistance to medical services of the US Armed Forces in RVN [Republic of Vietnam]." Each *USARV Medical Newsletter* offered dryly worded recommendations for preventing and treating infectious diseases from melioidiosis to malaria. Sprinkled throughout the *USARV Medical Newsletter*, however, were roughly sketched, pen and ink, cartoons that addressed topics from the need for safe drinking water to the importance of malaria discipline. Their purpose was to reinforce—with heavy doses of gallows humor—the sober jargon contained in the *Newsletters*' articles. This presentation, based on archival materials from the Vietnam Center and Archive at Texas Tech University, will examine the cartoons of the *USARV Medical Newsletter*. As our findings will show, the often crudely penned cartoons reflected not only the pressing issue of infectious disease among combat troops but also changing attitudes toward the war itself.

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INTERPROFESSIONAL EDUCATION IN A GLOBAL HEALTH COURSE

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Few global health experiences include intentionally-directed interprofessional training. We aim to prospectively evaluate the impact of a global health elective in facilitating interprofessional education (IPE) and promoting cultural sensitivity. We included in our study, medical and nursing students who participated in the 2015 and 2016 cohorts of the Nicaragua Global Health course. The course consisted of a 12-week curriculum conceptually divided into three phases: 1) pre-immersion preparation, 2) in-country immersion in Nicaragua, and 3) post-immersion reflection. The pre-immersion preparation phase included didactic lectures and small group discussions designed to introduce students to concepts of interprofessional collaboration. During the in-country immersion phase, students were organized into small-groups that participated in a variety of interprofessional activities. During the post-immersion phase, students also to prepare a poster presentation on patient education and teaching that was presented to students and faculty at our institution. Students filled out pre- and post-course surveys. We performed quantitative analysis on numeric data and qualitative analysis on open-ended questions. Of 39 total students enrolled in the course, 26 (18 medical and 8 nursing students) participated in the study and filled out the pre- and post-course surveys. Mean competency scores increased for all questions between pre- and post-course surveys, and of these, 5 of 7 reached statistical significance. Qualitative themes identified included: 1) the importance of understanding other team member's roles and relative strengths; 2) the value provided by the breaking down of traditional power dynamics between clinicians. Global health experiences represent a unique and under-utilized opportunity for facilitating IPE. Our global health course may be useful as a model for other educators seeking to prepare similar programs aimed at teaching the principles of IPE.

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TIME TO ADDRESS THE GROWING SYNDEMIC OF TUBERCULOSIS AND DIABETES: INDIA AS A MODEL HIGH BURDEN COUNTRY

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Tuberculosis (TB) is the top cause of death from a single infectious agent globally. The annual global incidence of 10.4 million TB cases in 2016 is alarming. Diabetes mellitus (DM) is an important comorbidity that complicates both treatment and clinical course of TB. Currently, India is the country with the highest incidence. A negative of India's fast-growing economy is that it is undergoing the epidemiological transition to noncommunicable diseases such as Diabetes. India launched an ambitious new strategy in 2018 to end TB by 2025. A successful strategy to achieve this goal should address co-morbid conditions that lead to poor outcomes in TB cases. We undertook this project with the aim of producing an in-depth needs assessment analysis of existing literature on TB-DM in India. Results of our analysis have revealed that TB-DM patients were more likely to experience poor treatment outcomes, longer times to sputum conversion, increased rates of adverse drug reactions, and increased drug. Prospective studies included in our analysis show that TB-DM patients were more likely to experience treatment failure (4.2% vs 0.7%, p=0.04) than TB-only patients. TB-DM patients were more likely to be sputum smear positive at the end of DOTS treatment (RR 3.9, 95% CI: 1.5-10.6, p=0.02). Time to sputum conversion was significantly longer at 64.2 days +/- 10.5 days in TB-DM patients vs 61.5 +/- 7.5 days for TB-only patients. An interesting finding was that TB-DM patients were more likely than TB- only patients to have rifampin resistant TB (27.8% vs 8.8%, p<0.01). Numerous molecular and cellular differences in individuals with co-morbid TB-DM were also found. In 2011 the WHO endorsed a bi-directional screening method for early diagnosis of TB-DM cases. Despite such efforts, India has the highest number of cases in the world with an estimated 29-44% of them being TB-DM cases. With better control of HIV and established HIV-TB programs, it is high time that TB programs pay attention to the growing epidemic of Obesity/Diabetes. Our results provide evidence for adopting comprehensive TB prevention and treatment programs that include important co-morbidities such as DM.

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BIOSAFETY CAPACITY BUILDING: A CASE STUDY IN PUBLIC-PRIVATE PARTNERSHIPS

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Biosafety capacity building has been a foundation to dozens of global health endeavors over the past several decades in various regions, many with limited success. Many shortcoming to realize sustainment of these efforts include transient engagement from international partners, inability of host countries and institutions to sustainably fund and manage resulting programs, challenges with respect to building human resources, and others. Private and public partners have much to offer in terms of capacity building and net results can be augmented when both partners work collaboratively with host nations and institutions. This presentation will detail a series of BSL2 to BSL3 tuberculosis diagnostic laboratory and program upgrades in different provinces of India as a result of a collaborative effort between private, public, and host nation resources. In short, laboratories of this nature had a strong need to increase TB diagnostic capacities, necessitating the inclusion of new liquid culture TB diagnostic systems. However, these new systems required more robust biosafety capacity. Our team led the upgrade of BSL2 laboratories to BSL3 laboratories in four locations. In the subsequent years, this program has expanded to over 20 laboratories. Through good partnership design, involvement of host nation resources, and a unique remote project management application, diagnostic capacity and accuracy of TB diagnostics expanded rapidly and cost-effectively. This case study will demonstrate critical decisions made during the project lifecycle that impacted both execution and resulting plans for sustainability. This presentation will also detail nuances of the public-private partnership that established a framework for close involvement of host nation resources to build internal human resources. Finally, this study will reveal both the lessons learned from the experience and the resulting successes.

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NUTRITIONAL STATUS AMONG CHILDREN UNDER FIVE AND ITS DISPARITY BETWEEN COMMUNITIES IN SAHEL REGION OF BURKINA FASO: A COMMUNITY-LEVEL INTERVENTION EVALUATION

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Stunting remains an important public health problem in Burkina and improving financial accessibility can help improve child nutritional status. The objectives of this study were to examine whether living in communities that were exposed to user fees removal combined with healthcare quality improvement and malnutrition management of malnutrition was associated with improved height-for-age z-score and decreased likelihood of stunting and severe stunting among children under five, four years after the interventions' implementation. We carried out an evaluative theory-driven, post-test-only design that included a control group and relied on a representative cross-sectional household survey conducted four years after the intervention onset in 41 communities from intervention district and 51 communities in comparison district. When comparing children living in the intervention district to children living in the non-intervention district, we determined no differences in terms of stunting [OR=1.13; 95% CI= 0.83 –1.54] nor in severe stunting [OR=0.99; 95% CI =0.76–1.26), nor in height of age z-score [(HAZ =-0.03; 95% CI: (-0.21–0.16)]. Our study demonstrated that most of the variance in stunting occurs at the individual level (variance =90.64% [95% CI= (86,62%–93,55%]. We also found that only 2% of the community-level variance of stunting was explained by the intervention in communities. In conclusion, this study provides important information to policymakers on the limits of targeting financial barriers at the community-level in addressing nutritional status at the individual level. In order to achieve better results in improving nutritional status, a population intervention should be designed with individual interventions specifically targeting the proximal determinants of malnutrition, as over 91% of the variance in nutritional status occurs at the individual level.

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ACHIEVING REGULATORY COMPLIANCE AT RESEARCH-NAÏVE MALARIA CLINICAL TRIAL SITES

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Malaria trials may be conducted in facilities with no previous research experience, serving poor, mobile communities. This was the context for a South African trial assessing the addition of low-dose primaquine to routine ACT malaria treatment. The World Health Organization has recommended this intervention to reduce malaria transmission in low transmission areas, without any G6PD testing. While available data suggest primaquine will not cause haemolysis in G6PD-deficient individuals, some have raised safety concerns. To provide local data to inform its potential licensing in South Africa, PRIM01 investigated the efficacy and safety of adding low-dose primaquine to artemether-lumefantrine for

symptomatic uncomplicated *Plasmodium falciparum* malaria. The trial was conducted within a quality management framework deemed suitable by pharmaceutical sponsors for higher risk trials, but designed as also relevant for lower risk field studies. This comprised over 30 standard operating procedures, a laboratory manual, role-specific training, job descriptions, and risk-based monitoring. Trial sites were selected in collaboration with the provincial Malaria Elimination Programmes and assistants employed to support clinic nurses who volunteered to help with the study. Staff at selected sites were overwhelmingly enthusiastic and dedicated to the success of this trial, however it was challenging to provide them with the required oversight due to their research-naivety, geographical location, limited staffing and infrastructure, and busy clinic schedules. More time than anticipated was needed for training in order to comply with Good Clinical Practice requirements, senior trial personnel had to be on-site much of the time, and ultimately only 2 sites could be supported. While this extended timelines, the quality management system and hard work of all involved resulted in a successful routine regulatory inspection. Though more burdensome than planned due to the context in which it must be conducted, this trial was at the required standard for unregistered medicines and has built capacity for further such trials in South Africa.

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LYMPHATIC FILARIASIS: COMPASSION AND THE ALLEVIATION OF SUFFERING AS A PUBLIC HEALTH PROBLEM

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The Global Plan to Eliminate Lymphatic Filariasis (GPELF) was conceived as having two pillars: one to interrupt transmission of the parasites that cause LF, and the other to alleviate the suffering of those with clinically significant manifestations of the disease, predominantly lymphedema, elephantiasis, and hydrocele. An estimated 40 million people worldwide suffer from these conditions, making LF the second leading cause of disability in the world. The World Health Organization requires that the Morbidity Management and Disability Prevention (MMDP) pillar must include a minimum package of care, including: 1) individual treatment to destroy any remaining adult parasites and microfilaria; 2) treatment for episodes of adenolymphangitis (ADL); 3) lymphedema management to prevent progression of disease and episodes of ADL; and 4) surgical management of hydrocele. For over two decades, the Notre Dame Haiti Program has supported the LF Clinic and Reference Center at Hôpital Sainte Croix in Léogâne, Haiti, currently the only site in the country that provides LF care. This clinic provides the minimum package of care described above; surgical management of hydroceles; as well as enhanced services. The latter include compression in the management of lymphedema, and a comprehensive Mental Health Initiative (MHI), whose aim is to reduce the social isolation and stigmatization of LF patients. Our MHI provides individual counseling, community peer support groups, a home visiting program, and a vocational training center to enable patients to better support their families financially. We will describe our comprehensive model of care, including both successes and challenges.

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EDUCATION, MENSTRUAL HYGIENE, AND FEMALE GENITAL MUTILATION: GENDER DISPARITY AMONG MAASAI YOUTH IN RURAL KENYA

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Girls' and women's empowerment has taken on increasing significance in the realms of politics and public health. With wider resources and funding available for interventions devoted to the welfare of girls and women, community-specific data are a crucial piece of the puzzle for implementation of sustainable programs. In Kenya, the Maasai are a minority ethnic group that suffer disproportionate rates of illiteracy, female genital mutilation (FGM), HIV, and other indicators of poor health. The aim of this project is to investigate the knowledge, practices, and attitudes of students in Chumvi, Laikipia County, a predominantly Maasai community, with specific emphasis on gender equity. 125 female and 101 male students ages 10-19 years from Chumvi Primary and Secondary schools were surveyed on FGM, HIV, safe sex practices, attitudes towards gender, and menstrual hygiene. Male-to-female student ratios and grade averages from the secondary school were also compared. 28% of female respondents reported missing class due to menstruation. Overall, 35% of female respondents reported they had experienced FGM, which increased to approximately 60% by the age of 17. However, this figure may underestimate the prevalence of FGM in the community, as girls may have felt ashamed to respond honestly. 18% of female students agreed with the statement: 'Girls should not go to secondary school because they should be getting married,' compared to 5% of male students. Female students were more likely to be unsure of the benefits of using condoms to prevent STIs and pregnancy than the males. Within Chumvi secondary school, there was no difference in the numbers of male versus female students or in their grade distributions, suggesting a lack of gender bias in the Chumvi educational system. Despite the challenges presented by language barriers and issues of self-reporting, these results help inform the efforts of organizations and community leaders to address the disparity between the male and female youth in Chumvi.

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KNOWLEDGE , ATTITUDES, AND PRACTICES OF PEDIATRIC PAIN MANAGEMENT BY NURSES AT A CHILDREN'S HOSPITAL IN LUSAKA, ZAMBIA

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Pediatric pain management is a frequently neglected area of child health, particularly in lower resource settings where nurses are the most likely clinicians to monitor, evaluate and treat pain. The general objective of our study was to assess the nurses' knowledge, attitude, and practices towards pain and pain management in children at Lusaka Children's Hospital, Lusaka, Zambia. We used a cross sectional sequential mixed methods study design to first survey a convenience sample of 40 nursing staff via the City of Hope Knowledge and Attitudes Survey Regarding Pain, followed up by three focus groups discussions, centred on pain and pain management. We used STATA 15 to analyze the survey for associations using correlations and two-sided Fisher's exact tests, and analyzed the qualitative data using content analysis. Overall the nurses' scored poorly, with a median score on the 43 item survey of 40.7%, (mean 41.5%, 95% CI 38.4%-44.6%). Neither self rated knowledge nor years of experience had any significant association with score, while higher nursing grade was positively and significantly correlated with higher scores (Corr 0.5192, P>0.001) and similarly a higher level of education was positively and significantly associated with a higher score (P= 0.038; Corr 0.3645, P>0.001). In the focus groups, nurses made negative statements about self reported pain, attributing patient and parent reports as attention seeking behaviors, yet at the same time the nurses expressed a positive desire to have training on pediatric pain management augmented by clear hospital policies and protocols. The overall findings suggest that nurses at a pediatric specialty hospital in subSaharan Africa were lacking across multiple areas for providing appropriate pain management. Ministries of Health should emphasize guidelines on pediatric pain management in pre-service curriculum and in-service trainings, and explore forming hospital pediatric pain management teams to address immediate patient needs while also educating and mentoring staff.

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DO BED BUGS REPRESENT A RISK TO CHAGAS DISEASE-ENDEMIC AREAS IN LATIN AMERICA?

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Arequipa is a southern Peru city with an urban infestation by *Triatoma infestans*, a triatomine insect vector of Chagas Disease. Over the past ten years, The Ministry of Health has held a vector control campaign. With less than 10% of the city remaining to treat, the control strategy is close to completion. However, in 2011 we recorded, for the first time, the presence of the common bed bug, *Cimex lectularius* in the city, and since then, the reports of infested houses by *C. lectularius* have been increasing each year. We are focused on determining the risk of transmission of *Trypanosoma cruzi*, the etiological agent of Chagas disease by *C. lectularius* in Chagas disease-endemic areas. We confirmed, under laboratory conditions, the vectorial competence of *C. lectularius*, to first acquire *T. cruzi* infection when fed on T. cruzi-carrying mice and subsequently to transmit the parasite to uninfected mice. We additionally showed that infection by *T. cruzi* does not affect the survival of bed bugs, another component of vectorial capacity. Additionally, we studied the survival rate of *C. lectularius* adults exposed to pyrethroid insecticide (deltamethrin), as well as the effect of insecticide on reproduction from colonies collected in different locations on the city and reared under laboratory conditions. We show that 10 days after exposition to insecticide, 42% (10 of 24) of colonies, present survival rates over 75 %, and that insecticide does not affect egg production of exposed females and later eggs hatching ($p = 0.88$). We discuss these outcomes in terms of the impact of bed bug infestation on Chagas disease control strategies in Chagas disease-endemic areas.

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A COMPARISON OF HOUSE EAVE ASPIRATION TO MOSQUITO SWARM SWEEP NET SURVEY AS MALE MOSQUITO COLLECTION METHODS

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Sample collections are essential to the study of disease vectors, and many methods have been developed for this. Most traditional malaria vector sampling techniques such as Human Landing Catches, indoor Pyrethroid Spray Catches and $C0_2$-baited traps bias collections towards female mosquitoes. Balanced vector sampling of both males and females is necessary for a more comprehensive understanding of vector dynamics. In some areas, swarm sampling (SWN), is considered the most efficient method for the collection of male mosquitoes. This method, however, requires labour, training and remuneration of part-time technicians as collectors. SWN catches are also influenced by local mosquito mating behaviour, environment and other co-occurring insect swarms. From previous field-tests of a range of techniques, the aspiration of house eaves (ASP-EAV) was identified as a promising male mosquito collection method for use at our field sites. During 2017, to assess whether ASP-EAV could be a viable supplementary or replacement method for SWM, we collected mosquitoes using both methods at three village sites located in the Mukono and Kayunga districts of Uganda. Three collections were made per season (wet and dry) in each village during which all identified swarms were collected using sweep nets and ten household eaves were aspirated using battery powered aspirators over a period of two days.1904 male mosquitoes were collected, of these, 1011 were collected by SWN and 893 by ASP-EAV. These data were analysed for seasonal variation in catch by method and comparisons were also made of the seasonal yield per man hour for each method. We found that ASP-EAV compared well to SWN in both seasons. While SWN caught more mosquitoes, the yield per man hour was lower than with ASP-EAV. The lower number of collectors required for ASP-EAV also reduced chances of sampler error affecting catches. We consider that ASP-EAV is a viable male mosquito collection method at our study sites and could also be an effective substitute for SWN in longitudinal vector abundance studies.

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A SYNERGISTIC BIOASSAY EVALUATION OF POTENTIAL RESISTANT MECHANISMS OF *ANOPHELES GAMBIAE* TO IVERMECTIN

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Despite remarkable success obtained with current malaria vector control strategies in the last 15 years, additional innovative measures will be needed to achieve the ambitious goals set for 2030 by the World Health Organization. New tools will need to assess insecticide resistance and residual transmission as key challenges. Endectocides such as ivermectin are drugs that kill mosquitoes which feed on treated subjects. Mass administration of ivermectin can effectively target outdoor and early biting vectors, complementing the still effective conventional tools. Although this potential approach has gained important attention, it is not free from potential development of resistance to ivermectin itself. The main aim of this work was to evaluate the potential role of xenobiotic pumps and cytochromes on ivermectin-induced mosquito mortality. We conducted an insectary-based randomized synergistic bioassay using membrane feeding to expose an *Anopheles gambiae* s.s to ivermectin with or without a battery of molecules affecting either the CYP3A4 or the P-glychoprotein. Seven drugs (or drug combinations) have been tested to assess the effect that the inhibition or induction of the said molecules can have on the ivermectin-caused mosquito mortality. In first place, dose-finding experiments were carried out to determine the 10-day LC_{50} of ivermectin. This was followed by synergistic bioassays combining ivermectin with different concentrations of the additional drugs. The main outcome was 10 day mosquito mortality determined by daily manual accounts. Survival analysis was done by Log-Rank test and Cox's regression. A dose-dependent synergism was found between ivermectin and CYP inhibitors like Voriconazole and Ritonavir. P-glychoprotein inducers did not show significant synergism. Other molecules like Cobicistat provided dose-dependent antagonism. Our data indicates that metabolic resistance to ivermectin could occur in the field.

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GEO-STATISTICAL ANALYSIS OF CONTINUAL ONCHOCERCIASIS TRANSMISSION IN AND AROUND AREAS RECEIVING BI-ANNUAL IVERMECTIN TREATMENT REGIMENS

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Ghana has scaled-up efforts aimed at eliminating human onchocerciasis, based solely on ivermectin chemotherapy, by 2025, a year set by the

London Declaration on Neglected Tropical Diseases (NTDs) and the World Health Organization Roadmap for NTDs. Thus, bi-annual ivermectin mass distribution was implemented, particularly in savannah villages showing sub-optimal responses to treatment by 2010, replacing the long-lived strategy of annual rounds of ivermectin treatment. We used entomological techniques and GIS-based geo-statistical tools to assess the impact of semi-annual ivermectin treatments on the number of *Simulium damnosum* s.l. flies harbouring third-stage infective larvae (L3) per 1000 parous flies from 17 selected onchocerciasis endemic communities along the middle savannah belt of Ghana which have been receiving bi-annual rounds of ivermectin treatment. Adult female *S. damnosum* s.l. were collected from the 17 communities, using human landing catches and analysed for *Onchocerca volvulus* infectivity and parity rates using manual fly dissection. A spatial geo-statistical analysis was performed with a GIS tool by interpolating vector infectivity rates using the inverse distance weighted (IDW) method. Raster maps were categorized into two according to start of ivermectin bi-annual treatment in 2010 and a six-year interval so as to obtain suitable maps for algebraic operations. In 2010 there was active transmission of *O. volvulus* in 11 of the 17 communities with vector infectivity rates far in excess of APOC's recommended threshold of 1 L3 per 1000 parous flies. However, after six years of bi-annual ivermectin distribution, 12 out of the 17 communities had zero vector infectivity rates with the other 5 communities having varying levels of vector infectivity rates, all above APOC's stipulated threshold. This geo-statistical approach is useful for delineating areas at risk of transmission despite intense chemotherapeutic interventions and is useful for decision-making in support of onchocerciasis control and epidemiology.

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MOSQUITO ELECTROCUTING TRAP: A SAFE ALTERNATIVE TRAPPING APPROACH TO MEASURE HUMAN EXPOSURE TO VECTORS COMPARE TO HUMAN LANDING CATCH IN SOUTHWESTERN BURKINA FASO

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Measuring human exposure to mosquito bites is a crucial component of surveillance for vector-borne diseases including malaria. For malaria vectors, the Human Landing Catch (HLC) remains the gold standard approach for direct estimation of this exposure. However, this method is risky as participants risk exposure to potentially-infected mosquito bites. Recently, a safer "Mosquito Electrocuting Trap" (MET) was developed to provide an exposure-free alternative to the HLC for measuring the human biting rate indoors and outdoors. Early prototypes of the MET performed well relative to the HLC in Tanzania, but it has yet to be tested in west Africa. Here we evaluated the performance of the MET relative to the HLC for characterizing mosquito vector population dynamics and biting behaviour in Burkina Faso. A longitudinal study was initiated in October 2016 within 12 villages in Burkina Faso where insecticide resistance levels are high. Host seeking mosquitoes were sampled monthly in each village over 18 months using the HLC and MET respectively. On each night of sampling, collections were made at 4 households, with METs being deployed inside and outside at 2 houses, and the HLC inside and outside at another two. The following night, trapping methods were switched between houses. Malaria vector abundance, species composition, sporozoite rate and location of biting (indoor vs outdoor) was recorded. A total of 34783 were female mosquitoes were collected in 251 days, in which *Anopheles gambiae* s.l. represented ~84%. The ratio of mosquitoes caught by the MET relative to the HLC was ~47% but varied between indoor (40.56% indoors) and outdoor settings (53.28% outdoors). The distribution of malaria vector species in MET and HLC collections was relatively similar (*An gambiae*: HLC: 40%; MET: 45%, *An coluzzii*: HLC: 60%, MET: 56%), as was their malaria infection rate (MET=5%, HLC=6%). The MET collected proportionately fewer mosquitoes than the HLC but remains safer both methods remained similar in predicting species composition and mosquito infection rate.

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DETECTION OF HUMAN BLOOD IN PERIDOMESTIC AND DOMESTIC KISSING BUGS (*TRIATOMA* SPP.) UTILIZING A RAPID FORENSIC TEST

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DNA- and proteomics-based techniques have been used to identify the vertebrate hosts upon which triatomines have previously fed. These procedures are time consuming, require access to a laboratory with sophisticated equipment, and trained personnel. The Rapid Stain Identification of Human Blood (RSID-Blood) is a lateral flow, immunochromatographic assay, used to detect as little as 1µL of human blood in forensic samples within 10 minutes after 1-2 hours of specimen preparation. To determine whether the RSID-Blood could be used to identify human blood within triatomines we conducted several experiments following the manufacturer's extraction protocol. All five laboratory-raised *Triatoma rubida* (100%) which fed on human blood through an artificial membrane feeding apparatus tested positive at 12 hours, 3, 5,7, and 14 days post-feeding. Laboratory-raised *T. rubida* having only fed on laboratory mouse (*Mus musculus*) blood within a 2-8 week period all tested negative (15/15) at various stages of engorgement. Peridomestic and domestic triatomines (*T. rubida, T. protracta, T. recurva, T. sanguisuga, T. gerstaeckeri, T. lecticularia*) collected across four states (Arizona, Texas, Louisiana, California) with visible blood meals during hindgut dissection were tested: 9/20 (45%) Arizona specimens, 4/7 (57%) Louisiana specimens, 9/21 (42%) Texas specimens, and the one California specimen were positive; with 23/49 (47%) of the total positive for human blood. Fecal drops (FDs) were also tested using RSID-Blood. Eight different laboratory-raised *T. rubida* having only fed on laboratory mouse blood provided eight FDs which all tested negative. Three triatomines that tested positive from our field collection, two of which were known to have fed on a human, provided three different FDs, all tested positive. In addition, laboratory-raised *T. rubida* that fed on human blood, provided two FDs, which were positive. Our results show that the RSID-Blood can detect human blood in triatomines and their fecal drops. This rapid test may have implications in triatomine fieldwork and other hematophagous vectors such as mosquitos, ticks, and biting flies.

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INTERACTION OF *RICKETTSIA FELIS* AND *WOLBACHIA* ENDOSYMBIONTS IN CAT FLEAS, *CTENOCEPHALIDES FELIS*

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Cat fleas (*Ctenocephalides felis*) have been identified as a biological vector and reservoir of *Rickettsia felis*. *Wolbachia* spp. are bacterial endosymbionts that are estimated to infect more than 60% of insect species, including cat fleas. The interaction of vertically transmitted endosymbionts within arthropods may influence the dissemination of pathogenic bacteria. However, the interaction between *R. felis* and *Wolbachia* spp. in cat fleas has not been examined. Thus, we hypothesize

that if *Wolbachia* endosymbionts compete with pathogens for stable vertical transmission events in the vector, then the presence of *Wolbachia* will influence vertical transmission of *R. felis* in cat fleas. In order to assess this interaction, fleas were separated and treated with different concentrations of tetracycline to remove *Wolbachia* from fleas. After treatments, fleas were allowed to mate and divided in to two groups. One group was exposed to *R. felis*, another group serving as a *R. felis*-uninfected control for 24 hours. Eggs were collected and allowed to develop to adults, and then the newly emerged adults were assessed for vertical transmission of *R. felis* by quantitative real-time PCR based on *R. felis* outer membrane protein B gene (*ompB*). Results show that the optimal concentrations of tetracycline were 1.0-2.0 mg/ml. Vertical transmission of *R. felis* and *Wolbachia* endosymbionts infected fleas was demonstrated and implied a possible influence of *Wolbachia* on the vertical transmission of this emerging rickettsial pathogen.

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PHLEBOTOMUS PAPATASI SALIVARY GLAND GENE DIVERSITY IN DISTINCT ECOTOPES OF EGYPT AND JORDAN

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Phlebotomus papatasi sand flies inject a host of pharmacological salivary proteins to assist with blood feeding and modulate host defenses. These salivary proteins have been studied for their role in cutaneous leishmaniasis disease outcome with different salivary proteins attenuating or exacerbating lesion size. Studies have shown that while co-administered sand fly saliva exacerbates *Leishmania major* infections in naïve mice, animals pre-exposed to saliva are protected, with the infection attenuated via a delayed-type hypersensitivity immune reaction. The immunogenicity of salivary components results in a hostile environment making it difficult for *L. major* to successfully establish an infection in pre-exposed individuals. These studies highlight the potential of the salivary components to be used as a vaccine. One protein in particular, *P. papatasi* salivary protein 15 (PpSP15) has been intensively studied due to its ability to protect mice against *L. major* challenge. The number of antigenic molecules included in vaccines is restricted thus emphasizing the role of population genetics to identify molecules, like PpSP15, that are not experiencing positive selection pressure. Functionally significant proteins conserved across populations and under purifying selection demonstrate great promise as a vaccine component. Three distinct ecotope study sites in Egypt (Aswan) and Jordan (Swaimeh and Malka) were chosen based on their elevation, rainfall, vegetation, differing reservoir species, and the presence or absence of *L. major*. The objective of this work was to analyze the intra- and inter-population diversity of nine of the most abundantly expressed salivary proteins including SP12, SP14, SP28, SP29, SP30, SP32, SP36, SP42, and SP44 and to predict their ability to elicit an immune response.

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GENETIC VARIABILITY OF TWO SPECIES OF *TRIATOMA* FROM COASTAL JALISCO, MEXICO

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The subfamily Triatominae contains hematophagous insects that serve as vectors for *Trypanosoma cruzi*, a single-celled parasite that infects mammals, including humans. Chagas disease, caused by infection with *T. cruzi*, is the most serious of the parasitic diseases in Latin America and is considered a neglected tropical disease. Triatominae display a high degree of morphological plasticity without clear genetic support. There are also clear cases where genetic variation is seen without distinct morphological traits. We collected a large sample of *Triatoma spp.* from Estación de Biología, Chamela, in coastal Jalisco, Mexico and examined the within-species genetic variability. Two species of *Triatoma* were collected, 61 *Triatoma bolivari* and 34 *Triatoma longipennis*. The specimens were sequenced for two non-conserved regions of DNA (internal transcribed spacer (ITS-1) and ITS-2) to examine the within-population genetic variability. *T. longipennis* is reported to have high genetic variation across Mexico, however within-population studies have not been undertaken. *T. bolivari,* a rare species of *Triatoma* only found in the dry tropical forest, is thought to have low diversity due to relatively low morphological differences between individuals. Though *T. bolivari* has previously been reported to be a sylvatic species associated with birds, we have found squirrel (*Sciurus colliaei*) to be the most common blood meal in wild-caught specimens. *T. bolivari* is attracted to lighting and has been found to have fed on human blood, suggesting a risk for domestication of this species. As rural populations increase and natural disasters such as hurricane Patricia cause population shifts, a better understanding of *T. bolivari* will be important for establishing adequate vector control.

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CHARACTERIZING THE VIROME OF *RHIPICEPHALUS MICROPLUS* TICKS FROM COLOMBIA THROUGH RNA-SEQ TECHNOLOGY

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Ticks (Ixodida) are hematophagous ectoparasites which harbor and transmit diverse virus species, some of which cause serious diseases with worldwide veterinary and human health impact. *Rhipicephalus microplus* is an important cattle tick species in Colombia, where it causes significant economic losses and despite its importance, its viral profile haven't been studied so far. Considering that Next Generation Sequencing technologies could provide a powerful means of studying viral diversity in ticks, RNA sequencing (RNA-Seq) was used in this study as a surveillance method for virus detection in *R. microplus*. Ticks were collected from Antioquia, in northwestern Colombia and pooled according to the collection site in 3 ticks per pool. RNA was isolated, libraries prepared and sequenced on Illumina HiSeq 4000 (TruSeq Stranded mRNA -PolyA) and NovaSeq 6000 (rRNA-depleted RNA) sequencing platforms. Raw sequence reads were filtered and trimmed according to their quality score (≥Q30). The clean sequence reads were assembled into contigs, which were compared and annotated. Taxonomic assignation of these contigs was achieved by successive searches using the BLASTX algorithm against a protein reference database. Manual annotations were performed using Artemis. The majority of viral contigs (n=30, 53%) were assigned to two putative

viruses: Wuhan tick virus 2 and Lihan tick virus (>98% sequence similarity). Both viruses were discovered in China during 2015 also in the *R. microplus* ticks. In addition, 5 contigs were similar (84-92%) to the Jingmen tick virus reported in China during 2014 in a *R. microplus* ticks pool, while the close relative of other 4 contigs was the Mogiana tick virus, isolated in 2011 from Brazil. This work will be continued with a more in-deep characterization of contigs carrying putative viral sequences. To our knowledge, this is the first report of the occurrence of these viruses in *R. microplus* from Colombia. Our results shed new light on the virus diversity of this tick species and provide the foundation for further studies into the evolutionary history and pathogenic potential of these intriguing viruses.

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VARIABLE EFFECTS OF MOSQUITO MIDGUT-EXPRESSED MICRORNAS FOR THE RESTRICTION OF WEST NILE VIRAL ORAL INFECTIVITY OF *CULEX QUINQUEFASCIATUS*

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The mosquito midgut epithelium serves as the initial site of infection of arboviruses, yet little is known about specific cell populations that dictate infection phenotypes of vectors. To identify mosquito cell biomarkers and assess specific roles of cell populations for midgut infectivity with West Nile virus (WNV), microRNA (miRNA) libraries were generated from C6/36 mosquito cells and dissected *Culex quinquefasciatus* (Cxq) midguts, respectively. Target sequences (three copies) for the twenty most highly expressed Cxq midgut miRNAs were incorporated into the 3'UTR of a WNV cDNA clone. Recombinant WNVs encoding these target sequences (WNV-miR) were generated and growth profiles compared to wildtype WNV (wt-WNV) in target C6/36 and non-target Vero cells. In C6/36 cells, variable inhibition levels were observed with WNV-miRs, ranging from complete to undetectable viral growth which largely correlated with cellular miRNA expression levels. In contrast, WNV-mir growth was indistinguishable from that of the wt-WNV in Vero cells, indicating undetectable off-target miRNA insertional effects. In Cxq orally exposed to WNV-miRs, target sequences for 17 of the top 20 midgut miRNAs resulted in complete loss of Cxq infectivity. Surprisingly, Cxq infectivity with the WNV-miR encoding the target sequence of the 6th highest expressed miRNA, miR-276-3p (65%), was indistinguishable from wt-WNV. The finding that miRNAs with lower expression levels could completely block WNV-miR oral infectivity in Cxq indicates that miRNA expression is unlikely to be uniform within the midgut and that select midgut cells with defined miRNA expression profiles likely serve as critical sites for initial oral infection with WNV.

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CHARACTERIZATION OF *WOLBACHIA* INFECTIONS FROM NATIVE AUSTRALIAN MOSQUITO VECTORS

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Wolbachia are maternally transmitted intracellular bacteria that naturally infect over 40% of all insect species. *Wolbachia* have become promising biocontrol agents against mosquito-borne diseases due to their unique effects on mosquito reproduction and immunity. However, the phenotypes mediated by *Wolbachia* vary between strains and host-pathogen systems. Discovery of novel *Wolbachia* strains is essential for broadening the application of *Wolbachia* for mosquito control. In this study we identified and characterized the anti-pathogen effects of natural *Wolbachia* infections in Australian mosquitoes. We identified three previously uncharacterized *Wolbachia* strains, in addition to the recently reported strain from *Aedes notoscriptus*, based on detection of *WSP*, *16s* and *FTSZ* genes. We established colonies of two local species, *Ae. notoscriptus* and *Culex sitiens*, and discovered a unique pattern of infection rates fluctuating between 15% and 60% in both colonies. Study on the maternal transmission of *Wolbachia* in *Cx. sitiens* revealed high transmission rates (99.3%) but low cytoplasmic incompatibility (9.9% mortality). We orally inoculated *Ae. notoscriptus* and *Cx. sitiens* with the alphavirus Ross River virus (RRV; $10^{3.31 - 6.48}$ and $10^{5.41 - 6.36}$ $CCID_{50}$ per mosquito, respectively). RRV infection rates varied between 17% - 85% for *Ae. notoscriptus* and 0% - 18% for *Cx. sitiens* with no significant differences between *Wol*$^{+}$ and *Wol*$^{-}$ mosquitoes. However, mean virus load in *Wol*$^{+}$ *Cx. sitiens* was 1000× lower than in *Wol*$^{-}$ mosquitoes (P = 0.0043), suggesting that natural *Wolbachia* infection suppresses virus proliferation in that species. We report on progress towards transinfecting cells (Aag2 and Aa20) and mosquitoes (*Ae. aegypti*) with *Wolbachia*, including oral inoculation trials. We observed substantial uptake of *Wolbachia* in the mosquito digestive tract by fluorescence microscopy however the midgut currently remains a barrier to dissemination. The behaviour of *Wolbachia* in new hosts can be difficult to predict, therefore transinfection and further characterization is required to determine if these strains can induce anti-pathogen effects.

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SHARED MOSQUITOES, SHARED PROBLEM: VECTOR SURVEILLANCE AS A TOOL FOR PUBLIC HEALTH DIPLOMACY

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The 2016 Zika virus outbreak highlighted the speed at which vector-borne pathogens can spread across countries and regions. While many affected countries shared common entomological risk factors, cross-border communication on vector surveillance or control was uncommon. To improve vector surveillance capabilities and enhance data sharing, binational exercises in vector surveillance were conducted on the island of Hispaniola. Beginning in March 2017, staff from the National Malaria Control Program in Haiti and the National Center for the Control of Tropical Diseases in the Dominican Republic received training in the use of the Epi Info Vector Surveillance application, a novel mobile tool for vector surveillance and automated data analysis. Teams from each country piloted the app and held a binational meeting in July 2017 to discuss results. During this meeting, both countries noted the ease of sharing and reporting data between countries using the app, and decided to explore how cross-border data sharing could be formalized. In November 2017, the programs met in the northern bordering towns of Dajabón, Dominican Republic and Ouanaminthe, Haiti to conduct a binational vector surveillance exercise. Five teams, each consisting of both Haitian and Dominican field workers, spent one day working in Ouanaminthe (47 households visited) and one day working in Dajabón (49 households visited) collecting entomological data using the mobile app. On the final day of the exercise, the collected data from both countries were simultaneously displayed and discussed, with a focus on the utility of collecting and analyzing the indicators in a standardized way. At the conclusion of the exercise, directors from both programs agreed to continue collecting and sharing their entomological data using this platform. In addition to improving and standardizing vector surveillance activities across Hispaniola, these exercises deepened the commitment for binational collaboration in addressing priority public health threats and strengthened the relationship between key stakeholders on both sides of the border.

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EVOLUTION OF RHS PROTEINS IN BLOOD FEEDING ARTHROPODS AND *WOLBACHIA*

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Members of the RHS protein family have been found in the saliva of mosquitoes and appear to be present in the genomes of several disparate groups of arthropods. Recent studies from bacteria have highlighted the likely function of RHS proteins as contact dependent growth inhibitors. Therefore, we hypothesize that RHS proteins act to influence the insect microbiome in certain insect tissues or act as immunomodulators of the vertebrate skin during blood-feeding. This research focuses on the RHS proteins of *Aedes albopictus*, *Cimex lectularius*, and *Wolbachia*. First we conducted phylogenetic and codon-bias analyses of the genomics-supported arthropod RHS proteins. We then determined the presence of an *rhs* gene in the *Cimex* genome, but not in its obligate *Wolbachia* symbiont, using long-PCR and tissue-specific qPCR analysis before and after antibiotic treatment. Using comparative analyses, we then identified the most logical target for functional studies, and RHS proteins are currently being examined through *in vitro* expression and analysis of their putative functional domains. Other ongoing studies are also investigating changes in RHS expression in the absence of *Wolbachia* in antibiotic treated *Cimex lectularius*, and we are working to silence RHS in *Cimex lectularius* to explore its effect on the life history and response to infection of *Cimex lectularius*.

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METABARCODING, A NEW APPROACH FOR THE COMPREHENSIVE STUDY OF *TRYPANOSOMA CRUZI* TRANSMISSION CYCLES AND TRIATOMINE BEHAVIOR

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Chagas disease, caused by the parasite *Trypanosoma cruzi*, is mainly transmitted to humans and other mammals by blood-sucking insects called triatomines. Establishing transmission cycles is key to understand the epidemiology of the disease, but integrative assessments of ecological interactions shaping parasite transmission are still limited. Current approaches also lack sensitivity to assess the full extent of this ecological diversity. We developed a metabarcoding approach based on next-generation sequencing to simultaneously identify triatomine gut microbiome, vertebrate feeding hosts, triatomine and parasite genetic diversity and their potential interactions. Using a sample of 15 *Triatoma dimidiata*, the main vector in the South of Mexico and Central America, we detected a dynamic microbiome, including 23 bacterial orders. Fourteen vertebrate species served as blood sources. Importantly, bugs fed on multiple hosts, with up to 11 hosts identified per bug, indicating very frequent host-switching. A high clonal diversity of *T. cruzi* was detected, with up to 20 haplotypes per bug. This analysis provided much greater sensitivity to detect multiple blood meals and multiclonal infections with *T. cruzi*, which should be taken into account to develop transmission networks, and characterize the risk for human infection, eventually leading to a better control of disease transmission.

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EFFECTS OF ESSENTIAL OILS OF *CHENOPODIUM AMBROSIOIDES* ON MALARIA AND ARBOVIRUSES MOSQUITO VECTORS IN MUHEZA TANZANIA

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Increase in insecticides resistance among mosquito population presents a great challenge to chemicals that are used for mosquitoes control in sub-Saharan African. Currently, there is no approved alternative insecticide to pyrethroid which is used in impregnated bed nets. This study aimed to determine effects of *Chenopodium ambrosioides* essential oils against malaria and arboviruses mosquito vectors. Insecticide susceptibility bioassays were performed according to the World Health Organization guidelines on 2-5 days old human biting mosquitoes. Each of the four species of mosquitoes were exposed to *C. ambrosioides* essential oils (10%) and two classes of insecticides commonly used for malaria vector control. Mosquito mortality rates (%) were determined after 24 hours post insecticide exposure. All mosquito species tested were susceptible to *C. ambrosioides* essential oils (10%). *An. gambiae* s.l. showed possible resistance to Permethrin (0.75%) and Deltamethrin (0.05%) with 93% and 92% mortality rates (%) 24 hours post exposure to insecticides. With Lambdacyhalothrin (0.05%), *An. gambiae* s.l. was resistance with mortality rate of 57%. *An. funestus* was susceptible to all insecticides tested. *Aedes aegypti formosus* was susceptible to all insecticides tested except DDT (4%). All insecticides tested were able to knock down 50% of all mosquito species (KDT50) exposed within 1 hour. *C. ambrosioides* essential oils (10%) took the shortest mean time to knock down 50% of all mosquitoes tested. The mean time ranged from 11.4 to 13.1 min. The mean time taken to knock down 95% of all mosquito species was 17.8 from *C. ambrosioides* essential oils (10%) in *An. gambiae* s.l. This study has revealed that, *C. ambrosioides* essential oils have demonstrated appreciably higher strong insecticidal effects on malaria and arboviruses mosquito vectors. Further studies are needed to determine the long-lasting insecticidal efficacy of essential oils extracts from *C. ambrosioides* for development of novel methods of controlling mosquito vectors.

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CYP6-Z4 (CYTOCHROME P450-6-Z4)-MEDIATED INSECTICIDE RESISTANCE IN *ANOPHELES STEPHENSI* AGAINST DELTAMETHRIN

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Anopheles stephensi is one of the major primary malaria vector found in Middle-East and South Asia region. In India, this is regarded as urban malaria vector. Control of malaria vectors relies mainly on insecticide-based vector control methods, which has become a challenging and formidable task due to rapid development of resistance against commonly used insecticides. Pyrethroid group of insecticides, which are still effective in India against most of the vectors, are currently choice of insecticides for the control of malaria vectors due to rapid killing action, low mammalian toxicity and degradability in nature. Although there is no report of pyrethroid-resistance in *An. stephensi* in India, we attempted to understand molecular basis of resistance by raising insecticide resistance in this mosquito in laboratory through selection pressure against deltamethrin. We hereby report evidence of Cytochrome P450-6-Z4 (CYP6-Z4)-mediated pyrethroid-resistance in laboratory colony of *An. stephensi*. Cytochrome P450 (CYPs)-Zs are important class of CYPs enzymes known to play a major role in resistance against pyrethroid group of insecticide, by metabolizing the insecticide at a higher rate in mosquitoes. Several CYP6-Z family genes like CYP6-Z1, CYP6-Z2, CYP6-Z3, CYP6-Z4 etc. are known to be overexpressed and responsible for pyrethroid resistance in various other anophelines. To understand the role

of CYP6-Z4 gene, cDNA was synthesized from total RNA of susceptible and laboratory selected deltamethrin-resistant strain of *An. stephensi* mosquito and a quantitative real-time PCR was performed by using gene specific primers. Quantitative real-time data shows that the relative amount of CYP6Z4 transcript was significantly higher in the deltamethrin-resistant strain of *An. stephensi* as compared to the susceptible strain which indicates possible role of this gene in pyrethroid resistance. Further study to characterize this gene in deltamethrin resistant and susceptible strain is under investigation. The study will help in understanding of molecular basis of pyrethroid resistance, which will be helpful in integrated vector management.

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EVALUATING THE EFFECT OF IVERMECTIN B_{1A} AND B_{1B} COMPOUNDS AGAINST THE MALARIA VECTOR *ANOPHELES DIRUS*

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Ivermectin (IVM) is a broad-spectrum medication used to treat onchocerciasis, lymphatic filariasis, strongyloidiasis and scabies. IVM is particularly potent against Anopheline *mosquitoes*, causing significant mortality, delayed re-feeding, and reduced fertility when ingested through a blood meal. IVM *in vitro* activity was assessed by spiking human blood with various concentrations of parent compound (IVM-spiked), blood feeding to *Anopheles dirus* and monitoring subsequent mortality to determine the lethal concentration that kills 50% (LC_{50}) of mosquitoes. IVM *in vivo* activity was assessed in a clinical trial wherein Thai volunteers were treated with IVM at 400 µg/kg (IVM-treated) and their venous blood was membrane fed to *An. dirus*. The *An. dirus* 7-day-LC_{50} of IVM-spiked blood was 56.90 [53.69 - 60.11] ng/ml, whereas IVM-treated blood had a 7-day-LC_{50} of 2.80 [2.65 - 2.95] ng/ml. These results indicate that metabolized IVM (IVM-treated) has a 20-fold greater lethal effect compared to IVM parent compound (IVM spiked). This suggests that IVM metabolized by humans may extend efficacy beyond that predicted from IVM parent compound alone. In nature, IVM is a mixture of two homologs, 22, 23-dihydroavermectin B_{1a} (IVM B_{1a}) and 22, 23-dihydroavermectin B_{1b} (IVM B_{1b}) at a ratio of >90% and <10% respectively. Both IVM B_{1a} and IVM B_{1b} are metabolized by similar pathways in the vertebrate. A recent study in snails demonstrated that the minor IVM B_{1b} is the lethal component, while the major IVM B_{1a} component had no lethal effect. Here we present for the first time, the *in vitro* impact of IVM parent compound, IVM B_{1a}, and IVM B_{1b} on *An. dirus* survival. These results will be used to guide discovery of which IVM metabolites possess mosquito-lethal effect.

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INSECTICIDE SUSCEPTIBILITY PATTERN AND BIOCHEMICAL ANALYSIS OF *PHLEBOTOMUS ARGENTIPES,* THE VECTOR OF LEISHMANIASIS IN SRI LANKA

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Phlebotomus argentipes is the known vector of *Leishmania donovani*, the causative organism of leishmaniasis. A major challenge of an effective vector control program is the development of insecticide resistance in the vector. Galgamuwa in North-Central Province and Pannala in North-Western Province of Sri Lanka were selected for sand fly collection. Identification of *P. argentipes* was confirmed using standard taxonomic keys. F1 progeny were exposed to different concentrations of DDT, malathion, deltamethrin and propoxur and LD_{50} was determined using mortality curves. Results were validated with the control mortalities using Abbott's formula. For biochemical analysis, esterase assay, glutathione S-transferase assay, oxidase assay & protein assay were performed with individually homogenized sand flies in 80µl of ice cold distilled water. Another set of population were homogenized in 50µl of ice-cold distilled water for acetylcholinesterase assay. The colony population was susceptible to concentrations of >0.6% DDT and >0.7% Malathion, >0.007% Deltamethrin & >0.015% Propoxur with no survivors after 24-hour recovery period. However, the insects were resistant to lower concentrations than the ones mentioned above & all flies were alive after 24 hour post-exposure recovery period. LD_{50} was determined as 0.6%, 0.7%, 0.007% and 0.016% for DDT, malathion, deltamethrin & propoxur respectively. Most of the flies had protein activity <0.3µmol min^{-1} mg^{-1}, esterase activity <1.00 µmol min^{-1} mg^{-1} and GST activity <0.4µmol min^{-1} mg^{-1}. All protein, esterase & GST activity were < 0.35 equivalent units of monooxygenase amounts. Over half of this population had <30% residual AChE activity in the presence of propoxur. However, 6.5% had >60% residual activity. Hence, the majority of the population is said to be the susceptible to the insecticides used in this study. Further research is being conducted to ascertain the genetic mechanism behind the susceptibility patterns of Sri Lankan *Ph. argentipes* to synthetic insecticides.

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ASSOCIATION OF F1534L KNOCKDOWN RESISTANCE (*KDR*) MUTATION WITH PYRETHROID RESISTANCE

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Aedes aegypti is a vector of several arboviral infections including dengue and chikungunya. In absence of specific treatment or vaccine available to control these arboviral infections, suppression of the vector population is the only option to control these infections. One of the potential methods for the control of this vector is the use of pyrethroid group of insecticides for space spray as well as personal protection measures. Knockdown resistance (*kdr*) is one of the mechanism of pyrethroid resistance occur due to mutation in the voltage gated sodium channel (target site of action), resulting in reduced sensitivity of target site to the insecticide. We carried out survey on presence of *kdr* mutations in *Ae. aegypti* in three metropolitan cities of India, i.e., Delhi, Bengaluru and Kolkata. Recently, we discovered a new *kdr* mutation F1534L in *Ae. aegypti* co-occurring with other *kdr* mutations S989P, V1016G and F1534C. The F1534L mutation is being recorded for the first time in *Ae. aegypti*. Mutations S989P and V1016G were in complete linkage disequilibrium and both mutations were having negative linkage disequilibrium with F1534C or F1534L. We established that the new mutation F1534L has significant protection against permethrin and deltamethrin. In the wake of protection conferred by this mutation on pyrethroid insecticides, it is of paramount importance to screen other populations of *Ae. aegypti* for presence of new mutation F1534L.

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INSECTICIDE RESISTANCE MECHANISMS INFLUENCE MOSQUITO LIFE HISTORY TRAITS AND BEHAVIOR

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Insecticide resistance (IR) in disease vectors is at a crucial tipping point. Resistant mosquitoes not only survive longer than their susceptible counterparts, but the associated resistance mechanisms can lead to significant alterations in key physiological functions. Do these changes influence vector behaviour and disease transmission? Exposure to different insecticides (REC-R to temephos, REC-M to malathion, REC-P to

permethrin), and resistance reversal (REC-U) were used to create multi-resistant strains of *Aedes aegypti* from Recife, Brazil. Mosquitoes were reared at two different larval densities to evaluate resource allocation using energetic resources, longevity and fitness. To assess whether IR mechanisms are associated with differing energy reserves, we measured lipid and glycogen content and body size at different ages. The effects of these mechanisms on further life traits were assessed using pupation and eclosion rates, sex ratio and longevity, mating efficacy, fecundity and egg viability. Generalised Linear Mixed Models were used to predict the effects of IR mechanisms on the energetic resources of mosquitoes, using the parameters day post-emergence, larval density and resistant strain. Our model showed an interaction between strain*density and strain*days with regards to lipid content, however no significant interactions where shown for glycogen. Of the strains tested, REC-R (larval exposure to temephos) contained significantly more lipids than all other strains at day 2 at both rearing densities, and significantly higher glycogen levels than all other strains on both days at both densities. Longevity assays showed REC-R females survived significantly longer than other strains. However, there appeared to be a fitness trade-off with fecundity, with REC-R exhibiting poor mating success, and poor fecundity in mated females. Male mosquitoes pupated and emerged one to two days before females, with REC-R adults emerging in greater initial numbers. Differences in sex ratio where observed to be more pronounced when reared under crowded conditions.

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EFFECTS OF DIFFERENT INSECTICIDE PRESSURES IN GENE EXPRESSION FOR TWO STRAINS OF *AEDES AEGYPTI* (CAYMAN AND RECIFE-R)

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Insecticide resistance in *Aedes* mosquitoes is challenging vector control measures for arboviruses. In *Ae. aegypti*, multiple insecticide resistance mechanisms have emerged independently across the globe for different insecticides. The effect of these resistance mechanisms on seemingly unrelated traits (pleiotropy) such as fecundity, immunity, behaviour or vector competence is poorly understood. Here, we perform a broad RNA-seq analysis in two strains of *Ae. aegypti* to infer the gene expression variation associated with different insecticide exposure or genotype within each mosquito strain. We established two sets of mosquito colony lines selected by different insecticide regimes across 10 generations. One set was based on the Cayman strain which contains knockdown resistant alleles (*kdr*) in the voltage gated sodium channel. A total of four new lines with resistance ratios between 6.5 and 213 were established by a combination of genotype and permethrin exposure. Whereas, the second set was based on the Recife-R strain that present metabolic resistance for temephos. In this strain, we establish two new lines exposed for two adulticides (malathion: RR = 3.1; and permethrin: RR = 9.9) and a third was unexposed for resistance reversal of temephos. Five biological replicates of each colony line and baselines were sequenced by RNA-seq. The characterization of molecular mechanisms of resistance indicates a combination of high expression of CYP9s with *kdr* mutations for Cayman resistant lines, while only one P450s (CYP6) was found upregulated for Recife-R permethrin line. Moreover, genes associated with temephos resistance (CYP6N12, CYP6M11, CYP6BB2) maintain a higher gene expression for Recife-R malathion line and a significant reduction for the Recife-R reversal line. Genes associated to pleiotropic effects are under annotation that suggest an upregulation of immunity genes in the Recife-R reversal lines. The differential expression associated with insecticide resistance would be discussed with the support of complementary insectary experiments.

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CHEMICAL COMPOSITIONS AND LARVICIDAL ACTIVITY OF ESSENTIAL OILS OF *CURCUMA ZEDOARIA* AGAINST THE DELTAMETHRIN RESISTANT OF *CULEX QUINQUEFASCIATUS*

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Culex quinquefasciatus is the important vector of several diseases to the human, including St. Louis encephalitis and lymphatic filariasis. The current way to eliminate the chain of transmission of the vector-borne diseases is to control the vector by using synthetic insecticidal chemicals. However, the continuous exposure of chemicals to the insect population has resulted in the development of resistant strains and is harmful to the environment. Therefore, the novel insecticide is required to reduce the number of diseases carrying vectors. Herbal essential oils have many biological properties with larvicidal effects and ecofriendly uses. Moreover, they have shown potential as natural insecticides. This study aimed to determine the chemical composition of essential oils extracted from *Curcuma zedoaria* fresh (FZEO) and dried (DZEO) rhizomes on *Cx. quinquefasciatus*. The larvicidal activity of FZEO and DZEO were tested against of the both deltamethrin resistant and susceptible strains of *Cx. quinquefasciatus* larvae. The chemical compositions of the oils were identified by GC-MS analysis. The main constituents in both oils were sesquiterpenes and monoterpenes. FZEO contained 37 compounds. AR-tumerone (23.66%), zingiberene (14.74%), β-sesquiphellandrene (10.64%) and eucalyptol (10.57%) were the major components. In contrast, DZEO contained 35 molecules. β-tumerone (16.69%), AR-tumerone (15.55%), eucalyptol (11.57%) and zingiberene (10.12%) were the major components. After 24h of exposition, both deltamethrin resistant and susceptible strains were responsive to the essential oils. FZEO showed a higher larvicidal efficacy against both strains compared to DZEO, in which the LC_{50} for resistant (LC_{50} = 36.19 ppm) and susceptible (LC_{50} = 33.19 ppm) strains are slightly higher than the DZEO, LC_{50} values of 37.30 and 36.32 ppm for both strains, respectively. The larvicidal efficacy of *C. zedoaria* essential oil to eliminate the insecticide resistant or susceptible strains of *Cx. quinquefasciatus* presents a promising new mosquito larvicide. The potential of *C. zedoaria* essential oil is undergoing to use as a natural larvicide.

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INSECTICIDE RESISTANCE STATUS OF *AEDES* MOSQUITOES IN GHANA

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This study investigated the insecticide resistance status of *Aedes* mosquitoes in Ghana. To determine phenotypic resistance status of *Aedes* mosquitoes, *Aedes* larvae were collected from five study sites (Accra, Tema, Ada-Foah, Tamale, and Damongo). These were raised to adults in the insectary and 2-5 days old adults were used to undertake WHO susceptibility bioassay. The test results showed that *Aedes* mosquito populations from all study sites were resistant to DDT (6.3 - 84%). Vectors showed resistance to deltamethrin in Tema (68%) and Tamale (85%) and suspected resistance in Accra (91.3%), Ada-Foah (93.5%) and Damongo (93%). Vectors showed resistance to permethrin in Accra (40.0%) and suspected resistance in Tamale (96.3%). *Aedes* mosquitoes showed suspected resistance to bendiocarb in Tema (95.0%) alone.

Aedes mosquitoes were susceptible to organophosphates in all sites. Morphological identification of the adult mosquitoes sampled in the sentinel sites showed that *Ae. aegypti* (93.7%) were the abundant species present in the sites, followed by *Ae. africanus* (6.0%) and *Ae. luteocephalus* (0.3%). The development of resistance by *Aedes* mosquitoes to pyrethroids is likely to have an operational impact on the efficacy of insecticide based vector control interventions.

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OPTIMIZATION OF MASS-REARING METHODS FOR *ANOPHELES ARABIENSIS* FOR STERILE INSECT TECHNIQUE APPLICATION

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Mass-rearing of *Anopheles arabiensis* mosquito is crucial for strategies that use sterile insect technique to suppress vector populations. Production at a large-scale requires the development of standardized rearing procedures to produce good quality males able to compete with wild males for mating with wild females. The aim of this study was to optimize rearing conditions to ensure high egg productivity of *An. arabiensis* in the mass rearing cages and the highest pupae production in the larval rearing unit. Mass production cages of two different volumes, two different sources of blood meal (bovine and porcine) and two different population densities (cages originally loaded with either 15,000 or 20,000 pupae) were tested and evaluated on the basis of eggs produced/cage or per female. Moreover, four different egg quantities (4,000; 5,000; 6,000 and 7,000) and two different water temperatures at the time of egg hatching (22 °C and 27 °C) were tested for effects on time to pupation, pupation and adult emergence rates, sex ratio and adult body size. Neither cage volume nor blood meal source affected egg production per cage or per female. However, increasing population density to 20,000 pupae had a negative effect on eggs produced per cage and per female. The data point out the negative impact of high larval densities (7,000 eggs/tray) and low water temperature (22°C) on *An. arabiensis* pupae production and adult size. With the current mass-rearing tools available at the Insect Pest Control Laboratory and within the tested range, 4,000 eggs per larval rearing tray hatched at a water temperature of 27°C are the optimal conditions, providing the largest number of pupae (105,000 pupae / larval rearing unit (rack) with subsequent large emerged males for release and females for eggs production. Moreover, 15,000 is the optimal number of pupae to be loaded into the *Anopheles* Mass production cages. These results are valuable information to be taken into consideration when mass-rearing *An. arabiensis* for SIT programmes, and others relying on large scale production of mosquitoes.

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FITNESS COSTS OF TWO PYRETHROID RESISTANCE MECHANISMS IN *AEDES AEGYPTI*: CYP-MEDIATED DETOXIFICATION AND *KDR*, INDIVIDUALLY AND IN COMBINATION

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Aedes aegypti, an important vector of many human diseases, is a serious threat to human health due to its wide geographic distribution and preference for living near humans. Insecticides, especially pyrethroids, are still the primary means to control adult *Ae. aegypti* in endemic areas, especially during disease outbreaks, which has led to its extensive use over the past decades. Consequently, pyrethroid resistance is now found worldwide and is a major obstacle to the control of medically significant arthropod pests. Resistance alleles often have a fitness cost in the absence of insecticides. Understanding these costs and evolution of resistance is critical in integrated resistance management practices for it helps to determine how quickly resistance will be lost under field conditions after pesticide application has ceased. Our goal is to understand how the different resistance mechanisms impact mosquito fitness both independently and in combination. As a first step, such a study is best conducted in a controlled environment with minimal variables. We did this using a life table analysis to measure developmental time, survival, fecundity, egg viability, and body size. In *Ae. aegypti*, the two main mechanism of pyrethroid resistance are mutations in the *voltage-sensitive sodium channel* (*Vssc*) and enhanced cytochrome P450 (CYP)-mediated detoxification. To compare the fitness costs of the different mechanisms without the interference of other genetic variations, we isolated three pyrethroid resistant strains of *Ae. aegypti* that are congenic to the susceptible Rockefeller (ROCK) strain, but containing the different resistance mechanisms. CYP+KDR:ROCK contains both CYP-mediated resistance and *Vssc* mutations S989P+V1016G (*kdr*); KDR:ROCK contains only *kdr* and no CYP-mediated resistance; and CYP:ROCK contains only CYP mediated resistance and no *kdr*. Our preliminary results show that CYP-mediated resistance causes significant fitness costs regardless of the presence or absence of *kdr*. The implications of these results to the control of *Ae. aegypti* will be discussed.

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EVALUATING THE EFFICACY OF MINI DOUBLE NET TRAP (MDN) FOR SAMPLING HOST SEEKING MOSQUITOES

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Effective sampling tool that do not expose collectors is essential for monitoring malaria transmission. Human landing catch is considered as a gold standard but it exposed human being to both malaria and non-malaria pathogens. We have introduced a mini-double net trap as a replacement of HLC due to ethical and protection issues. The MDN with different sizes of the outer layer were tested to observe which height is suitable for collecting more mosquito species. The trap was designed with different sizes of the outer layer, i.e. 20cm, 50cm, 80cm from the ground and one with the holes on the side, allocated 100m apart. A Latin square design was used to evaluate the MDN trap. A total of 2,004 mosquitoes were collected using a MDN trap. The trap with holes on the side caught 468 (23.3%), MDN-20cm caught 505 (25.1%), MDN-50cm caught 535 (26.7%) and MDN-80cm caught 496 (24.7%). The MDN trap with the length of 50cm had significantly higher number of *Anopheles arabiensis* than the standard height MDN-20cm [Relative rate, RR=1.37, 95%CI (1.02 - 1.85), *P<0.05*]. MDN-80cm had fewer catches of *An. arabiensis* compared to MDN-20cm, [RR=0.88, 95%CI (0.68 - 1.14), *P=0.*337]. There is not significantly differences in the number *An. arabiensis* capture by MDN with side holes compared to MDN-20cm [RR=1.05, 95%CI (0.767 - 1.44), *P=0.*759]. The number *An. funestus* captured by MDN-50cm, MDN-80cm and MDN with side holes were not significantly different with the MDN-20cm [RR=0.33, 95% CI (0.06 -1.69), *P=0.*184), [RR=0.82, 95%CI (0.24 - 2.82), *P=0.*754) and [RR=0.98, 95%CI (0.30 - 3.20), *P=0.*979) respectively. We can therefore conclude that, MDN trap with the outer layer measuring 50cm from the ground can be considered as the most suitable options for collecting host-seeking mosquito species especially the most abundance vectors, *An. arabiensis*.

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INSECTICIDE RESISTANCE IN *ANOPHELES ARABIENSIS* POPULATIONS FROM DAKAR AND ITS SUBURBS: ROLE OF TARGET SITE AND METABOLIC RESISTANCE MECHANISMS

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Malaria is still a major public health problem in Senegal particularly in Dakar, where recurrent flooding occurring since 2005 complicated the epidemiology of the disease. Despite, the effort deployed to control the disease, high resistance of vectors to insecticide make hypothetical the country ambition to pre-elimination. This study was conducted from 2013 to 2015 to estimate the burden of the insecticide resistance and associated mechanisms in *An. arabiensis* populations from the flooded areas of Dakar the capital city of Senegal. *Anopheles* larvae and pupae were collected by dipping method from natural breeding sites. Bioassays were carried out using WHO test kits and CDC bottle for unfed, 3-5 days old adults mosquitoes. Detection of metabolic resistance was realised using CDC bottle with synergists. Molecular identification of An. gambiae complex species and kdr molecular genotyping was perform by PCR using respectively methods described previously. All populations tested were resistant to all insecticides families except Organophosphates . The presence of metabolic resistance like glutathion-S-transferases (GST) and cytochrome P450 (CYP450) was found. Molecular identification revealed the presence of *An. arabiensis* only. Kdr genotyping showed the presence of L1014F mutation (Kdr-West) and L1014S (Kdr-East). This L1014S mutation was found at high frequencies in almost all districts surveyed, and in association with L1014F. In conclusion, results showed the contribution of both target-site and metabolic mechanisms in conferring pyrethroid resistance to *An. arabiensis* from the flooded areas of Dakar suburbs. This vector is main malaria vector in the Cap Vert Peninsula, due to the presence of permanent larval habitats so-called "Ceane" gardening pits, especially during the dry season, as reported previously. Our findings indicate the need for close monitoring of the urban *An. arabiensis* populations to implement a suitable insecticide resistance management system to preserve core insecticide-based vector control tools in the flooded area of Dakar.

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SUSCEPTIBILITY OF FIELD-COLLECTED *AEDES AEGYPTI* (L.) TO CONVENTIONAL INSECTICIDES IN COASTAL KENYA

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Aedes aegypti are the vectors for many important arboviruses in Kenya, and control options are limited. We evaluated the susceptibility of field populations of nonblood-fed *Aedes aegypti* mosquitoes to four conventional insecticides: permethrin, 0.75%; deltamethrin, 0.05%; bendiocarb, 1%; and fenitrothion, 1%; according to WHO standard procedures. We also determined the efficacy and residual activity of a water dispersible formulation of the biolarvicide *Bacillus thuringiensis var israelensis* (Bti) (VectoBac® WG) under semi-field conditions during the dry and wet seasons. Three concentrations of larvicide in 2-L plastic trays were used in this study: 1×, 10× and 20× the manufacturer's recommended dosage (8mg/L, 80mg/L and 160mg/L respectively). Bioassays of adults showed full susceptibility to permethrin and fenitrothion. A low level of resistance was observed for deltamethrin and bendiocarb with 86 and 84% mortality, respectively. Knockdown rate for the first 20 min was notably higher (>90%) for both deltamethrin and permethrin than for bendiocarb and fenitrothion (<10%). Bti was effective under semi-field conditions for an average of 16 days at 8 and 80mg/L and for 22 days at 160mg/L, with 100% mortality during the dry season. In the wet season Bti efficacy at 80 and 160 mg/L lasted for 22 days. The results of this study suggest a need to monitor for developing resistance along the Kenya coast in order to recommend suitable adulticides and dosages for *Ae. aegypti* control, especially given the recent outbreaks of dengue and chikungunya.

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THE RELATIONSHIP BETWEEN KNOCK-DOWN RESISTANCE (*KDR*) MUTATIONS METOFLUTHRIN INSECTICIDE TREATMENT IN MEXICO

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The application of any insecticidal vector control intervention requires an understanding of the susceptibility of the target population. Knock-down resistance (*kdr*) mutations in voltage-gated sodium channel (VGSC) genes protect insects against DDT and pyrethroids. In Mexico, pyrethroids are used extensively, in domestic aerosols and by public health authorities, particularly during arbovirus outbreaks. As a result of heavy selection pressure, V1023I and F1565C *kdr* mutations are common in the dengue, chikungunya and Zika vector, *Aedes aegypti*. Volatile pyrethroids like metofluthrin are currently being considered for deployment indoors to prevent mosquito biting. These compounds have lethal and behavioral effects on mosquitoes and appear to retain their efficacy against *Ae. aegypti* carrying *kdr* mechanisms. A prototype metofluthrin "emanator" is being tested as part of a USAID-funded trial in experimental houses and an urban field trial. A genomic analysis of local Mexican strains by double-digest Restriction-site Associated DNA sequencing (ddRAD-seq) revealed selective sweeps around the VGSC gene and P450 gene family, confirming that local strains harbour potentially protective mechanisms against pyrethroids. More specifically, we are monitoring 4 common *kdr* mutations using a cost effective High Resolution Melt (HRM) analysis. Of the mosquitoes characterized to date, there are no S996P mutations in the "super-kdr" region of domain II but an increased frequency of V1023I and F1565C *kdr* mutations in the S6 of domain II and III in mosquitoes surviving metofluthrin treatment. The impact of these mechanisms on *Ae. aegypti* survival in experimental houses will help us interpret the spatial and temporal impacts of metofluthrin in the context of the *kdr* patterns observed during the larger urban field trials. The extent to which these mechanisms differentially affect the lethal impacts and biting behaviors of metofluthrin will also be discussed. Results gained from free-flying mosquitoes with a subset of *kdr* mutations suggest that behavioral effects can be maintained even when mortality is reduced.

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PLANT-DERIVED EDIBLE OILS AS EMERGING LARVICIDES FOR YELLOW FEVER MOSQUITO, *AEDES AEGYPTI* (DIPTERA: CULICIDAE) CONTROL

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Aedes aegypti is the primary vector for dengue, yellow fever, chikungunya and Zika viruses. Plant-derived edible oils are important sources of insecticides that can provide an eco-friendly, efficient and affordable tool for controlling *Ae. aegypti* populations around homes in resource-limited settings. The objective of this study was to evaluate the larvicidal activity of plant edible oils against *Ae. aegypti* larvae. Plant edible oils were purchased from local food stores in Champaign County, Illinois,

USA and tested for their larvicidal activity against *Ae. aegypti* larvae in the laboratory. Bioassays were performed using late third instar larvae and replicated 4 times at concentrations ranging from 500 to 4,000 ppm, with 10 larvae per replicate. For each oil, the lethal concentration killing 50% of larvae (LC_{50}) was determined using probit analysis and compared with its linoleic acid concentration. In addition, stage-specific susceptibility of larvae and oviposition deterrence of selected edible oils were also tested. Among the 13 edible oils tested, hempseed oil was the most toxic, with an LC_{50} of 342.2 ppm, followed by sesame and pumpkin seed oils (661.4 ppm and 829.1ppm), respectively. Moreover, the efficacy of the oils is correlated with percentage content of linoleic acid (R^2= 0.52). The fatty acid profiles of 4 edible oils revealed that oils with high linoleic acid composition are more toxic to *Ae. aegypti* larvae. Larval exposure to 100% linoleic acid yielded a much lower LC_{50} value (94.23ppm) compared to the most toxic oil (hempseed, 342.2ppm), a strong indication that linoleic acid may be the active principle in the oils. Third and fourth instar larvae experienced higher % mortality compared to first and second instar larvae ($p < 0.05$), indicating that they are more susceptible to the edible oils tested. On oviposition deterrence, all the oils tested reported an effective repellency of ≥ 63%. These results demonstrate that some plant edible oils are toxic to *Ae. aegypti* larvae and are very promising in creating new, safe, effective and affordable larvicides for controlling *Ae. aegypti* populations around homes in resource-limited settings.

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NEAR-INFRARED SPECTROSCOPY TO DETECT MALARIA PARASITES IN *ANOPHELES GAMBIAE* S.S

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During the past decade there has been a substantial reduction in malaria incidence and related mortality. A few countries have announced plans for malaria elimination, setting ambitious goals for areas so far faced with perennial transmission. Elimination demands efficient programs together with real-time surveillance. Although vector control interventions, such as LLINs and IRS, have been the driving force behind the reduction in malaria cases, surveillance of vectors is restricted by shortage of entomologists and high costs of analyzing large number of mosquito samples. Near-infrared spectroscopy (NIRS) is used to measure the light in the near-infrared region that is absorbed by a mosquito; the spectra depend on its external and internal composition. It is non-destructive, high-throughput and requires no reagents. This study aimed at investigating if NIRS can be used to determine if a mosquito is infected with malaria sporozoites. For this purpose, *Anopheles gambiae* s.s (Keele strain) mosquitoes were infected in the laboratory using cultured *Plasmodium falciparum* parasites. These were kept in insectary conditions for 14 days along with mosquitoes that were given an uninfected blood meal on the same day. After 14 days mosquitoes were killed and scanned using NIRS. Parasite infection was quantified using qPCR. A calibration was developed using 69 uninfected mosquitoes and 69 sporozoite-infected mosquitoes with different infection loads. The calibration was then used to predict an independent set of samples composed of 22 uninfected and 68 sporozoite infected mosquitoes. Results showed the method predicted the independent samples with 100% sensitivity and 88% specificity (22/22 accurately predicted as uninfected; 66/68 accurately predicted as uninfected). The adoption of this new high throughput method to identify infected mosquitoes could deliver a cheap and efficient approach to malaria vector surveillance in endemic regions as well as in regions facing disease importation. Validation with wild mosquito infections is on-going and will verify the applicability of the method to the field.

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IMPORTANCE OF AN EFFICIENT LARVAL DIET FOR *ANOPHELES DARLINGI* TO *PLASMODIUM VIVAX* IN THE PERUVIAN AMAZON

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Anopheles darlingi, the main malaria vector in the Neotropics, was recently colonized under laboratory conditions. Hence it was necessary to optimize larval feeding because the environmental conditions in juvenile stages, such as food availability, impact the survival and reproduction and their susceptibility to *Plasmodium*. The high cost and lack of availability of commercial larval food in the Peruvian Amazon limits our ability to produce mosquitoes of high quality for assays such as transmission blocking or sporozoite production. In this study, we investigate the effectiveness of different larval diet mixtures with local accessible ingredients to promote more affordable and sustainable mosquito production. We evaluated five mixtures: Puripaiche (contains 45% crude protein), LabDiet (23%), Nutrafin (32%), Whiskas (31%), and Conejina (18%). Life tables and wing length (adult body size) were recorded to analyze the effect of foods on larval development and adult emergence. Three- and four-day-old adults were provided with *P. vivax*-infected blood, and on the 7th day post feeding, oocysts were counted in the midgut. All diets resulted in a larval survivorship >70%, with the exception of Conejina, for which the larval survival was 6%. Similarly, the mean time to adult emergence was 24 days (CI: 22-27) for all diets except for Conejina, for which the mean time to emergence was 30 days. Significant differences ($p<0.05$) were found in the wing length of adult mosquitoes: Puripaiche, Nutrafin and LabDiet produced mosquitoes with larger wing lengths (2.79mm, 2.73mm and 2.70mm respectively), whereas Whiskas (2.66mm) and Conejina (2.59mm) yielded smaller mosquitoes. There was no significant difference in the susceptibility to *P. vivax* between diets, with a mean of 12.85 oocysts/mosquito for Puripaiche, 10.75 for LabDiet, and 10.17 for Nutrafin. Overall, Puripaiche, Nutrafin and LabDiet are promising larval diets for the rearing and efficient production of adult mosquitoes.

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GENDER DIFFERENCES IN THE IMMUNE RESPONSES AGAINST *AEDES AEGYPTI* SALIVARY PROTEINS

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Aedes aegypti is one of the most well-characterized mosquito species due to being the primary vector of Dengue, Chikungunya, and Zika in tropical areas. During mosquito blood feeding, a salivary exudate is injected into the host skin to allow the mosquito to successfully uptake blood. Mosquito salivary secretions cause allergic reactions and induce significant antibody responses in humans. Specifically, levels of IgG and IgM antibodies increase in human populations with continued exposure to high numbers of mosquitoes. It has been proposed that antibody responses and vector-transmitted disease infections show some gender-dependent variation in humans due to sex-related physiology and differences in rates of exposure. However, there is little understanding about the influence of gender on antibody response in humans with continued exposure to *Ae. aegypti*. This study provides a secondary analysis of data measuring the levels of antibodies against *Ae. aegypti* saliva in human populations from dengue endemic areas of Colombia. We aim to establish whether there are gender-dependent and age-related antibody responses to mosquito exposure and associations with the presentation of dengue fever infections. Our results showed higher levels of IgG antibodies in males before traveling to dengue-endemic areas (p=0.0463) and higher

IgM antibody levels in females after mosquito exposure and throughout the follow-up period. We also observed a discrete decline in the antibody levels (IgG and IgM) with age, which suggests that age related responses are independent of gender. Moreover, we found a significant positive correlation between the IgG levels against dengue fever and 37/38kDa proteins (r=0.6272, p=0.0217) in females with active DENV. Our results suggest that sex and age should be taken into account when interpreting serum IgG and IgM levels.

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THE *ANOPHELES FARAUTI* HABITAT AND ITS ASSOCIATION WITH LARVAL DENSITY AND ADULT FITNESS

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The fitness of adult malaria mosquitoes, defined by size and survivorship, is influenced by density-dependent processes at the larval stage, and simultaneously by density-independent (environmental) processes. Understanding the roles of density-dependent and independent influences is essential for predicting the response of mosquito populations to larval control. Evidence for the existence of density dependent effects on *Anopheles farauti*, the primary vector in the Solomon Islands will be presented together with data on the physical, chemical and biological characteristics of larval habitats and their association with productivity. Methods: Human landing catches were carried out across the Solomon Islands with the wings of the collected mosquitoes measured to determine size variation within the adult population. As well as adult collections, the aquatic stage was also investigated looking at the chemical, physical, and biological factors associated with the presence or absence of the *An. farauti* larvae as well as the productivity of each potential habitat. Size variation was confirmed within the adult mosquito population, and significant factors found that act on the larvae with water temperature (P<0.0001*), pH (P=0.0002*), nitrate (P<0.0001*), ammonia (P=0.0190*), and phosphate (P<0.0001*) associated with increases in larval density. However, there does not appear to be any single dominant factor associated with habitat utilisation and productivity. In conclusion, a lot of information is unknown regarding the aquatic stages of anophelines in the Solomon Islands. This is especially evident for *An. farauti* which is concerning considering the impact it has on human health due to its association with malaria. Looking at the relationship between the larval habitat, larval density, and adult fitness could help influence future control methods to complement the use of insecticide treated nets for malaria control.

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PRIMATE MALARIA TRANSMISSION IN THE ABSENCE OF HUMAN HOSTS: INVESTIGATING MOSQUITO VECTOR ECOLOGY IN THE LOWER KINABATANGAN WILDLIFE SANCTUARY, SABAH, MALAYSIAN BORNEO

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A significant outbreak of the primate malaria *Plasmodium knowlesi* in humans has occurred in Sabah, Malaysian Borneo. The competent vector *Anpheles balabacensis* is responsible for human infections, arising as spill-over events from wild macaque reservoir hosts. Little is known about the ecology of *Plasmodium knowlesi* transmission within reservoir primate hosts in undisturbed forests. Elucidation of these transmission systems is crucial to assess whether human infections could be prevented by interrupting transmission in macaques. With the objective of characterising the macaque malaria transmission cycle, we aimed to determine the abundance and diversity of potential malaria vector species in the vicinity of macaque troops and to identify circulating *Plasmodium* infections of *P. knowlesi* and other primate malaria species. Here we trialled Mosquito Magnet Independence Traps (MMIT) to sample mosquitoes host seeking near macaque sleeping sites within the Lower Kinabatangan Wildlife Sanctuary, Sabah from August to November 2017. This reserve contains high numbers of macaques but very few humans, making it appropriate for studying the monkey-monkey malaria transmission cycle. For 30 nights, a thermal imaging camera was used to select trees for the positioning of one MMIT at a tree hosting sleeping macaques and one MMIT at an unoccupied (control) tree each night. Higher mosquito abundances were found at macaque roosts (n=5489) than at control trees (n=5065). *Anopheles* comprised 4% of collections, including three species: *An. balabacensis*, *An. barbirostris* and *An. donaldi*, previously implicated in malaria transmission. Their combined abundance was higher at control (n=233) than macaque trees (n=124). *Anopheles balabacensis* was more abundant at macaque trees (n=13) than at controls (n=2) and only one *An. balabacensis* was infected with the primate malaria *P. inui*. The low abundances of primate malaria vector species and low *Plasmodium* infection rates were surprising, and specifically that *P. knowlesi* was not detected. Ongoing work screening macaque stool samples will confirm the intensity of *P. knowlesi* in macaques in this area.

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AEDES AND *ANOPHELES* MOSQUITO CO-CONCURRENCE: IS THERE ANY IMPACT ON HUMAN IMMUNE RESPONSE AGAINST PATHOGENS?

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Dengue and malaria are among the principal mosquito transmitted diseases. Although, malaria incidence has significantly decreased in the last decade, there is an alarming increase of pathogens transmitted by *Aedes* mosquitoes including Dengue virus. Although, *Anopheles* and *Aedes* prefer very different breeding sites, adults of booth species are often captured in endemic areas, during surveillance efforts. Also, the concept of malaria - dengue co-infection is starting to get more attention in recent years due to an increase in cases in tropical regions. Our preliminary study using 32 samples collected in two different regions of Colombia revealed a significant positive correlation between antibodies against salivary proteins from *Ae. aegypti* and *An. albimanus* in people living in areas endemic for both malaria and dengue in the Caribbean. However, this correlation was not observed in the Pacific Coast, where *An. darlingi* and *An. nuneztovari* are the main vectors of malaria and the presence of *An. albimanus* is uncommon. Interestingly, antibody levels against the salivary proteins and the correlation between them was age dependent with lower antibody levels in younger populations. Correlation between antibodies against the specific pathogens and its vector was more complicated. We observed a negative but significant correlation between IgG against *An. albimanus* and the CSP in older populations (>26 years old) suggesting the potential impact of human-vector contact intensity in immunity against malaria. However, we did not observe a relationship between antibodies against the CSP and salivary proteins in younger populations. In the case of dengue, we found a significant correlation between antibodies (IgM or IgG) against all serotypes and IgG against *Ae. aegypti*, except for DENV4, which is the least transmitted serotype in Colombia. In conclusion, people in tropical areas are exposed to both *Anopheles* and *Aedes* mosquitoes in

any given time increasing the possibility of malaria-arboviral co-infections. More studies are needed to establish the relationship between vectors of different diseases and impact of immunity in disease transmission.

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DRIVING FORCE AND IMPACT OF URBANIZATION ON THE ECOLOGY OF *AEDES* MOSQUITOES IN YELLOW FEVER AND DENGUE CO-ENDEMIC AREAS IN COTE D'IVOIRE

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Aedes mosquito-borne arboviruses have increasingly occurred in rural and urban settings of Africa. We explored the effect of urbanization on *Aedes* ecology along a rural-to-urban gradient in yellow fever (YF) and dengue (DEN) co-endemic areas in Cote d'Ivoire. *Aedes* eggs, larvae and adults were sampled using ovitraps, larval surveys and human-baited double-net traps in rural, suburban and urban areas from January 2013 to December 2014. *Aedes* breeding sites were characterized, and species identified. A total of 51,439 specimens of *Aedes* mosquitoes belonging to 20 species (*Ae. aegypti*, *Ae. africanus*, *Ae. albopictus*, *Ae. angustus*, *Ae. apicoargenteus*, *Ae. argenteopunctatus*, *Ae. dendrophilus*, *Ae. fraseri*, *Ae. furcifer*, *Ae. haworthi*, *Ae. lilii*, *Ae. longipalpis*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. palpalis*, *Ae. stokesi*, *Ae. unilineatus*, *Ae. usambara* and *Ae. vittatus*) were sampled. The highest *Aedes* species richness was found in rural (18 species), followed by suburban (7 species) and urban (3 species) areas. Conversely, *Aedes* showed higher abundance in urban (n = 51,439; 50.7%) compared to suburban (32.6%) and rural (16.7%) areas. *Aedes*-positive breeding sites were more abundant in urban (2,136/3,374; 63.3%) than suburban (1,428/3,069; 46.5%) and rural (738/2,423; 30.5%) areas. Breeding sites were mainly industrial (i.e., tires, cans and water receptacles), traditional (i.e., clay-pots) and natural (i.e., tree holes and fruit husks) containers in urban, suburban and rural areas, respectively. *Ae. aegypti*, *Ae. dendrophilus*, and *Ae. vittatus* bit humans in rural (4.48 bites/person/day), while *Ae. aegypti* inflicted 99.7% of bites in urban (15.73 bites/person/day) areas. *Ae. aegypti* was the dominant species and displayed bimodal daily feeding cycles in all areas, with stronger magnitude in urban areas. In Cote d'Ivoire, urbanization shifts *Aedes* ecology by restricting of wild *Aedes* species in rural and favoring *Ae. aegypti* in urban areas. Data suggested that while *Aedes* wild species act as bridge vectors of YF and DEN viruses in rural, *Ae. aegypti* raises the risk of inter-human transmission of arboviral diseases in urban areas.

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SPATIAL RISK OF URBAN EXPOSURE TO *ANOPHELES* AND *AEDES* MOSQUITO BITES IN AFRICA USING SALIVARY ANTIBODY-BASED BIOMARKERS

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Urban settings often present low densities of mosquito vectors which do not allow to accurately assess the risk of arthropod-borne diseases based on entomological parameters. This study aims to evaluate the spatial risk of both malaria and arbovirus transmission in a northern urban area of Senegal, West-Africa, using antibody-based biomarkers of human exposure to Anopheles and Aedes mosquito bites. A cross-sectional study was undertaken between August and September 2014 (rainy season) in four urban districts (UDs) of the city of Saint-Louis, Senegal: Leona (LEO), Ndioloffène (NDI), Guet Ndar (GND) and Pikine Sor Diagne (PSD). In each UD, dry blood spots were performed in 809 children aged 6-59 months and ELISA method was used to evaluate IgG antibody (Ab) responses to both gSG6-P1 (Anopheles) and Nterm-34kDa (Aedes) peptides of respective mosquito saliva. The median of IgG response levels to both gSG6-P1 and Nterm-34kDa salivary peptide varied significantly according to UDs and were lower in LEO compared to PSD, GND and NDI ($p<0.0001$). Heat maps of IgG responses to both salivary peptides indicated variations in the spatial distribution of the intensity of Ab responses inside UDs. There were no hot spots of malaria transmission risk (areas with children presenting a high IgG intensity) in LEO. Hot spots of malaria were mainly located in the northern part of NDI and GND, and in the southern part of PSD. As for the risk of arbovirus transmission, there were no hot spots in LEO and PSD. Hot spots of arbovirus transmission risk were located in some patch in the north of NDI and were dispersed throughout the UD of GND. Our results demonstrate that hot spots of both malaria and arbovirus transmission risk actually exist in northern parts of NDI and GND. This highlights that a targeted fight against mosquitoes in these hot spots could be effective against all mosquito-borne diseases. Antibody-based biomarkers could then help national control programs to target and prioritize vector control strategies in areas with common risk of malaria and arbovirus transmission.

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LARVAL ENVIRONMENT INFLUENCES MICROBIOTA OF CONTAINER DWELLING MOSQUITOES

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Mosquitoes host a community of microbiota that influence their growth, survival and susceptibility to pathogens. These microbiota are known to vary markedly between individuals but our understanding of the factors that influence this variation is still limited. The aim of this study was to determine how the larval environment influences the microbiome of two container-dwelling mosquito species, *Aedes triseriatus* and *Ae. japonicus*. Larvae of the two mosquito species were sampled from tire and tree-hole habitats at South Farms and Trelease Woods study sites in Champaign, Illinois, and their microbiome characterized through MiSeq sequencing of the 16S rRNA gene. Approximately 66% of bacterial operational taxonomic units (OTUs) were shared between mosquito larvae and water samples from the larval environment. *Dysgonomomanas* and an unclassified genus from family *Comamonadaceae* were the dominant bacterial taxa in *Ae. triseriatus* larvae and water samples respectively, but no clear dominance of any bacterial taxa was observed in *Ae. japonicus* larvae. Overall, there was significantly higher number of OTUs observed and predicted in mosquito larval samples compared to water samples. Bacterial OTU richness was significantly higher in *Ae. japonicus* tire samples from both study sites compared to *Ae. triseriatus* tire and tree-hole samples from South Farms or water samples from South Farms. NMDs plots based on Bray-Curtis distances revealed a clear separation of *Ae. japonicus, Ae. triseriatus* and water samples indicating that the bacterial communities differed by sample type. The findings of this study reveal that *Ae. japonicus* and *Ae. triseriatus* harbor distinct bacterial communities some of which are likely acquired from the larval environment.

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HOST PREFERENCES AND ACTIVITY RHYTHMS OF *ANOPHELES* MOSQUITOES IN CAMBODIA

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The main vector control tools against malaria mosquitoes have been the scaling up of indoor-oriented vector control tools, despite the fact that Southeast Asian mosquitoes populations mainly feed early and outdoors. In Cambodia, the main persistent reservoirs of transmission appear to be located outside of villages, in forests. This study aimed at better characterizing *Anopheles* vector bionomics to inform novel vector control tools development. Mosquitoes were sampled hourly over a 24h period during the rainy and the dry season in three types of sites: villages, forest habitats surrounding the villages, and deep forest. Inherent mosquito host preference was determined using odour-baited traps set side by side in a choice arrangement, releasing either human or cow odors. This allows calculation of the anthropophily index (AI): number of *Anopheles* mosquitoes caught in the human-baited trap over the total number of mosquitoes caught in both traps. A total of 3803 *Anopheles* mosquitoes were caught, with most of them at night, although 25.9% were caught during daytime. Overall *Anopheles* mosquitoes caught in the deep forest were significantly more anthropophilic (AI rainy season=26.5%; AI dry season=32.9±3.1%) than mosquitoes caught in the villages (AI rainy season=4.3±1%, P<0.0001; AI dry season=6.3±4.9%, P<0.0001) or in the forests surrounding the villages (AI rainy season=5.3±1%, P<0.0001; AI dry season=16.5±5.5%, P<0.0001). When considering females collected in the human-baited traps only, similar numbers were collected during the two rainy seasons, with 79.5% collected in the deep forest sites. These results strengthen the hypothesis that forests are the main risk areas for human malaria transmission, and highlight the importance of daytime biting behavior as a potential source for transmission. Molecular biology analyses are ongoing to determine malaria prevalence and transmission risks. In the current context of malaria elimination in Greater Mekong Subregion, there is an urgent need to develop vector control tools adapted to forest transmission settings in order to effectively break malaria transmission.

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SPATIAL DISTRIBUTION OF PCR -IDENTIFIED MALARIA VECTORS IN CROSS RIVER STATE, NIGERIA

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Anopheles gambiae sensu lato complex describes a group of seven morphologically indistinguishable members, comprising the main species responsible for malaria transmission in tropical and subtropical Africa. The diverse biological characters of these sibling species as well as their behavioral variations are responsible for the difference in their ability to transmit malaria and susceptibility to pyrethroids. This highlights the need for precise mapping of their spatial distribution to monitor and evaluate malaria threat levels, and to enhance effective implementation of integrated vector control strategies. The present study aimed at developing a GIS- based overlay on the spatial patterns of PCR-identified *A. gambiae* complex species collected from four sites in Cross River State; Calabar and Akpabuyo in southern, Yakurr in central and Ogoja in Northern region representing three different ecological zones. Trapping was conducted every other month from October 2015 until June 2016, covering both dry and rainy seasons, using CDC-UV traps and pyrethrum spray catch. *Anopheles* complex species were identified using molecular techniques based on differences in the rDNA region between species and the molecular forms of *A. gambiae s.s* specimens. A total of 1,386 female *A. gambiae* mosquitoes were collected and identified. DNA was extracted from legs of each specimen and species identification determined by multiplex PCR using specific primers. The molecular forms of *A. gambiae s.s* were determined by RFLP. Results indicated dominant occurrence of *A. gambiae s.s.* (99.2%) across the four sites in comparison to *A. arabiensis* which was detected in small number (0.8%). Out of 1,375 *A. gambiae s.s* specimens, 78.2%, 20%, and 1.8% were *A. coulzzii, A. gambiae* and hybrid forms respectively, a finding that contradicts other studies. *A. coulzzii* is predominant in guinea-savannah and rain forest ecological zones mainly during rainy seasons, while *A. gambiae* is prevalent in mangrove rain forest during dry season. These results provide important insights for strategic planning of malaria control programs in Nigeria.

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MOON PHASE EFFECT ON MOSQUITO VECTORS OF WEST NILE VIRUS IN MADAGASCAR: BIODIVERSITY, ABUNDANCE, HOST ATTRACTIVENESS AND FEEDING RATES

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West Nile Virus (WNV) infection occurs throughout Madagascar. Its epidemiological cycle involves horses, human, birds and mosquitoes. Our entomological data shows unexpected information on mosquitoes vectors diversity and biology that relates to the collection methods. This study highlights the effect of lunar cycle that has not been previously considered in previous studies in Madagascar. During 2017, the influence of the two lunar phases (full *versus* new moon) on mosquito populations was analyzed in a farm located in the surroundings of Antananarivo city, Madagascar. Each month, mosquito collections were performed twice: one night during the full moon and one during the new moon. Six light traps were used: three indoors (in horse's box stall, in a house, in a cowshed), while three outdoors (near a pigsty, near a chicken coop, near a water point). During 24 night catches, 36,448 specimens belonging to 23 species were collected with *Culex antennatus* (64%) and *Cx. quinquefasciatus* (30%) the most abundant species. *Cx. antennatus* was mostly collected in traps associated with domestic animals while *Cx. quinquefasciatus* in trap placed in house. Each month, the total number of females caught during new moon was 1 to 3,5 times higher than those caught during full moon (ANOVA; F=34.4, DF=3, P<0,05). Larger numbers of mosquitoes, driven mainly by *Cx. antennatus*, were collected during the new moon in the three outdoor traps; and inversely during the full moon in the cowshed. This new moon effect was observed in the house but driven mainly by *Cx. quinquefasciatus*. Lunar phase did not influence the abundance of mosquitoes in horse's box stall and the variation of mosquitos' diversity. The total number of fed and unfed females followed (F=0.709, DF=39, P>0,05) the same pattern than the abundance of mosquitoes collected in the farm. The lunar cycle has an effect on mosquito abundance and host attractiveness and might vary according to the mosquito species. This lunar effect and the location of traps should be taken into consideration for one target species during entomological investigations aiming at unraveling West-Nile virus transmission when using light traps.

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RELATIONSHIP BETWEEN MICROCLIMATE AND ENVIRONMENTAL VARIABLES AND MOSQUITO ABUNDANCE IN RURAL ECUADOR

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Mosquito abundance is an important predictor of mosquito-borne illnesses, such as dengue fever. *Aedes aegypti* mosquitos, which spread the dengue virus, have a close relationship with humans, often living only within urban environments. Fine-scale relationships between climate, environment, and mosquito populations in this setting are poorly understood. Using mosquito traps fitted with microclimate sensors, we

determined the relationship of these microclimate and environmental factors with host-seeking mosquito abundance, within a small town (approximately 5500 people) in rural Ecuador over a twelve-month period. Data were analyzed with a negative binomial (all-species mosquitos) or binomial logit (proportion of female *Aedes aegypti*) model with generalized estimating equations. Temperature and relative humidity were variable even at small spatial scales; temperature maximum and minimum relative humidity exhibited the widest range of values across the sampling timeframe. For all-species mosquito abundance, median temperature (effect=-0.23, p=0.0071), minimum relative humidity (effect=0.31, p=0.0017) and median relative humidity (effect=-0.70, p=0.0095) had the strongest associations, but temperature variance and maximum temperature also had statistically significant effects. Seasonality and urbanicity levels also influenced all-species mosquito abundance. Among the sites with any mosquitos, the odds of a captured mosquito being a female *Ae. aegypti* were increased by 45% for each degree increase in median temperature (p=0.006), with seasonality also strongly affecting these odds (p=0.0001). This is one of few studies of microclimate, environment, and mosquito abundance, and may provide important insights into global prediction modeling, as well as local prevention and control efforts.

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ENVIRONMENTAL AND ENTOMOLOGICAL SURVEYS TO DETECT BREEDING SITES OF *AEDES* SPP. IN SALVADOR, BRAZIL

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Larvae and pupae of *Aedes aegypti* and *Ae. albopictus,* the vectors of dengue, chikungunya and Zika viruses in Brazil, are found in natural and artificial water-storage containers, but also in other breeding sites outside human dwellings. During October 2016 we conducted a cross-sectional entomological and environmental study in a residential medium-high socioeconomic level area (0.08 km^2), located in Pituba neighborhood, Salvador, Brazil to describe and estimate the relative contribution of different breeding sites inside and outside human dwellings. We detected 448 breeding sites within private areas, of which 238 (53.0%) held water and 7 (1.6%) contained immatures [7 (1.6%) with *Ae. aegypti*, 1 (0.2%) with *Ae. albopictus*, and 2 (0.4%) with *Culex* spp.]. Adult mosquitoes were captured in 27 (23.3%) of the 116 surveyed properties: *Ae. aegypti* in 20 (17.2%) and *Culex* sp. in 11 (9.5%); no adult *Ae. albopictus* were captured inside the properties. In public areas, we surveyed 108 catch basins, of which 43 (40.0%) contained water, 10 (9.3%) contained immatures [2 (1.9%) with *Ae. aegypti*, 2 (1.9%) with *Ae. albopictus*, and 8 (7.4%) with *Culex* spp.], and 11 (10.1%) contained adult mosquitoes [*Ae. aegypti* in 3 (2.8%), *Ae. albopictus* in 3 (2.8%), and *Culex* spp. in 4 (3.7%)]. We also installed 34 ovitraps in a third of the properties, and found immatures of *Ae. aegypti* in 10 (29.4%) and of *Ae.albopictus* in 3 (8.8%) traps. *Aedes* spp. eggs were detected in 22 (64.7%) of the traps. Our findings demonstrate that catch basins in Salvador accumulate water and serve as larval development sites and as adult resting areas for *Ae. aegypti* and *Ae. albopictus*. Furthermore, breeding sites outside the houses serve as an important source for domestic *Ae. aegypti* infestations. Consequently, determining the rate of house level infestations based only on the presence of larvae in the properties (as is done in Brazil), underestimates the true level of infestation. We suggest that additional surveillance measures targeting capture of adult mosquitoes inside houses are needed. Even more importantly, we emphasize the need to include proximate public areas in *Aedes* surveillance programs.

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MODELING THE POTENTIAL DISTRIBUTION OF *AEDES AEGYPTI* IN THE CITY OF CORDOBA, ARGENTINA

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Aedes aegypti, an urban mosquito, is involved in the transmission of numerous viruses, including dengue, chikungunya and Zika. In Argentina *Ae. aegypti* is the main vector for dengue virus, involved in several outbreaks expanding from northern to central areas since 2009. In order to evaluate potential vector-borne diseases transmission in Córdoba city, Argentina, we aim to identify the environmental, socio-economic and demographic factors driving the distribution of *Ae. aegypti* larval presence through spatial analysis in the form of species distribution models (SDMs). A maximum entropy species distribution model was developed to identify the relationship between known occurrences of *Ae. aegypti* and 11 factors. The model identified suitable breeding areas for *Ae. aegypti*, as location with high Human population density, in close proximity to vegetation, and near water channels. The model goodness of fit was good, with an area under the receiver operating characteristic curve (AUC) greater than 0.8 and presented a fair extrapolation capacity (average test AUC>0.75). Results from this work will allow for target surveillance and prevention measures, as well as for mosquito management within areas of high habitat suitability for the species.

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ZIKA AND DENGUE VIRUS INFECTIONS IN PREGNANT MOTHERS IN GRENADA

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While Zika virus (ZIKV) poses a significant threat to pregnant women and their infants, 80% of infections are asymptomatic. The objective of this study was to document the impact of maternal symptomatic and asymptomatic ZIKV infections on infants in Grenada. During the ZIKV outbreak in 2016, a cohort of pregnant women in antenatal clinics were enrolled, surveyed and blood samples were collected to test for ZIKV and dengue virus (DENV) infection by PCR and pGOLD immunoassay (IgG and avidity). From April to November 2017, 140 offspring of the women were followed and examined. 268 mothers were newly enrolled whom were pregnant or gave birth during the outbreak. Infant assessments consisted of physical examinations, anthropometrics, and serum collection for ZIKV and DENV pGOLD IgG testing. In total, 383 mothers and 388 infants were assessed postpartum. Thirty-five (9%) mothers reported ZIKV symptoms during pregnancy; 16%, 47% and 28% in their 1st, 2nd and 3rd trimesters, respectively. Of the 388 infants, 8% tested positive for DENV IgG, 1% for ZIKV IgG, and 0% had dual exposure. Twenty-three (5.9%) severely and 35 (9.0%) mildly microcephalic cases were discovered. Of the original antenatal cohort, 7 mothers were ZIKV PCR confirmed (5%) at

the prenatal visit: 3 were symptomatic and 4 were asymptomatic. Seven other mothers were viremic for DENV and all were asymptomatic. IgG pGOLD testing revealed that 99% (80/81) had previous DENV exposure, 84% (68/81) had ZIKV exposure, and 83% (67/81) had dual exposure. Most viremic mothers had positive IgG results: 86% (6/7) for ZIKV IgG and 100% for DENV IgG. Avidity testing revealed that 97% of mothers had ZIKV exposure 6 months prior, whereas only 29% had recent DENV exposure. Of the 68 ZIKV-exposed mothers, only 2 of their offspring (3%) were positive for ZIKV IgG. Of the 80 mothers with previous DENV exposure, all of their offspring were DENV IgG positive. DENV and ZIKV transmission are co-occurring and prevalent in Grenadian mothers and are causing asymptomatic infections that may put infants at risk unknowingly. Additional testing will corroborate microcephaly and its association with timing of ZIKV exposure.

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JUVENILE HORMONE REGULATED MICRORNA-276 IN *AEDES AEGPYTI* FATBODY

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MicroRNAs are a class of small non-coding RNAs (20-23 nucleotides in length) that play a critical role in gene silencing in eukaryotic organism. Our previous studies characterized the role of miRNAs in blood digestion and lipid secretion in *Aedes* mosquitoes. Inhibition of both of these microRNAs results in biological defects that prevent egg production and reproduction. In adult female mosquitoes, Juvenile Hormone (JH) regulates the cascade responsible for tissue development that is required for the reproductive cycle following acquisition of a blood meal. Currently, the involvement of microRNAs during the post-eclosion (PE) developmental stage are unknown. MicroRNA-276 is an insect specific microRNA and has been shown to be abundant across multiple tissues. A recent study in D. melanogaster characterized the role of mir-276 in circadian rhythms through its regulation of timeless. In *Aedes aegpyti*, JH III activation of kh-r1 and hairy occurs in a light-dependent circadian rhythm during PE development. Here we explore the regulatory role of mir-276 in the fatbody during tissue maturation with the goal of disrupting proper circadian rhythms as a means to inhibit JH III activation to prevent mosquito reproduction. We demonstrate that mir-276 is a JH responsive microRNA through both an *in vivo* JH III topically assay to artificially induce the JH mediated response and through dsRNAi knockdown of the key receptor, methoprene-tolerant, to artificially prevent the JH response. Next, we used a combination of in silico and *in vivo* predictions from our previously published Ago1 CLIP-Seq dataset to create a high-confidence list of mRNA targets for mir-276. We then validated if mir-276 was capable of binding to these targets *in vitro* using a dual luciferase assay in S2 cell line. Finally, we performed antagomir injections to systemically reduce mir-276 levels during the PE developmental stage and measured gene expression of key targets in the JH III pathway. While our results indicate mir-276 is regulated by JH III, the miRNA does not inhibit key components of the JH pathway. It is likely that mir-276 plays another role in regulating gene expression in *A. aegypti*.

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ENTOMOLOGICAL PATTERN OF MALARIA TRANSMISSION IN THE HIGHLAND FRINGES OF MADAGASCAR:RESULTS FROM A LONGITUDINAL ENTOMOLOGICAL SURVEY

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Knowledge on malaria transmission characteristics in a given area is critical for efficient malaria control guidance. Data on malaria transmission in the Malagasy Highland Fringes (MHF), where mosquito bed-nets are deployed as the primary vector control strategy, is scarce. Entomological data were collected in three sites, three times a year from 2014 to 2016. Field investigations were fitted to the period before, during and after the usual malaria transmission peak. For each survey, mosquitoes were collected hourly by human landing catch from dusk to dawn. During three consecutive nights, five houses per site were sampled indoors and outdoors. Human Biting Rates (HBR) and sporozoite index (SI) were calculated. A total of 2,585, 1,503 and 4,528 *Anopheles* were collected in Ihosy, Maevatanana and Tsiroanomandidy, respectively. In Ihosy, *An. arabiensis* was the most abundant vector (70.6%) while in Maevatanana it was *An. coustani* (17.2%) and *An. gambiae* (15.6%). In Tsiroanomandidy, *An. coustani* (36.3%) and *An. arabiensis* (30.4%) were the most abundant vectors. Nightly 12h collections showed that in Ihosy, *An. arabiensis* was actively biting outdoors (75.0%), with a peak of activity around midnight. In Maevatanana and Tsiroanomandidy, malaria vectors were actively biting outdoors too (>60.0%) but did not show a clear biting peak. In Ihosy, the highest HBR for *An. arabiensis* was observed in February, before the transmission peak. In Maevatanana and Tsiroanomandidy, the highest HBR for *An. coustani* was observed in April during the transmission peak. In Ihosy, the SI value of *An. arabiensis* was 0.38% while in Maevatanana and Tsiroanomandidy, the SI value of *An. coustani* were 0.39% and 1.70%, respectively. These results on malaria vector abundance and behavior could explain the persistence of malaria transmission in the MHF areas. Due to the observed exophagic behavior of these vectors, additional control measures should be explored to reduce the malaria burden. In light of its abundance, the possible role of *An. coustani* as a primary malaria vector in the MHF should be further investigated by integrating studies on its vector competence.

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THE FEEDING ECOLOGY OF *AEDES ALBOPICTUS* ON LONG ISLAND, NEW YORK

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The Asian tiger mosquito, *Aedes albopictus*, is one of the most invasive species in the world and is an important vector of more than 20 viral pathogens, including dengue and Zika viruses. In the Northeastern USA, *Ae. albopictus* is expanding its range and is now detected in New York through the Hudson River Valley. Unfortunately, there is limited knowledge of the feeding ecology of the species in this new context, which is critical to understanding local disease transmission and optimizing control strategies. This study investigates both the blood feeding and sugar feeding tendencies of *Ae. albopictus* in rural and urban settings in Long Island. Mosquitoes were collected using a combination of vegetation aspiration, resting boxes and BG-sentinel traps. Blood meals were identified to species and host populations were enumerated to calculate forage ratios and infer host preference and feeding plasticity, the extent of which is highly variable in the published *Ae. albopictus* literature. Sugar feeding of non-engorged female and male mosquitoes was tested using three different methods: 1) cold anthrone to detect all sugar feeding 2) thin layer chromatography to detect secondary metabolites unique to honeydew feeding and 3) cellulose staining of gut to detect plant tissue feeding. Structural equation modeling was conducted to determine the correlation between the proportion of sugar positive mosquitoes collected with plant species in bloom, vegetation density, temperature, humidity, and precipitation. This provides valuable information because little is known about the sugar feeding habits of *Ae. albopictus,* despite on-going drives to develop attractive toxic sugar baits to control this species. Together, the two main components of this study will give a comprehensive picture of the adult feeding ecology of *Ae. albopictus* on Long Island and will inform local mosquito control departments about disease transmission risk and avenues for control.

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IDENTIFICATION OF MOSQUITO BLOOD SOURCE USING MID-INFRARED SPECTROSCOPY

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Identification of mosquito blood meals is essential for monitoring vectorial capacities and overall importance of these mosquito species in transmission of human pathogens. Currently, for the best approaches for mosquito blood meal identification involve either ELISAs or PCR, both of which are time consuming, expensive, laborious and require many reagents. However, non-molecular techniques such as infrared technology are promising alternatives. Near-infrared spectroscopy has already been proven to detect mosquito age and parity status, but the mid-infrared spectra offers greater resolution for such analyses. We used mid-infrared spectroscopy combined with statistical regressions to identify blood meal sources of laboratory-reared mosquitoes *Anopheles gambiae s.s.* mosquitoes, which had fed directly on either humans, bovine, chicken or goat blood. Blood fed mosquitoes were anesthetized using chloroform and preserved dry in silica gel. A partial least square regression (PLS) method was used to analyze the spectra and predict the unknown samples. The data set was divided into two; a training set with 120 samples, and a test set with 30 samples for each PLS model. Four PLS models, one for each blood meal source, were developed, where human blood meals were compared against: bovine, chicken, goat or all the three hosts together. From the initial results, the human *vs.* bovine model had overall prediction accuracy of 88.3%, human *vs.* chicken gave 93.3%, human *vs.* goat gave 88.3%, and human *vs.* other hosts gave 83.3 accuracy. Our preliminary conclusion is that approach has greater potential for high-throughput determination of mosquito bloodmeals in field surveys. Once fully calibrated, it could vastly improve studies on human-vector interactions and malaria epidemiological surveys, as it is easy to use, cost and time efficient.

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PUPAL PRODUCTIVITY OF LARVAL HABITATS OF *AEDES AEGYPTI* IN MSAMBWENI, KWALE COUNTY KENYA

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Dengue is endemic in Kenya with recent studies suggesting continuous low transmission year round. Frequent outbreaks occur, especially on the coast. *Aedes aegypti*, the primary dengue vector in Kenya, breeds predominantly in water storage containers and discarded tires. We estimated habitat-specific pupal productivity of *Ae. aegypti* in a south coastal Kenyan village. A total of 78 different water storage containers and discarded tires were inspected daily for *Ae. aegypti* larvae and pupae for 30 days. Containers were categorized as drums, tires, pots, small domestic containers (SDC) or 'others'. Small domestic containers (SDC) included small plastic food containers, tins, bottles, plates, cans, cooking pots (sufuria) and jars. 'Others' included; buckets, jerrycans, polythene bags, fallen leaves, coconut shells, hoof prints, drains, gutters, septic tanks, shoes, cisterns, sinks and animal feeding containers (AFC). Water management behavior including container covering, uses, filling and emptying, and habitat characteristics including size and movability were recorded. Overall, drums and tires were the most productive habitats accounting for 83% of all pupae collected. A poisson regression analysis showed that compared to SDC, the risk of finding pupae was 18% higher in drums, but comparable for the other habitats types. Pupae were more abundant in containers that were larger (15%), were stable (17%), and those that contained water with no particular purpose (60%). In the absence of an effective antiviral and vaccine, control of the immature mosquitoes remains the most viable prevention method. Vector control interventions targeting primarily drums and tires in the study region could likely produce a positive impact on local dengue transmission.

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EARLY AND LATE NIGHT HUMAN LANDING PERIODICITY OF *AEDES AEGYPTI* FEMALES IN URBAN AND RURAL SITES IN KENYA

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Aedes aegypti, the main vector for yellow fever, dengue, chikungunya and Zika viruses, is generally known as a daytime biting mosquito. Hence, there is scarcity of information about the extension of its biting activity into the night. This study aimed to determine the extension of *Ae. aegypti* biting activity into the early and late night hours in Kenya. Hourly human landing catches were conducted quarterly for three consecutive days from 4:30 am to 9:30 pm, indoors and outdoors and rural and urban areas in Kenya from June 2017 to March 2018. A total of 440 *Ae aegypti* mosquitoes were collected: 134 (30.5%) in the morning hours whereas 306 (69.5%) in the afternoon hours. Bi-modal biting activity was observed: the morning peak started from 4:30 am (with the peak between 6:30 am to 7:30 am) to 12:30 pm whereas the afternoon peak started from 12:30 pm (with the peak stretching from 2:30 pm to 7:30 pm) to 9:30 pm. A total of 91 (20.7%) *Ae. aegypti* mosquitoes were collected at night (including dawn and dusk): 24 (5.5%) from 4:30 am to 6:30 am and 67 (15.2%) from 6:30 pm to 9:30 pm. Out of these, more mosquitoes were collected outdoors (76, 83.5%) than indoors (15, 16.5%) and more mosquitoes were collected in the urban site (77, 84.6%) than the rural site (14, 15.4%). The *Ae. aegypti* night blood seeking behaviour observed in this study has epidemiological implications as it extends the period for the risk of arboviruses like dengue and chikungunya transmission in Kenya. Furthermore, appropriate vector control measures can be designed to coincide with this extended *Ae. aegypti* night biting behaviour.

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ABUNDANCE AND BEHAVIOR OF *AEDES AEGYPTI* IN TWO CITIES WITH HIGH TRANSMISSION OF ZIKA AND DENGUE IN ECUADOR

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Aedes aegypti is the main mosquito vector of the three viral diseases of high burden in the Americas: Zika, Dengue and Chikungunya. Vector control remains the primary control strategy for these diseases, but the deployment of such strategies is hampered by the lack of robust surveillance data on mosquito vector abundance, behavior and infection rates within major *Foci* of transmission. The recent outbreak of Zika throughout South America highlighted the urgent need for such data, particularly in outbreak settings outside of Brazil where knowledge of local vector ecology is limited. This study aimed to tackle this knowledge gap by investigating the ecology and virus infection rates within 2 cities that were

substantial *Foci* of Zika transmission in Ecuador: Quinindé and Portoviejo. Starting in November 2016, an 8-month longitudinal study was conducted to characterize the abundance, habitat preferences and viral infection rates in *Ae. aegypti* populations within urban and suburban areas. Mosquito samplings were carried out at the intra and peri-domestic areas with BG sentinel traps and Prokopack aspirators. *Ae. aegypti* was significantly more abundant in Portoviejo than in Quinindé. Mosquito preference for resting sites greatly differed between the two cities: in Portoviejo, mosquitoes tended to be six times more abundant inside than outside houses; while in Quinindé, indoor and outdoor abundance of resting mosquitoes was very similar. There were no significant differences in mosquito abundance between urban and suburban areas from both cities, which denotes the importance to carry out surveillance control in suburban areas as much as it is currently being done in urban areas. Preliminary results from molecular analysis confirms the presence of Zika and Dengue virus in *Ae. aegypti* in both urban and suburban areas. These results provide a clear picture of how mosquito ecology is different in two cities with high arbovirus transmission rates within the same country. Therefore, we highlight the importance that arbovirus prevention and vector control programmes should be adjusted to the local conditions.

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ACCEPTANCE AND WILLINGNESS-TO-PAY FOR ARBOVIRUS VACCINES IN GUATEMALA: A CROSS SECTIONAL SURVEY IN RURAL GUATEMALA

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Arboviruses including dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) cause significant morbidity in Latin America. With multiple arbovirus vaccines in development, better understanding of community attitudes and acceptability for these vaccines is needed. In September 2016, a cross-sectional survey assessed arbovirus knowledge, attitudes, vaccine demand and willingness to pay (WTP) at the conclusion of a DENV/norovirus surveillance study in a rural Guatemalan community with high arbovirus endemicity. Factors associated with vaccine demand and WTP were assessed with regression analysis. Among 564 surveyed households, DENV knowledge was high. There was high concern for all three arboviruses, particularly CHIKV. Overall vaccine attitudes were positive with <5% identifying significant barriers to, doubting or refusing previous vaccination. At 50% and 75% efficacy, 75% and 88% of respondents wanted arbovirus vaccines, respectively. DENV vaccine demand at 50% efficacy was associated with increased housing density, non-health-post vaccination location, older children and medical source for information. For each vaccine, 52-55% of respondents were WTP $0-$3.40, while 16-17% were WTP ≥$6.81. WTP at $3.40 and $6.81 levels for all vaccines was associated positively with parental education but negatively with good dengue knowledge. History of purchasing and identifying barriers to vaccines were associated with WTP ≥ $6.81. In conclusion, demand for potential DENV, CHIKV, ZIKV vaccines is high at 50% and 75% efficacy in this Guatemalan community. Associated factors could be leveraged to optimize arbovirus vaccine implementation. Overall low WTP suggests government subsidization may be necessary in resource-poor regions, though a small private market may be supported.

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PHOSPHORYLATION OF VEEV CAPSID IS REGULATED BY PP1 AND PKC

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Venezuelan equine encephalitis virus (VEEV) is an arthropod borne New World alphavirus. It can cause disease in humans and is characterized by a febrile illness that may progress into encephalitis. The capsid protein of VEEV is a structural protein that binds to the vRNA and interacts with the membrane bound glycoproteins. Capsid has auto-proteolytic activity during translation as well as several non-structural roles during the course of infection. Here we demonstrate that capsid phosphorylation is mediated by the host phosphatase Protein Phosphatase 1α (PP1α) and the host kinase Protein Kinase C delta type (PKCδ). Inhibition of PP1α causes decreased viral titers starting at 8 hours post infection. Confocal microscopy showed that PP1α appears to colocalize with capsid and this relationship is confirmed through co-immunoprecipitation. Mass spectrometry showed that capsid protein is phosphorylated on T93, T108, S124, and T127. Co-immunoprecipitation confirmed the phosphorylation status of capsid and that dephosphorylation by PP1α is necessary for capsid:vRNA binding. In an effort to identify the kinase responsible for this phosphorylation, phosphorylation site prediction software suggests that a member of the PKC family of kinases is responsible for the phosphorylation event. Furthermore, PKCδ, but not other PKC family members, co-immunoprecipitates with VEEV capsid whilst chemical inhibition or siRNA knockdown of PKCδ causes a decrease in viral titer. Finally, in cells transfected with the VEEV structural polyprotein and treated with the PKCδ inhibitor, Rottlerin, there is less phosphorylation of VEEV capsid compared to the solvent control. These data suggest that PKCδ is potentially the kinase that is responsible for the phosphorylation of capsid. Further studies are being performed to confirm this hypothesis as well as to determine the importance of phosphorylation during VEEV infection. These data indicate that the phosphorylation status of VEEV capsid is important during infection and that inhibition of capsid phosphorylation could be a potential target for therapeutics and vaccine development.

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PROBABLE INTRADOMICILIARY TRANSMISSION OF CHIKUNGUNYA VIRUS DURING THE 2015 OUTBREAK IN YUCATAN, MEXICO

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In the second half of 2015, a chikungunya outbreak took place in Yucatan, Mexico with over 1600 laboratory-confirmed cases. Chikungunya is primarily a mosquito-borne disease but can also be transmitted mother-to-child in pregnant women. Our aim in this study was to determine the probable intradomiciliary transmission of chikungunya and which age group and gender was most affected. We conducted a verbal survey of all patients (or their parents in the case of minors) at the O´Horan general hospital who had a clinical diagnosis of chikungunya fever between July-December 2015. The survey included questions about travel, daily mobility, exposure and contact with the vectors inside the household (as a proxy for probable intradomiciliary transmission), and cohabitation with infected individuals within a ten-day-period prior to the onset of symptoms. 803 patients were diagnosed with chikungunya at the O´Horan hospital and all of them completed the survey (participation 100%); 59% were female. Median age was 23.3 (range:1-77) years-old, while 751 (93.5%) reported spending most of the day inside their homes along with the presence of mosquito vectors inside their houses or yards. 239 (30%) mentioned that they cohabitated with a relative who had chikungunya <10 days prior

to their infection, of them, 215 (90%) were housewives who reported having taken care of a chikungunya infected relative. Female adults aged 20-44 were the most frequently affected group (n=151, 19%). In conclusion, Probable intradomiciliary transmission accounted for 30% of chikungunya infections in this study population. Women aged 20-44 were the most affected group in this study population. Mosquito-borne prevention campaigns in endemic regions could emphasize the importance of preventing mosquito bites and isolation of infected individuals in the household. We utilized the Epidemiologic chikungunya surveillance survey developed at O´Horan General Hospital in Merida, Mexico as a data source.

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AN OUTBREAK OF CHIKUNGUNYA: TRACKING THE EPIDEMIC THREAT IN SALVADOR, BRAZIL

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In Salvador, Bahia, the most common arboviruses are Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV), although others, such as Yellow fever (YFV), Oropouche (OROV) and Mayaro (MAYV), which can also cause acute febrile illness are expanding their distribution in Brazil. As part of a study of the changing arboviruses scenario in Salvador, we conducted an investigation of 50 reported cases of febrile patients with signs and symptoms compatible with arboviral infections in a poor neighborhood in northeast Salvador between April 15 and June 25, 2017. Blood samples were collected from 45 of the 50 cases; 17 were tested by RT-PCR for CHIKV, ZIKV, DENV, YFV, OROV and MAYV, 45 by ELISA IGM for CHIKV, and 31 by ELISA IgM for DENV. Chikv infection was detected in 70% (35/45) of the cases by RT-PCR (8) or ELISA IgM (27). Half (51%) were male (mean age 32 years). Clinical disease was mild and self-limiting in most cases, not requiring hospitalization. All positive cases presented myalgia, most reported pain in multiple joints and about half developed pruritus and rash. Sequencing revealed that the CHIKV strain responsible for the outbreak belonged to the ECSA genotype, and grouped phylogenetically with the strain isolated from humans in Bahia during the 2015 outbreak and from *Ae. aegypti* and humans in 2016 in several Brazilian states. DENV IgM was detected only in 3 cases, of which 2 also presented CHIKV IgM. A total of 125 adult mosquitoes were aspirated inside houses of 21 cases, with *Cx. quinquefasciatus* being the most abundant (79.2%; 69,7. female) followed by *A. aegypti* (20.8%; 30.8% female). Mosquitoes were tested by RT-PCR, and all were negative for arboviruses. With the current epidemiological scenario in Brazil, investigations of arboviral outbreaks presents a serious public health challenge due the similar clinical symptoms associated with infection. This , complicates epidemiologic surveillance, as well as potential control strategies. In our study, we detected a new outbreak by CHIKV ECSA genotype in a slum community in the periphery of Salvador, showing that four years after the introduction of CHIKV in Brazil, its epidemic potential remains high.

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A NOVEL QUANTITATIVE PCR ASSAY FOR THE DETECTION OF ROSS RIVER VIRUS

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With over 5,000 infections annually, Ross River virus (RRV) is the most common mosquito-borne disease in Australia. RRV is a mosquito-borne alphavirus endemic to Australia, Papua New Guinea, and parts of the South Pacific. Symptoms of RRV infection include fever, fatigue, and debilitating polyarthritis that is known to persist for long periods of time. RRV is transmitted by a broad range of mosquito vectors, with laboratory transmission confirmed in at least ten species of *Aedes* and *Culex* mosquitoes. In the absence of any comprehensive diagnostic tests and appropriate testing facilities, RRV was believed to be maintained in zoonotic cycles of transmission involving macropod marsupials. The 1979-80 outbreak of RRV infection in the Pacific and the availability of comprehensive diagnostic facilities in Australia, provided strong evidence for cycles of human-mosquito-human transmission without a requirement for intermediate vertebrate hosts. The re-appearance of RRV transmission in Fiji and the large human population born since the 1979-80 outbreak (and so susceptible to RRV infection) highlights the need for a sensitive and specific surveillance system able to detect future outbreaks of RRV infection in this region. To this end, we have designed and validated a real-time quantitative polymerase chain reaction (RT-qPCR) assay that specifically detects RRV at high sensitivity, and differentiates RRV from other alphaviruses, including the genetically similar Getah virus. Mosquitoes and mosquito fecal/excreta samples collected during an ongoing field study in American Samoa were screened for RRV using this assay in order to further validate the use of this screening method in the surveillance of RRV in endemic regions. Our results support the use of this assay for the detection of RRV at low viral concentrations in mosquito vectors through the use of our molecular xenomonitoring platform.

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INVESTIGATION OF *IN VITRO* INFECTIVITY OF CHIKUNGUNYA IMMATURE VIRUS PARTICLES IN MAMMALIAN AND INSECT CELL LINES

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The recent worldwide outbreak caused by chikungunya virus (CHIKV; *Alphavirus, Togaviridae*) has shown that despite being first isolated in the 1950s, this infection still has several points to be elucidated, not only regarding the correct diagnosis but also the treatment and prevention of this disease. This virus, in spite of a short viremia produces a disease characterized by high fever, myalgia, rash and arthritis/arthralgia; the latter may persist for months or years, causing a major public health impact. The aim of this work was to characterize the immature virus particles of CHIKV, addressing both structural and infectious aspects in both insect and mammalian cells. Immature particles were produced in LoVo cells, which lacks furin activity, necessary for viral maturation. Electron microscopy has shown that immature particles are significantly larger than mature virions (in average, 120 and 75nm, respectively). Mammalian (Vero, HeLa and LoVo) and insect (C6/36) cells were infected at a multiplicity of infection of 0.1 with both immature and mature particles, supernatants were collected at different times post-infection and titrated by plaque assay in Vero cells. Despite presenting a delay in replication, immature particles infected the cells, with the exception of the LoVo lineage, probably due to furin deficiency, reaching viral load values similar to those of mature particles. The presence of viral progeny in LoVo cells was only observed when infected with mature particles and the viral load decreased as the infection progressed. These results demonstrate that, different from what was previously believed, immature particles can infect cells and probably cause a disease, but the success of this infection is dependent on the furin protease.

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INVESTIGATING THE EFFECTS OF CHIKUNGUNYA IMMUNITY ON MAYARO VIRUS DISEASE AND EPIDEMIC POTENTIAL IN THE NEW WORLD

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Mayaro virus (MAYV) and chikungunya virus (CHIKV) are evolutionarily closely related members of the Semliki Forest virus antigenic complex. Similar to CHIKV, MAYV causes an acute febrile illness that can include debilitating, and often persistent arthralgia. MAYV emergence is typically sporadic and geographically restricted, but recent outbreaks and isolations indicate the virus remains a public health concern. Given the disease similarities and the phylogenetic and serological relationship between these viruses, we hypothesize that prior CHIKV immunity may affect MAYV pathogenesis and epidemic potential. To this end, we pre-exposed immune-competent (C57/B6J) and immune-compromised (A129) mice to wild-type CHIKV, two CHIKV vaccines (CHIKV/IRES and EILV/CHIKV), and a live-attenuated MAYV vaccine (MAYV/IRES), and then challenged with MAYV. We observed strong cross-protective effects against MAYV disease for mice pre-exposed to wild-type CHIKV, and moderate but significantly less cross-protection in CHIKV/IRES vaccinated mice. Interestingly, EILV/CHIKV vaccination resulted in enhancement of MAYV disease; however, further studies are warranted to investigate the mechanism underlying this singularity. Immunity to other alphavirus and flavivirus controls provided no protection against MAYV disease or viremia, suggesting the observed cross-protection between MAYV and CHIKV is a result of the close evolutionary relationship between these viruses and not the result of a non-specific immune response to a subsequent viral infection. Mechanistic studies suggest neutralizing antibodies alone can facilitate protection from development of MAYV viremia, but T-cells have a cumulative effect for diminishing MAYV disease. Further experiments such as adoptive transfers are being conducted to determine the degree of cell-mediated cross-protection in C57/B6J and A129 mice. In summary, our data suggest CHIKV immunity may impact the epidemic potential of MAYV and possibly reduce associated disease.

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RE-EMERGENCE OF CHIKUNGUNYA VIRUS IN COASTAL KENYA 2017-2018

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Chikungunya virus is an alphavirus which belongs to the Togaviridae family; it's transmitted by *Aedes aegypti* mosquitoes. It causes chikungunya fever, a febrile condition associated with severe joint and muscle pains, fever and headaches. Between 2004 and 2005, Kenya experienced its first outbreak of Chikungunya in Lamu County at the coastal region with over 13,500 cases. Since then, passive surveillance had not shown circulation of the virus. In mid-December 2017 the Arbovirus/VHF laboratory at KEMRI received eight serum samples from hospitals in Mvita and Kisauni sub-counties in Mombasa. After testing for different arboviruses, the results showed that 6/8 were positive for Chikungunya, 5/8 by RT-PCR and 1/8 by IgM ELISA. In January 2018, a team from KEMRI was sent to Mombasa to conduct further investigations to determine the magnitude of the chikungunya outbreak. Hospital visits were made and patients selected based on their clinical presentations. Any patient presenting with sudden onset of fever, headache muscle and joint pains was considered for sample collection. A total of 239 human blood samples were collected from various hospitals in the six sub counties of Mombasa, and shipped to Nairobi for testing. Samples were also received from different parts of the coastal Kenya including Lamu, Taita Taveta and Kilifi, leading to a total, 361 human samples being collected. Of these samples 191/361 (53%) were female patients while the age range was from 6 months to 73 years. The samples were processed and tested using RT-PCR for Alphaviruses and Flaviviruses. In total, 138 out of 361 (38.2%) tested positive for Chikungunya virus. Likoni sub-county had the highest positivity at 55.3% followed by Mvita sub-county with 48.4%. These findings show that chikungunya virus was the cause of the outbreak, and that it's reemerging in the coastal Kenya after more than a ten year lull. Results showed that 38.2% who were positive for chikungunya were within the viraemic phase of the disease. Community awareness campaigns on the public health effects of the virus and preventive measures including vector control need to be instituted to prevent future outbreaks.

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MOLECULAR EPIDEMIOLOGY OF CHIKUNGUNYA VIRUS FROM ECUADOR DEMONSTRATES TWO INTRODUCTIONS IN 2013 AND THE E1-K211E MUTATION, SUGGESTING PRESENCE OF ENHANCED FITNESS TO *AEDES AEGYPTI*

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Chikungunya virus (CHIKV) was first reported in the Americas in 2013, and by the end of 2014 the first cases were described in Ecuador. In Ecuador, *Ae. aegypti* is the primary vector of CHIKV; the presence of *Ae. albopictus* has not been officially reported in the country. Here, CHIKV positive samples were first detected in 2015 from an active surveillance study in Machala, Ecuador, and viral genomes from a subset of the positive samples were sequenced. The 25 full genomes from Ecuador were aligned to all publicly available CHIKV full genomes and a maximum likelihood phylogenetic analysis revealed that they belonged to the Asian lineage of CHIKV. Unsurprisingly, the Ecuadorian genomes clustered within the American sub-lineage. Here, the genomes from Ecuador were found in two well-separated clades. One clade was most closely related to a virus sampled in 2014 in Panama, and the other one to viruses from USA and Nicaragua, sampled in 2014 and 2015. This suggested two separate introductions of CHIKV into Ecuador. TreeTime analyses revealed that both these introductions happened around the same time, Aug-Oct 2013, suggesting that once CHIKV arrived in this region, it flowed into Ecuador from several different geographical locations. All genomes from Ecuador, like the most of the Asian lineage genomes, did not have any *Ae. albopictus* adaptive mutations. Instead, they possessed the E1-A98T mutation, which restricts positive selection of the *Ae. albopictus* adaptive E1-A226V substitution, thereby hampering the emergence of enhanced infection within this vector. However, genomes from Ecuador had E1-K211E mutation, which induces enhanced fitness to *Ae. aegypti*. It remains to be seen how these mutations affect CHIKV adaptation and spread, highlighting the importance of continued surveillance in this region, especially as Ecuador appears to be vulnerable to import of CHIKV from several different locations.

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DYNAMIC SPATIOTEMPORAL FEATURES OF EPIDEMIC ROSS RIVER VIRUS IN SOUTH EAST QUEENSLAND, AUSTRALIA

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Ross River virus (RRV) is responsible for the most widespread and frequently reported mosquito-borne disease in Australia. The virus transmission dynamics are complex, involving multiple vector and host species. Despite regular outbreaks across varied ecological settings of Australia, as well as Pacific Island countries, the factors which drive transmission risk are largely unknown. This study investigated spatiotemporal influences on changing RRV epidemiology across three major cities of South East Queensland (SEQ) where a large outbreak occurred in 2015: Brisbane, Gold Coast and Sunshine Coast. Notification data for Ross River virus in SEQ were obtained from the Queensland Department of Health for the years 2001-2015. Spatiotemporal and time series seasonal decomposition methods were employed to compare the distribution of RRV incidence in the three regions. RRV cases were found to persist across all seasons and years, with peak incidence occurring in the autumn months: March in Brisbane, and April in both Gold Coast and Sunshine Coast. Among the three areas, the highest annual incidence rates were consistently observed in Sunshine Coast, with a mean annual incidence rate of 73/100,000 (range: 33-149), followed by Brisbane (39/100,000; range: 15-134) and Gold Coast (33/100,000; range: 11-117). RRV spatiotemporal patterns were highly variable in each location across the years, and between coastal and inland suburbs. Our findings indicate that although RRV disease is strongly seasonally-influenced in SEQ, the specific influences on disease occurrence likely vary at the local level, including the density and distribution of both vectors and vertebrate hosts (human and animal), as well as the role of built and natural environments. This study uses spatiotemporal analysis of human notifications as a baseline, with a view to further assessing the ecological drivers of disease risk.

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INDIVIDUAL AND CONTEXTUAL RISK FACTORS FOR CHIKUNGUNYA VIRUS INFECTION ON RÉUNION ISLAND: A MULTI-LEVEL MODELLING STUDY

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Vector borne diseases are driven by multiple individual and contextual factors - which are often analyzed separately. Previous studies on chikungunya risk factors have highlighted the importance of the environment. We used multilevel modelling to investigate the importance of context during a chikungunya epidemic while taking into account both individual and contextual level risk factors. We explored a dataset of 2,242 subjects from a serosurvey conducted in La Réunion completed with spatialized aggregated data at infra-communal (IRIS) level including sociodemographic and climate variables. The importance of context was estimated using the Median Odds Ratio (MOR), the interval Odds Ratio (80% IOR) and the Sorting Out Index (SOI), three indicators used in social epidemiology. Risk factors for chikungunya were identified using mixed-effect (random intercept) logistic regression models, to disentangle contextual and compositional effects (i.e., the possible correlation of individual observations within a same spatial unit). Our multilevel analysis revealed no substantial compositional effect but confirmed the importance of the environment in the risk of infection (MOR 1.57). The most important contextual factors explaining spatial heterogeneity in chikungunya prevalence were the maximum temperature of the month before the infection (OR 3.39), the pluviometry of the preceding month (OR 2.08), the altitude of dwelling <500m (OR 2.26), and the proportion of individual residences >50% (OR 2.00). The pluviometry of the current month (OR 1.71) and vegetation cover >40% (OR 1.36) contributed slightly to the spatial heterogeneity as evidenced by 80% IOR and lower SOI values. Individual factors identified after measuring contextual heterogeneity were low education level (OR 1.93), unemployment (OR 1.47), living in an individual house (OR 1.86) or in a neighborhood at risk (OR 1.37) and the absence of window screens (OR 1.57). This study suggests the interest of using multiple data sources and multilevel modelling for identifying chikungunya risk factors while helping the understanding of contextual heterogeneity of seroprevalence.

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GENOMIC CHARACTERIZATION OF DENGUE VIRUS TYPE 2 FROM FEBRILE PATIENTS SEEN IN MALINDI DISTRICT HOSPITAL, KENYA DURING THE 2017 DENGUE FEVER OUTBREAK

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Dengue virus (DENV) is a mosquito-borne *Flavivirus* and the etiological agent of dengue fever (DF). It is the most important arbovirus-affecting humans in terms of clinical cases and geographical expansion. The last 5 decades have seen an unprecedented increase in the number of DF outbreaks in sub-Saharan Africa and Kenya in particular. In May 2017, the coast province was hit by a DF outbreak that was characterized by severe morbidity. In the present study we examine the DENV genomes associated with this outbreak to determine their genotype and their genome characteristics. Serum samples testing positive for DENV2 with PCR were used to obtain the whole genome sequence. Total RNA was extracted and reverse transcribed to cDNA for library preparation. The libraries were then sequenced on the Miseq platform. Resulting sequence reads were assembled and annotated in CLC Genomics Workbench v 8.5. Genome alignments were conducted with the Muscle and phylogenies analyzed with MrBayes v3.2 and MEGA v7. Complete polyprotein gene sequences were assembled with a total length of 10,176 bp. These encoded a typical DENV-2 polyprotein consisting of three structural proteins: capsid, membrane glycoprotein precursor and envelope protein, as well as seven nonstructural proteins, the NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The closest polyprotein gene homologues were DENV2 from India, accession numbers (KY427084 and JX475906 isolated from Humans in 2010 and 2009 respectively) with 98% nucleotide identity. There was minimal intra-strain sequence variation indicating the clonality of the strains. Phylogenetic analysis revealed that the study strains clustered with the Cosmopolitan DENV2 genotypes from Asia. This is the first description of the complete polyprotein coding gene from DENV2 from Kenya. The sequences contribute to understanding the epidemiology of DF outbreaks in Kenya. The virus responsible for the 2017 outbreak was determined to be DENV2 of the cosmopolitan genotype. More work is required to understand the molecular differences between DENV that are detected routinely in Africa and those detected during outbreaks.

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DENGUE VIRUS SEROTYPE-1 CIRCULATION IN NEPAL DURING 2016 OUTBREAK

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Dengue is an emerging disease in Nepal. The objective of this study is to present a comprehensive picture of the dengue outbreak in 2016 and to classify the prevailing serotypes. The demographic information was collected along with the clinical evaluation of the patients. Serum samples were collected from suspected dengue fever cases. The samples from fever cases <7 days duration were tested for dengue NS1 antigen employing Panbio (Australia) NS1 ELISA kit. Serum samples of ≥7 days fever were tested for dengue-specific IgM antibodies by MAC ELISA test kit. Serotyping of 1779 serum samples was done by dengue-specific reverse transcriptase polymerase chain reaction (RT-PCR) followed by nested PCR with serotype-specific primers. The number of dengue cases in 2016 clearly out-numbered the dengue cases in 2010-2013. The outbreak occurred during June to November-2016 signifying increased disease transmission in the monsoon and post-monsoon periods with a climax during September. There were 3473 confirmed patients visited/ admitted to hospital. The results showed that the affected patients were primarily adults with a mean age (± standard deviation) of 41.23 (± 35.75) years. The major clinical features of the patient were fever (100%), headache (71.3 %), rashes (11.3%), retro-orbital pain (23.5%), vomiting (23.4%), joint pain (32.1%) and thrombocytopenia (85.7%). Serotyping of 1779 serum samples confirmed the circulation of dengue virus type 1 in Nepal. Our results suggest that the dengue outbreak in Nepal during 2016 was predominantly due to dengue virus type 1 and there is a sudden shift in dominant prevailing serotype of the virus from the 2013 epidemic. It is an evidence of the circulation of serotype 1 after 2006 dengue episode in Nepal. The government should take strict action to control the disease and its vector to combat against an upcoming epidemic with severe outcomes.

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LIMITING GLOBAL WARMING TO 1.5 DEGREES AND DENGUE FEVER IN LATIN AMERICA

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At the Paris climate conference (COP21) in December 2015, governments agreed to aim to keeping increases in global-mean temperature to well below 2°C above pre-industrial levels, and to aim to limit it to 1.5°C. It is believed that human health would largely benefit from limiting global warming to 1.5°C compared against a business-as-usual scenario. Whether this assumption is true, and what is the magnitude of the associated benefits is fundamentally unknown. An understanding of the potential public health impacts at different levels of warming is essential for effective public health planning and decision-making. Here, we used dengue in Latin America as a study case to predict the health benefits of restricting global warming to 1.5°C. A climate-driven statistical dengue model and five different global circulation models to represent a range of global-mean temperature assumptions were used to this aim. We demonstrate that future global warming is likely to significantly amplify dengue transmission in Latin America if no action for limiting its effects are taken. We project that limiting global warming to 2°C could reduce dengue by about 3 (1-7) million cases annually towards the end of the century compared with a business-as-usual scenario that warms by 3.7°C. Assuming that global warming is further limited to 1.5°C, an additional decrease in dengue cases of 1 (0-1) million per year is observed. We also project that the geographical expansion of the disease towards areas where incidence is currently low would be significantly lower undear a scenario where global warming is limited to 1.5°C. Thus, constraining the level of warming to 1.5°C would lead to significant public health benefits for the region.

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MOSQUITO SALIVA HAS PROFOUND EFFECTS ON THE HUMAN IMMUNE SYSTEM

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Mosquitoes transmit viruses that collectively cause over 1 million deaths each year. Not only are mosquitoes responsible for transmitting these diseases, but mosquito saliva has also been shown to enhance pathogenesis of several arboviruses (dengue, Zika, West Nile, etc) in experimental mouse models by modulating the immune response away from a Th1 (anti-viral) immune response and towards a Th2 (anti-parasite) immune response. However, these mouse studies are unable to yield a complete picture of how mosquito saliva affects the human immune system. Therefore, we investigated how mosquito saliva affects humanized NOD/SCID/IL-2$\gamma^{-/-}$ mice (huNSG mice), which are reconstituted with human CD34+ stem cells to recapitulate the human immune system. Hu-NSG mice were bitten by uninfected mosquitoes, and changes in immune cell populations in multiple tissues at multiple timepoints compared to unbitten controls were assessed using flow cytometry. Serum cytokine concentrations were assessed via multiplex bead array. Mosquito saliva alters the frequencies of natural killer T cells, CD4/CD8 double positive T cells, regulatory T cells, monocytes, and macrophages. When analyzed in conjunction with the cytokine results, these results indicate that mosquito saliva caused a mixed Th1/Th2 immune response in human immune cells. Th2 immune responses are known to inhibit aspects of Th1 (anti-viral) immune responses and may contribute to enhanced viral pathogenesis in infected animals. Next, we investigated how the mosquito salivary protein sialokinin impacted the human immune response by exposing hu-NSG mice to mosquito bites from sialokinin knockout mosquitoes. The results showed a shift towards a Th1 immune response. Thus, the absence of sialokinin may prevent mosquito saliva from initiating a Th2 response. These studies are significant because they show that the mouse immune response and human immune response to mosquito saliva are different and highlight the importance of using appropriate models to study human immune responses.

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METABOLIC ACTIVITY AS A SURROGATE MARKER OF IMMUNE CELL ACTIVATION FOLLOWING VACCINATION

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The identification and quantification of lymphocytes activated in response to vaccination is a critical first step in characterizing the efficacy of any candidate vaccine platform. Traditionally, this has been accomplished by monitoring the upregulation of defined activation markers on circulating lymphocytes by flow cytometry or by measuring the production of effector cytokines following *ex vivo* stimulation. However, there is significant variability in both the kinetics and magnitude of activation marker up-regulation and cytokine production following stimulation, even within the same type of lymphocyte. Therefore, traditional methodologies for identifying activated lymphocytes may offer a significantly skewed perspective on exactly which immune cells are responding to vaccination or natural infection. Herein, we examine the utility of measuring the metabolic activity of circulating lymphocytes as a marker of activation following immunization with a live-attenuated dengue vaccine. Unlike any

other marker, metabolic upregulation is a universal indicator of lymphocyte activation which can be detected quickly after stimulation and persists throughout the functional stage of an immune response. We observe that assessing the metabolic activity of both *in vitro* and *in vivo* stimulated human T cells is a high effective method for evaluating activation status and identifying antigen-reactive clones. We further characterize and contrast the functional and clonotypic diversity of viral-reactive T cells identified by either conventional methodologies or metabolic parameters using single-cell RNA sequencing. Collectively, these data suggest that metabolic activity is a highly informative parameter of T cell functionality that has multiple applications in the characterization of vaccine-elicited immunity.

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DECREASED INCIDENCE OF DENGUE, CHIKUNGUNYA, AND ZIKA INFECTIONS IN CHILDREN THROUGHOUT BELO HORIZONTE, BRAZIL: SURPRISING FINDINGS FROM A PILOT PROJECT IN A REFERRAL EMERGENCY DEPARTMENT IN 2017

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Infections with chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV) viruses are a significant public health concern throughout Brazil, although the true incidence and demographic drivers for disease transmission remain to be fully described. This pilot project was created to establish a baseline understanding of arboviral disease incidence in urban Brazil and the major contributing factors to disease transmission. Patients aged < 13 years and >1 year were enrolled into the prospective study during January-April 2017 in the emergency department of Hospital Odilon Behrens, a large referral center in Belo Horizonte, Brazil. We enrolled patients based on the inclusion criteria of fever plus one or more prominent symptoms of arboviral infection. A blood sample was obtained from each patient and was tested for ZIKV, CHIKV, and DENV RNA by RT-PCR. The patients and their families also completed a questionnaire to obtain demographics and information regarding disease transmission risk factors. A total of only 22 patients were enrolled in the study during the four months, a significant drop in the incidence of suspected arboviral disease compared to prior years. Of the 22 patients enrolled, zero tested positive for ZIKV, DENV, and CHIKV by RT-PCR. While a drop in DENV infection was anticipated due to an outbreak the 2 years prior to the study, this proved to be an extraordinary reduction in the number of confirmed cases and suspected arboviral infections during 2017. Such a drop is possibly due to improved public health efforts, cross-protection between arboviruses, and improved mosquito control. More research is required further elucidate the major contributors to the decreased incidence of arboviruses throughout Brazil in 2017 compared to prior years.

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LABORATORY INVESTIGATIONS OF DENGUE AND CHIKUNGUNYA VIRUS INFECTIONS IN GHANA

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Dengue Virus (DENV) and Chikungunya Virus (CHIKV) are globally important causes of human disease which constitute part of the known arthropod-borne viruses (arboviruses). These viral agents co-circulate with overlapping clinical symptoms in endemic areas. In Ghana there is a dearth of data on DENV and CHIKV contrary to what pertains in other parts of the world. Thus, the study seeks to identify and characterize DENV and CHIKV and other endemic arboviruses in selected health facilities in Ghana. Whole blood collected from eligible patients was investigated for immunological markers, IgM and IgG by ELISA as well as the detection and amplification of viral nucleic acids if present. From April 2016 to March, 2017, 513 clinical specimens were collected of which 60% were females. Of the samples collected, 22.7% and 53.7% had anti-DENV IgM and IgG antibodies respectively while 14.3% and 32.4% had anti-CHIKV IgM and IgG antibodies respectively. No viral RNA has been detected for both viruses by RT-PCR. Most clinical samples were received from the Greater Accra and Ashanti regions. The proportion of anti-DENV IgM positives in the Ashanti region was 10 times higher than in the Greater Accra region while anti-DENV IgG positives was 3 times higher in Greater Accra region. Therefore, there is a higher probability of a recent infection of Dengue in the Ashanti region. There are significant differences in the abundance ratio of anti-CHIKV IgM and IgG antibodies between the two regions. These data indicate exposure to DENV and CHIKV and suggest possible circulation of these and other arboviruses in the country which may be contributing to febrile illnesses. There is the need for a nationwide surveillance to identify the overall prevalence of DENV and CHIKV infections.

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FIELD-DEPLOYABLE PCR SYSTEM FOR RAPID DETECTION OF DENGUE VIRUS INFECTION

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Dengue virus (DENV) infection, a mosquito-borne disease, is a major public health problem in tropical countries. Point-of-care DENV detection with good sensitivity and specificity enables timely early diagnosis of DENV infection, facilitating effective disease management and control particularly at regions of low resource. POCKIT™ Dengue Virus Reagent Set (GeneReach Biotech), a reverse transcription-insulated isothermal PCR (RT-iiPCR), is available to detect all four serotypes of DENV on the field-deployable POCKIT™ system, which is ready for on-site applications. In this study, analytical and clinical performances of the assay were evaluated. The index assay did not react with 14 non-DENV human viruses, indicating good specificity. Compared to the US CDC DENV-1-4 Real Time RT-PCR (qRT-PCR), testing with serial dilutions of virus-spiked human sera demonstrated that the index assay had comparable detection endpoints with the 4 serotypes separately. Excellent reproducibility was observed among repeat tests done by six operators at three sites. In clinical performance, 195 clinical sera collected around Kaohsiung city in 2012 and 21 DENV-4-spiked sera were tested with the RT-iiPCR and qRT-PCR in parallel. The 121 qRT-PCR-positive (11 DENV-1, 78 DENV-2, 11 DENV-3, 21 DENV-4) and 95 qRT-PCR-negative samples were all positive and negative by the RT-iiPCR reagent, respectively, demonstrating high inter-rater agreement (100%; $CI_{95\%}$, 98.81 ~ 100%; $\kappa = 1$). With analytical and clinical performance equivalent to those of the reference qRT-PCR, the index PCR assay on the field-deployable system can serve as a highly sensitive and specific on-site tool for DENV detection. Application of this point of care testing to Solomon Islands conformed to the local needs are under evaluation.

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DETECTION OF DENGUE AND ZIKA VIRUS ANTIBODIES AMONG SUSPECTED MEASLES CASES IN KENYA

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Dengue (DENV) and Zika (ZIKV) viruses are emerging and re-emerging arboviruses of public health importance worldwide. DENV has been associated with recent multiple outbreaks primarily in coastal and north-eastern regions of Kenya in 2011 to 2016.Serological surveys carried out in the country indicated the presence of ZIKV antibodies. We sought to determine the possible circulation of these viruses among patients seen and sampled at health facilities in Kenya as suspected measles cases for laboratory investigation at the measles reference laboratory. Archived serum samples collected between 2008-2014 from health facilities in Nairobi, Taita-Taveta, Tana River, Mombasa, Kilifi, Kwale, Lamu, Mandera, Garissa and Wajir counties, were randomly selected from the bio-banked samples. The samples were tested for IgM and IgG antibodies against DENV using ELISA commercial kits whereas ZIKV IgM was tested using CDC in-house MAC-ELISA.DENV and ZIKV positive samples were further tested for neutralizing antibodies against 4 flaviviruses: DENV, ZIKV, Yellow Fever (YFV) and West-Nile Virus (WNV) by PRNT. Out of the 392 samples, 6.1% tested positive for DENV IgM, 10.9% tested positive for DENV IgG. The highest prevalence for DENV was observed among those aged 14 years and above and those sampled from Nairobi County between 2008 and 2009.1% tested positive for ZIKV IgM with the highest number of cases sampled from Nairobi County .The prevalence of ZIKV varied significantly with age ($p<0.001$).WNV was most prevalent in those sampled in Garissa and Nairobi. Evidence of past YFV exposure was observed in those sampled in Garissa, Nairobi and Taita-Taveta counties. However, nothing is known about their YFV vaccination status. Results show that these flaviviruses may contribute significantly to the burden of disease. Awareness is recommended among clinicians on the potential of arboviruses to cause measles-like febrile illnesses among patients and the need for additional diagnostic support to determine the true burden of these diseases.

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DENGUE HOSPITALIZATIONS IN BRAZIL: ANNUAL WAVE FROM WEST TO EAST AND RECENT INCREASE AMONG CHILDREN

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Dengue is the most common arthropod-borne viral disease and a global health threat due to its presence in almost every tropical region and its alarming incidence increase within the last decade. In Brazil, the number of dengue epidemics has increased dramatically in the last 15 years. To identify factors associated with higher numbers of hospitalizations attributed to dengue in Brazil, we analyzed the seasonal patterns in hospitalization incidence across the different states and compared these with the corresponding climatic patterns. Therefore, we collected information from the public database of hospital stays provided by the Brazilian Unified Health System for the period from 1998 to 2015, and data on mean monthly vapor pressure, temperature, number of wet days and precipitation for the years 2001 to 2014 from the Climatic Research Unit of the University of East Anglia. Fourier analyses were used to characterize the seasonal patterns of dengue hospitalizations and climate in each state. The relative contribution of each age group to the total number of hospitalizations per month and state was also examined, and the cubic spline model was used to detect inter-annual trends. We discovered that the seasonality of dengue hospitalizations in Brazil has a clear zonal gradient that is characterized by the progression of primary peaks from West to East during the first half of the year, which may be associated with the increased vapor pressure and rainfall during this period, leading to increased mosquito abundance and activity. We also found that the proportion of children among hospitalized individuals was especially high during the peak outbreaks in 2007/2008 and 2010. This may be due to the emergence and spread of the new DENV-2 Southeast Asian genotype lineage II since 2007, which has probably arrived from the Caribbean and may have caused an increase in incidence and severity of the disease, particularly among children. Our findings may allow health systems to improve control interventions and contribute to reducing dengue morbidity and mortality by using integrated vector control in conjunction with early diagnosis and prompt supportive care.

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IMPACT OF DENGUE VIRUS INFECTION ON MOSQUITO OVARY GENE EXPRESSION AND EGG PRODUCTION

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Dengue virus (DENV) is a mosquito-borne flavivirus that causes significant human disease and mortality in the tropics and subtropics. There has been a recent global trend of increased epidemic activity, and dengue infection is considered a serious emerging health problem worldwide. There is no specific therapeutic agent available against dengue virus, and safety issues have continually hampered vaccine development. A novel approach is to develop new preventative measures through an increased understanding of the interactions between the virus and its mosquito vector. Previous studies have suggested that pathogen infection can alter reproductive fitness in mosquitoes, although the majority of these experiments were done using *Anopheles* mosquitoes. It has also been proposed that a reduction in either fecundity/fertility may be an adaptive strategy of the pathogen to increase mosquito survival, although studies remain to be comprehensively undertaken. There is limited evidence to suggest that arboviral infection of *Aedes* can also alter fecundity; thus, further studies on both the effects of DENV infection on fecundity/fertility and the mechanisms behind these effects are required. A comprehensive understanding of how dengue virus impacts fecundity and fertility can enhance our knowledge on pathogenesis in the vector and inform on epidemiology and transmission forecasts. Our hypothesis is that infection with dengue virus alters both gene expression and egg production in the ovaries of *Aedes* mosquitoes. We found that DENV-infected mosquitoes have altered egg production (fecundity) and altered gene expression in ovary tissue, as measured by RNA-Seq analysis, compared to uninfected mosquitoes. We used qRT-PCR analysis to confirm the altered expression of 10 genes with significant changes in expression during infection in mosquito ovaries, and examined the role of these genes during infection for subsequent analysis. Here, we report on the identity and putative roles of genes that have altered expression in the ovaries of dengue virus infected mosquitoes.

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USING PAIRED SEROLOGY AND SURVEILLANCE DATA TO QUANTIFY DENGUE TRANSMISSION AND CONTROL DURING A LARGE OUTBREAK IN FIJI

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Dengue is a major health burden, but it can be challenging to examine transmission dynamics and evaluate control measures because outbreaks depend on multiple factors, including human population structure, prior

immunity and climate. We combined population-representative paired sera collected before and after the major 2013/14 dengue serotype 3 outbreak in Fiji with surveillance data to determine how different factors influence dengue virus (DENV) transmission and control in island settings. Using surveillance data from 25,494 suspected cases of dengue during 2013/14 and paired sera collected from 263 participants in 2013 and 2015, we analysed the roles of age, environmental factors, geography, pre-existing immunity, climate, and control measures in shaping observed patterns of infection and reported cases. The serological survey suggested that the 10-19 year old age group had the highest risk of acquiring infection (56%, 95% CI: 44-68%), whereas reported disease incidence per capita was highest in the 20-29 age group, many of whom were experiencing their second DENV infection. We did not find strong evidence that demographic or environmental risk factors were linked to seroconversion. Joint mathematical modelling analysis of surveillance data and the serological survey suggested that temperature-driven variation in transmission could not fully explain the sharp decline in cases during April/May 2014. However, there was evidence of an additional reduction in transmission in March 2014, coinciding with a large vector clean-up campaign, which may have contributed to the outbreak decline. By identifying key factors that influence DENV transmission dynamics, these results could also help inform forecasts of outbreaks and evaluation of control measures in other settings.

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ANTIBODIES AGAINST DENV: REVEALS FROM A NOVEL NEUTRALIZATION ASSAY

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Dengue is the most prevalent arboviral disease affecting humans. The illness is caused by dengue viruses (DENV). Although a vaccine is recently licensed its efficacy against some viral serotypes is limited, despite apparent protection as measured by Plaque Reduction Neutralization Test (PRNT), the conventionally accepted correlate of protection. We hypothesize false-positive results of PRNT are obtained from non-neutralizing antibodies. We received from collaborators 12 human-derived DENV-specific monoclonal antibodies (mAbs), each of these therapeutically potential mAbs neutralize DENV in the PRNT. We developed an alternative assay, named VIremic Blood Neutralization Assay (ViBNA), to re-characterize the neutralization profiles of these mAbs. ViBNA exploits the use of DENV drawn from acutely infected dengue patients, and considers the biologically relevant endpoint of mosquito infection. Results highlighted a significant discrepancy between assays, with seven out of the 12 mAbs failing to neutralize any DENV serotypes in ViBNA. The results support our hypothesis and have implication for the failure of PRNT to predict the efficacy of dengue vaccine. In addition, antiviral therapy is not available for dengue. Administration of DENV-specific antibodies is considered a potential antiviral approach to reduce disease severity, the symptomatology, and the duration of illness. With ViBNA, we identified five neutralizing mAbs as candidates of antivirals for dengue. A subgroup of these mAbs was diluted and tested for minimum concentrations that mediated DENV neutralization with ViBNA. These concentrations varied between mAbs, ranging from 0.1 µg/mL to 0.4 µg/mL. Results of mAb-dilution experiments have direct implications for the design of dengue clinical trials.

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INVESTIGATING THE FIRST OUTBREAK OF DENGUE HEMORRHAGIC FEVER IN THE GREATER DARFUR, WESTERN SUDAN

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Dengue fever is one of the most rapidly spreading diseases around the world with confirmed presence in 128 countries. An estimated 390 million dengue infections are reported annually with 3.97 billion people at risk of infection worldwide. Dengue in Sudan has historically been confined to Eastern Sudan. Here, we are reporting the first outbreak of the disease in Darfur, western Sudan. A cross-sectional community-based study has been designed to investigate an outbreak of a hemorrhagic fever-like disease in the five states of greater Darfur with a concentration on the internally-displaced population of refugee camps. Blood samples were successfully obtained from 204 individuals out of 560 suspected-cases who visited the health clinics of 29 refugee-camps in East, West, South, North and Central Darfur states between August 2015 and February 2016. All suspected cases presented with fever, 98.6% of cases with bleeding, 52.9% with a headache 48.2% with joint pain, and the mortality rate was 18.2%. IgM ELISA and Polymerase Chain Reaction (PCR) analyses confirmed 32 (15.7%) cases of dengue fever, 6 (2.9%) cases of West Nile virus (WNV) and 3 (1.5%) cases of Crimean-Congo hemorrhagic fever (CCHF). No cases of Zika virus (ZIKV), yellow fever (YF), chikungunya virus (CHIKV), or Rift Valley fever (RVF) were detected. Our analysis showed that most of infections were children and young adults, and *Aedes aegypti* to be the dominant suspected vector in the area with an incidence of 25-86% in water containers. This is the first report of dengue outbreak in Greater Darfur, which seems to be associated with the recent massive human population movement and socioeconomic changes in this war-torn region of Sudan.

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EMERGENCE AND CO-CIRCULATION OF NEW GENOTYPES AND LINEAGES OF DENGUE VIRUSES DURING 2012-13 AND 2014-15 EPIDEMICS IN SOUTHERN INDIA

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High genetic diversity is demonstrated among dengue virus (DENV) serotypes, however, strains circulating in India are not well characterized. We genetically characterized DENV strains among dengue-infected children from 2012-13/2014-15 outbreaks in Bangalore, in southern India. Acute-phase blood samples from children with clinically suspected dengue were collected. DENV serotypes were detected using RT-PCR targeting *Capsid-preMembrane (C-prM)* and *Envelope (Env)* regions; *C-prM* of DENV strains from 99 patients (33 DENV-1, 32 DENV-2, 27 DENV-3 and 7 DENV-1) were sequenced and aligned using ClustalW in BioEditV7.1.9, and compared with 207 GenBank sequences. Phylogenetic analysis was performed by maximum-likelihood method using Kimura 2-parameter model with 1000 bootstrap replicates in MEGA6. In 2012-13 (n=113), DENV-3 (44, 38.9%) and DENV-2 (43, 38.1%) predominated; DENV-1 was less common (22, 19.5%) followed by DENV-4 (1, 0.9%). In 2014-15 (n=499), DENV-1 (329, 65.9%) predominated, followed by DENV-2 (97, 19.4%), DENV-3 (36, 7.2%) and DENV-4 (10, 2.0%). Co-infections with multiple serotypes were seen in 2.7% (n=3) and 5.4% (n=27) in 2012-13 and 2014-15, respectively. Among 7 DENV-1 isolates

from 2012-13, 6 were genotype III (GIII) and 1 belonged to genotype I (GI). The pattern changed in 2014-15 where, 21 were GI and 5 were GIII among 26 DENV-1 isolates. Out of total 32 DENV-2 isolates, 9 clustered with previously reported GIV lineage B strains (GIVb) from India and the remaining 23 clustered separately (GIVc). All 27 DENV-3 sequences were GIII and all the 7 DENV-4 isolates were lineage C of GI (GIc) which further diverged into two clades. This is the first report of a major outbreak due to re-emergence of DENV-1 GI, circulation of new lineage (GIVc) of DENV-2 and two clades of DENV-4 GIc in India, which may explain the severe dengue outbreaks in 2014-15. Our findings highlight the rapidly changing epidemiology of dengue in southern India and the likelihood of its impact on transmission dynamics and disease severity. This study emphasizes active surveillance of DENV strains in predicting outbreaks and for aiding vaccine and anti-viral trials.

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SEROLOGICAL EVIDENCE OF FLAVIVIRUS CIRCULATION IN HUMAN POPULATIONS IN NORTHERN KENYA: A RISK ASSESSMENT 2017

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Yellow fever (YF), dengue (DEN), Zika and West Nile (WN) are important reemerging mosquito-borne flaviviruses. Outbreaks have been reported in Kenya; YF (1992-95), Dengue (2011-2018) and there is concern of reemergence or importation of Zika virus. The potential threat of spillover remains high following recent reports of Flavivirus outbreaks in countries neighboring Kenya and thus, the need for human exposure assessment in border areas. We screened human serum samples collected through a cross-sectional survey in village clusters from two northern Kenyan Counties (West Pokot and Turkana), both bordering Uganda, South Sudan and Ethiopia by serological detection of IgG antibodies against DEN virus using commercial IgG Enzyme Linked Immunosorbent Assay (ELISA) kits (InBios) and virus neutralization for YF at a dilution of 1:20 by plaque reduction neutralization test (PRNT). Sera reactive for YF or DEN were titrated by 90% PRNT for 4 flaviviruses (YF, DEN, Zika and WN viruses) to rule out cross-reactivity. Overall, 29.5% (259/877) of the samples showed seropositivity for DEN virus by ELISA. Employing the criterion for 4-fold greater titre 50 (5.7%) were seropositive for YF virus, 36 (4.1%) for WN, 6 (0.7%) for Zika and one (0.1%) for DEN virus. 21.8% (90/413) of samples from Turkana County neutralized with one of the three flaviviruses (11% with YF, 9% with WN, 0.2% with Zika viruses), nine were classified as indeterminate. In West Pokot County, 2.6% (12/464) were positive with one of the 3 flaviviruses (1.3% with YF, 1.1% with Zika, 0.2% with DEN viruses). The odds of Flavivirus infection was about 11 times higher in Turkana than W. Pokot (OR=10.5, 95% CI= 5.59-21.38). These results suggest Flavivirus exposure in areas of Northern Kenya, bordering epidemic *Foci* of neighboring South Sudan, western Uganda and Omo River Valley in Ethiopia. The presence of YF, WN and Zika antibodies in the population may point to previous vaccinations (YF) or undetected cases. Focused case-based surveillance will be needed to determine accurately the burden and for implementation of preparedness strategies to counter potential threats posed by these viruses.

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DENGUE VIRUS INFECTS TYPE II PNEUMOCYTES AND HEPATOCYTES AND INDUCES AN INFLAMMATORY RESPONSE IN TARGET ORGANS OF FATAL CASES

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In Colombia, dengue disease is a major public health problem, with 60% of its population at risk of infection and a case mortality rate of 3%. The pathogenic mechanisms underlying the severe manifestations of dengue, such as hemorrhage, raise in vascular permeability and shock that can lead to death are still unknown. Studies on the histopathology of fatal cases are crucial for the understanding of this mechanisms. To describe the immunohistopathology of dengue in Colombia, 46 cases of fatal dengue were evaluated; in each case, paraffin-embedded tissues of liver and lung were prepared. To confirm the infection of dengue virus we performed qRT-PCR. The presence of dengue antigen was evaluated by immunohistochemistry. For the characterization of the cellular infiltrates, we performed hematoxylin/eosin and immunohistochemistry staining for the detection of CD3, CD4, CD8 and CD68 cells. We confirmed the presence of dengue virus in 8 cases by RT-PCR, and in 12 cases by immunohistochemistry. In the lung, the antigen was found infecting macrophages, type II pneumocytes and endothelial cells; the presence of alveolar edema and hemorrhage was the most common finding, followed by abundant mononuclear cellular infiltrate, T cells were the major infiltrating cellular subpopulation, located in septum and perivascular regions, macrophages were mainly located in alveoli. The liver was the most affected organ, with macro and micro steatosis, edema zones and vascular congestion, and infiltrates of T cells mainly in portal zones, the dengue antigen was found infecting macrophages, endothelium and hepatocytes. We found that dengue virus infects not only macrophages and endothelial cells but also type II pneumocytes and hepatocytes, which can contribute to the alveolar collapse and hepatic dysfunction respectively. In most cases, the infection induced edema and hemorrhage in liver and lung, with an abundant infiltration of T cells. In liver was prominent the presence of steatosis, compromising most of the cases. These results provide evidence to support the contribution of dengue-induced immunopathology in the multiorganic involvement of fatal dengue.

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DISTRIBUTION OF DENGUE CASES IN KISUMU CITY AND ENVIRONS

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Dengue is the most common mosquito-borne viral infection with an expanding global distribution. But, little is known on its distribution in Kenya. In this study our main aim was to characterize the spatial distribution of dengue cases in Kisumu city and its surroundings. From January 2014-June 2018 children aged 1-17 years having acute febrile illness in our study facilities were enrolled in a cohort and tested for dengue using reverse transcription polymerase chain reaction (RT-PCR). For febrile children with dengue RT-PCR positive results, residences were mapped and location information recorded. We mapped 82 of 100 DENV PCR positive cases. At least 20 sub-locations in Kisumu city and its surroundings had one positive RT-PCR dengue result. The city alone had cases in at least 11 of 25 sub-locations. Of the 20 sub-locations that had positive results, 4 sub-locations had more than 3 dengue cases while the others had 1-3 cases. Of the 82 cases mapped, ~55% (45/82) occurred

within Kisumu city, while~45% (37/82) occurred outside. Dengue cases occur both within the city and in its surrounding locations, with most cases coming from the city. This study points to areas with high transmission of dengue, and results can be used to target vector control measures.

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DENGUE VIRUS SEROTYPE 2 DETECTED IN FEBRILE CHILDREN IN A PERI-URBAN AREA OF ACCRA, GHANA

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Accurate diagnosis of non-malarial febrile illnesses remains a big challenge in many malaria endemic countries due to nonspecific clinical presentation and diagnostic limitations. This often results in presumptive treatment of malaria for most febrile episodes. To obtain a snap-shot of the pathogens causing febrile illnesses in Ghana, a hospital-based cross-sectional study was conducted out at out patients departments of two hospitals in Ghana. A total of 166 blood samples from febrile children aged 1-14 years were screened using a customized multi-pathogen real time PCR-based TaqMan probe-array card which simultaneously detects 26 pathogens, including three protozoa, seven bacteria and 16 viruses. All children were physically examined and blood or urine cultures were performed at the request of attending clinician. *Plasmodium falciparum* was the predominant pathogen detected in the children (36.0 % of samples tested). *Salmonella enterica* Typhi (0.6%), *Rickettsia* spp.(3.0), *Coxiella burnetii*, (0.6) and HIV-1(0.6) were also detected in a few of the samples. A notable observation was the detection of dengue virus (1.2%) in two children who were 3 and 14 years old. A sequence analysis of the dengue RNA revealed a close relationship with dengue serotype 2 implicated that was implicated in outbreak in Burkina Faso in 2016. One of the dengue-positive samples was also positive for *Plasmodium*, providing a vivid demonstration of the complex etiology of AFI in malaria-endemic areas. Pathogens were not identified in 58% of the samples tested. In conclusion, the result highlights the importance of dengue as a cause of acute febrile illness and underscores the need of diagnostic testing for dengue and other flaviviruses in malaria endemic areas, in order to facilitate control, including the prevention of nosocomial transmission

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ASSESSING THE INTERCONNECTION BETWEEN PSYCHOSOCIAL DISTRESS, IMMUNE RESPONSE, AND DENGUE VIRUS IN MACHALA, ECUADOR

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Aedes aegypti (*Ae. Aegypti*), the mosquito that transmits diseases such as dengue virus (DENV), chikungunya, yellow fever and Zika virus infection is now present in nearly every tropical and subtropical region of the world. Almost one billion people around the world are at risk of contracting a mosquito-borne illness and DENV is considered the most common arboviral disease impacting humans. In Latin America, the incidence of DENV has increased from 16.4 cases per 100,000 in the 1980s to 71.5 cases per 100,000 from 2000 to 2007. Biological factors, social stressors, anthropogenic environmental modifications and/or ecological changes such as natural disasters can influence and increase the risk of dengue transmission. Understanding how these factors intersect are essential to explaining how social-biological processes influence disease onset. Research on DENV virus has largely been conducted separately in bench and in public health environments. Little research exists, however, that evaluates the intersection of the social-biological process and how it influences disease progression. We are implementing the Medical Ecological Framework, a novel analytical framework, to propose a study that evaluates the relationship between distress and immune responses to DENV with two specific aims: 1) Describe how distress influences the production of inflammatory cytokines in response to infection. 2) Describe the social conditions, exposure, and experiences related to mosquito-borne diseases from an ethnographic point of view in two different, ecologically-diverse communities of Machala, Ecuador and to seek evidence on how these factors influence stress-related behaviors. We expect to provide new understandings of the intersection of social-biological processes that occur in communities susceptible to mosquito borne-illness. In addition, we seek to implement theories and models of community organization and community building by assuring inclusive and integrated participation across community sectors in the planning process and to build consensus on what can and should be done based on the communities' unique assets and needs.

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IS THERE A ROLE FOR DEFECTIVE DENGUE VIRUS PARTICLES IN DENGUE EPIDEMIOLOGY?

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There is ongoing debate about the relative contributions of the virus and the host in the pathogenesis of dengue. As obligate parasites, dengue viruses (DENV) have a vested interest in not killing their host before they have been transmitted to a new one. Furthermore, DENV dissemination is constrained by the restricted movement of its common *Aedes* mosquito vectors. We have identified sub-genomic RNA in DENV from patients, and in cell culture, composed of short lengths of RNA from the 5′ and the 3′ ends of wild type (WT) DENV genomes and demonstrated that these suppress DENV replication *in vitro*. While some of these sub-genomic RNAs contain all elements necessary for their replication, others do not. We proposed that virions containing these defective genomes, reduce viraemia, attenuate disease and enable viraemic patients to remain mobile and therefore increase exposure to more mosquito vectors. It is not known how these defective genomes are generated, whether they can be transmitted between vectors and hosts, how effective they are in inhibiting virus replication and whether they can interfere with the replication of other viruses. Our *in vitro* data suggests that defective DENV genomes are generated in both vertebrate and invertebrate cells by a variety of mechanisms. For instance, several contain no common junctions between 5′ and 3′ elements of the sub-genomic RNA, while a subset contain a small conserved junction site suggesting targeted recombination. Transfection of the sub-genomic DENV RNA into cells infected with the DENV serotype from which it was derived, or with a range of other flaviviruses, resulted in decreased yields of extracellular WT virus. While each DENV infection appears to produce a unique spectrum of defective genomes, it is not clear whether these compete with each other as well as with the WT genome for the intracellular DENV replicative machinery and whether different defective DENV genomes interfere with the replication of wildtype genomes in the same manner. Defective interfering DENV provide mechanisms to modulate both disease severity and virus transmission.

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IDENTIFICATION OF SYNONYMOUS CONSTRAINT ELEMENTS IN THE POLYPROTEIN OF DENV-2 HAPLOTYPES FROM METAGENOMIC SEQUENCE DATA

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Despite high mutation rates of the dengue virus (DENV), there are parts of its genome that are under unusually high evolutionary constraint. These regions, called synonymous constraint elements (SCEs), are highly conserved and known to be signatures of overlapping function. This study aims to systematically find SCEs across multiple sequences of DENV to identify probable elements that may have overlapping roles in the viral

life cycle. Metagenomic sequencing was done on 14 serum samples from patients presenting with acute febrile illness. Finding Regions of Excess Synonymous Constraints (FRESCo) analysis tool was used to systematically identify SCEs across DENV haplotype sequences of the structural and non-structural (NS) gene regions. Significant SCEs were identified at the 3' half of the envelope (E) and NS5 gene. While the E-gene is under constant selective pressure due to its roles in host-virus interaction, this study has seen parts of it that are highly conserved. Although parts of the NS5 gene harbor putative functional elements involved in viral replication, other SCEs identified here which has not yet been described elsewhere can serve as initial information to do site-directed mutagenesis studies. The study demonstrates the feasibility of systematically identifying SCEs using haplotype alignment from metagenomic viral sequences. Finally, the highly conservative nature of SCEs may serve as excellent targets for antiviral drug designs.

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DENGUE IN SOUTHEASTERN BRAZIL: A LARGE OUTBREAK FOLLOWED BY A THREE-YEAR LOW INCIDENCE PERIOD. OBSERVATIONS FROM A PROSPECTIVE COHORT STUDY

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Brazil is the largest contributor to the number of reported dengue cases worldwide. However, those figures may not reflect the true burden of the disease. Accurate data on dengue incidence are necessary for the evaluation of novel control interventions. A cohort study was set up to determine the incidence of dengue in a mid-level endemicity town, Araraquara, state of Sao Paulo, Brazil. A cluster randomized sample of children and adolescents from 2 to 16 years of age was selected and invited to participate. Parents or legal guardians who agreed to have their child enrolled provided a written consent. A baseline blood sample was collected for dengue serological diagnosis. Families are contacted weekly for fever surveillance. If the participant reports a febrile episode, the study nurse visits the household to collect a blood sample for dengue diagnosis. Acute cases are confirmed according to their PCR and NS1 results. Confirmed dengue cases undergo full medical examination. Yearly blood samples are collected for serology. Participants were recruited from Sept 2014 to March 2015; 3,514 participants were enrolled in the cohort. A large dengue outbreak occurred in 2015. There were 290 confirmed cases among the participants in 2015 and 14 in 2016 (cumulative incidence of 8.3% and 0.48%, respectively). All cases were confirmed as DENV1. No confirmed cases were diagnosed in 2017 and none have been confirmed in 2018. Dengue seroprevalence was 13.7% at baseline, 30.9% after one year, 30.8% in the following year, and 33.4% in 2018. The incidence of inapparent infections among previously dengue naïve subjects was 14.4% in 2015/16, 4.6% in 2016/17 and 5.3% in 2017/18. The state of Sao Paulo faced its largest dengue outbreak ever in 2015. Since then, incidence has decreased markedly. No symptomatic cases have been detected among the cohort participants in the past two years, although asymptomatic cases still occurred. While these numbers reflect the national trend of reduction in the number of dengue cases, local factors such as vector density, urban infrastructure, population immunity, circulating serotype, and others, play a role in the transmission dynamics.

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COMMUNITIES ORGANIZED TO PREVENT ARBOVIRUSES: COPA - A PROSPECTIVE COMMUNITY STUDY TO ASSESS OCCURRENCE OF ARBOVIRAL INFECTION AND EVALUATE VECTOR CONTROL INTERVENTIONS IN PONCE, PUERTO RICO

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The public health response to the spread of arboviruses such as dengue, chikungunya, and Zika has been hampered by a lack of effective interventions to prevent human infections. Vector control programs with substantial community participation can improve acceptance and sustainability, and complement vertical approaches. Identifying effective vector control strategies to control current and emerging arboviruses requires conducting robust epidemiologic evaluations in urban communities with infection and disease in humans as the outcomes evaluated. The purpose of this study is to implement a community-based platform for arbovirus surveillance and research. We will recruit a community cohort to assess arboviral infection incidence and prevalence. Through active surveillance we will establish incidence and etiology of acute febrile illness. We will monitor entomological parameters and we will ultimately evaluate the feasibility and effectiveness of integrated vector control strategies to reduce human arboviral infection. Fourteen groups of communities with high rates of arboviral disease in southern Puerto Rico were identified using surveillance data and constitute the study clusters. We aim to recruit 250 participants per cluster or 3,500 individuals in total. A sero-survey and questionnaire to assess knowledge, attitudes, and vector control practices will be repeated annually, after the initial baseline, for at least five years. We aim to measure a difference of 50% in arboviral infection incidence between intervention and non-intervention clusters over the course of the study period. After the first year of data collection, prevalence rates will be calculated for each cluster. Clusters will be paired based on baseline prevalence rates, and intervention and control status will be randomly assigned. The incidence rate in each cluster will be assessed through annual testing for arboviral infections. Comparisons will be made between the intervention and control clusters using paired t-tests to assess differences between groups. In this presentation we will outline the study design and plans for evaluation of interventions.

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DENGUE KNOWLEDGE, ATTITUDES AND PRACTICES AND THEIR IMPACT ON COMMUNITY BASED VECTOR CONTROL IN RURAL CAMBODIA

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The World Health Organization global strategy for dengue prevention aims to reduce mortality rates by 50% and morbidity by 25% by 2020. The adoption of integrated vector management approach using community-

based behavior change methods tailored to the local context is one of the recommended strategies to achieve these objectives. Understanding local knowledge, attitudes and practices is therefore essential to designing culturally appropriate strategies to fit each local context. A Knowledge, Attitudes and Practices survey in 600 randomly chosen households was administered in 30 villages in Kampong Cham, Cambodia which is one of the most populated provinces of Cambodia. KAP surveys were administered to a sub-sample of households where an entomology survey was conducted (1200 households), during which *Aedes* larval/pupae and adult female Aedes mosquito densities were recorded. Participants had high levels of knowledge regarding the transmission of dengue with 96.7% identifying mosquitoes as the dengue vector, and 95.5% able to identify at least one breeding site. *Aedes* breeding and biting prevention methods were similarly high. The majority of participants (97.5%) believed they were at risk and that dengue transmission is preventable (77.8%). However, self-reported vector control practices did not match observed practices recorded in our surveys. No correlation was found between knowledge and observed practices. An education campaign regarding dengue prevention in this setting with high knowledge levels is unlikely to have any significant effect on practices unless it is incorporated in a more comprehensive strategy for behavioral change, such as the Communication for Behavioral Impact method, which includes appropriate theoretical models, creates enabling environment and ensures active community participation in dengue to bring about sustained behavioral changes in target communities.

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QUALITATIVE ASSESSMENT TO UNDERSTAND COMMUNITY'S ACCEPTANCE, PREFERENCES AND SUSTAINABILITY OF GUPPY FISH (*POECILIA RETICULATA*), PYRIPROXYFEN, AND COMMUNITY ENGAGEMENT FOR DENGUE CONTROL IN KAMPONG CHAM, CAMBODIA, 2016

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Dengue is the most rapidly spreading mosquito-borne viral disease in the world, and Cambodia has one of the highest per-capita incidence rates in the region. Without a widely available vaccine or therapeutics, vector control is the most effective way to fight dengue. Increasing resistance to commonly used insecticides has been reported and alternatives are needed. Three inventions recently trialed in Cambodia include guppy fish, a controlled release pyriproxyfen (PPF) matrix (Sumilarv® 2MR), and Communication for Behavioral Impact (COMBI) activities. The trial explored community perception, acceptability, and willingness to pay for vector control tools through a qualitative assessment. Using a purposive sampling technique, nine in-depth interviews and 12 focus groups discussions (FGD) were conducted at community and health center level. A thematic analysis approach was adopted during data analysis. Free listing and pile sorting were performed to identify and rank all vector control tools available in their community. The majority of individuals (62.5%) preferred guppy fish due to ease of use/rearing, quick reproduction and propensity to eat larvae. FGD participants remarked *"We love guppies as they are attractive, easy to keep, and clean the water."* Respondents were willing to pay 100-500 riel ($0.02-0.1 USD) for a pair of guppies. The next commonly preferred method was PPF (35%) due to its long-lasting effectiveness, convenience and easy maintenance. However, some concerns over the persistent presence of larvae existed. *"We know PPF works well as fewer mosquitoes are around, however, we are afraid the presence of larvae may contain parasites that can spread the disease".* These concerns can often be overcome through health education. Additional concerns about access, availability and affordability of PPF were raised by FGD participants. The least preferred methods included mosquito coils, and chemical sprays due to the smell and health concerns. Results showed that in the presence of well-developed COMBI activities it was possible to achieve high acceptance and demand for guppies and PPF.

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BUILDING ECOLOGY INTO MODELS TO PREDICT ARBOVIRUS DYNAMICS

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Understanding the ecological conditions that enable arboviruses to emerge and circulate is critically important for understanding disease dynamics, predicting future outbreak events, and breaking transmission cycles. Climate factors (e.g., temperature, rainfall, and humidity), land cover type, and microhabitats can influence vector abundance, distribution, and transmission potential by affecting a variety of vector traits, including survival, reproduction, and development. Using dengue and chikungunya as examples, we will discuss how we are incorporating empirically-derived relationships of climate, land cover, and microhabitats into mathematical models to understand their impacts on the abundance of the *Aedes aegypti* mosquito vector and disease transmission. We will also discuss how we are incorporating many data sources to improve predictions for specific locations, including the use of data from in situ climate loggers, satellites, field entomology studies, and laboratory-confirmed disease cases for urban and rural locations within Kenya. These models can help us predict disease transmission in the near future and understand how global climate change is likely to shift transmission in space and time in the longer term. Further, these results have implications for understanding where other *Aedes aegypti*-vectored diseases could circulate.

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USE OF A RANDOM FOREST MODEL CONTAINING AUTOCORRELATION, POPULATION, WEATHER, CLIMATE, VEGETATION, AND LAND USE PREDICTORS TO FORECAST DENGUE FEVER CASES AT A CITY LEVEL IN CHINA UNDER IPCC CLIMATE CHANGE SCENARIOS

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Dengue Fever is a vector borne disease that occurs primarily at tropical latitudes. In recent years the incidence rate in many countries has been increasing, and it is thought that as the effects of climate change are realized that this will continue. We then used a random forest model to forecast cases under climate change pathways and identify key areas of risk. The model contained autocorrelation, population, weather, climate, vegetation, and land use variables and was used to predict cases across China. Daily averaged estimates of weather variables were obtained from the IPCC. We used the RCP 4.5 and RCP 8.5 pathways. The dataset was then aggregated to a weekly timescale. Several time periods were selected from the forecast data and the model used to predict cases at the City level. The projections of case counts were converted to basic incidence rates and the difference between these and current incidence rates calculated. For China there was a slight decrease in the minimum temperature and a slight increase in the relative humidity and maximum temperature projected throughout the year compared to the current observations. Wind speed was projected to be either similar or increased

over current observations, dependent on City. Total Rainfall showed increases mid-year. The model used for predictions attained a good fit with the observed data. When projected, seasonal patterns and differences between Cities were observed. The incidence rate plots showed that the areas of risk moved further to the north and west; with areas of highest incidence remaining in similar areas to that currently observed. As the climate warms the risk areas for Dengue Fever in China spread further west and north as more areas become suitable, but the majority of cases remain in key population centres.

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ANTIVIRAL INVESTIGATION, HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS AND PHYTOCHEMICAL PROFILING OF *BRYOPHYLLUM PINNATUM* (*ODAA OPUO, ABAMODA*) AND *VISCUM ALBUM* (*AWURUSE, AFOMA*)

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Despite tremendous progress in human medicine, no drugs exist for the complete treatment of these viral diseases. This study was designed to investigate the antiviral potentials of two medicinal plants available locally in South Eastern, Nigeria. Fresh leaves of *Bryophyllum pinnatum* (L) and *Viscum album* (L) were collected from Lagos, South Western, Nigeria. Extraction of the plant materials was done with methanol using the soxhlet extractor and concentrated using the rotary evaporator. Measles, polio, and yellow fever viruses were isolated from their respective vaccines, while herpes simplex virus-1 was isolated from a positive HSV male case. The toxicity profile showed that the minimum non-toxic concentration (MNTC) of *B. pinnatum* (L) was 0.016 µg μL^{-1} with an IC_{50} of 0.063 µg μL^{-1} while that of *V. album* (L) was 0.063µg μL^{-1} and IC_{50} of 0.313 µg μL^{-1}. Result of the antiviral analysis showed that two of the viruses were susceptible to *B. pinnatum* (L) and *V. album* (L). While HSV-1 was sensitive to *B. pinnatum* (L), MV was susceptible to *V. album* (L) at the concentrations of 0.016 µg μL^{-1} (IC_{50} 0.004 µg μL^{-1}) and 0.063µg μL^{-1} (IC_{50} 0.031 µg μL^{-1}; IC_{50} 0.039 µg μL^{-1}) respectively. Polio and yellow fever viruses were resistant to both plants extracts at all the concentration tested. Result of the phytochemical of both plants revealed the presence of various secondary useful metabolites. In conclusion, this study has shown that the solution to measles and herpes simplex-1 viral diseases could be found in the forest zones of Nigeria.

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ZIKA VIRUS PREVENTION: US TRAVELERS' KNOWLEDGE, RISK PERCEPTIONS, AND BEHAVIORAL INTENTIONS - A NATIONAL SURVEY

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Limited data exist about US travelers' knowledge, risk perceptions, and behaviors related to the Zika virus (ZIKV). Using an internet research panel, in March 2017 we surveyed 1,202 Americans in the continental United States and Puerto Rico who planned to travel to a Zika-affected country, state, or US territory in 2017. We compared levels of knowledge and perceived risk of ZIKV, and intentions to practice ZIKV prevention behaviors across respondents from 3 regions: Puerto Rico, at-risk states, and other states. Over 80% of respondents correctly understood that a person could acquire ZIKV through a bite from an infected mosquito, and over 64% of respondents knew that a pregnant woman could pass the virus to her fetus. Less than half of respondents from at-risk states and other states knew that ZIKV could be transmitted sexually, as compared with three-quarters of respondents from Puerto Rico. Compared with respondents from at-risk and other states, respondents from Puerto Rico were the most knowledgeable for almost all types of knowledge assessed. Knowledge about post-travel precautions was low across all 3 regions. Differences in perceived risk and intentions to practice specific prevention behaviors also varied among regions. Significant gaps exist in US travelers' knowledge about how to prevent ZIKV transmission both during and after travel. Input and collaboration from the travel industry, health care providers, and the media are needed to help educate travelers about how to prevent ZIKV infection and transmission.

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ZIKA VIRUS SCREENING IN THE KENYAN OLYMPIC TEAM ATTENDING THE 2016 OLYMPIC GAMES IN BRAZIL

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The 2016 Olympic Games happened at the time of heightened fears of Zika virus (ZIKV) that was causing microcephaly in newborns in Brazil. To avert or track introduction of ZIKV in Kenya, the Ministry of Health developed a public health response that involved screening of the Kenyan contingent before and after traveling to Brazil. Of the 92 team members that were screened, all but one tested negative for ZIKV IgM and IgG. The sero-positive individual had high IgM serum titers before and after travel to Brazil. When tested for potential antibody cross-reactivity to other flaviviruses that have been reported in Kenya, the sample showed high IgM cross-reactivity to West Nile, Tick-Borne Encephalitis and Yellow Fever Virus. Our data supports the low risk predictions of acquiring ZIKV that were made before the Games and will help inform risk assessments for U.S. military personnel traveling to endemic regions under similar circumstances in the future.

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ZIKA VIRUS UPREGULATES INTERFERONS AND PRO-INFLAMMATORY CYTOKINES IN HUMAN PROSTATE CELLS

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Zika virus (ZIKV) is an ssRNA Flavivirus, traditionally known to cause Zika fever but recently linked to Guillain-Barré Syndrome and microcephaly. While ZIKV is typically transmitted by *Aedes* species mosquitoes, sexual transmission has also been reported. Secreted ZIKV RNA has been detected in the semen of infected males up to 6 months after initial infection, suggesting that the virus may persist in the urogenital tract and be sexually transmitted for an extended period of time. However, little is known about ZIKV persistence mechanisms that may mediate long-term urogenital tract replication and delayed sexual transmission. We recently demonstrated ZIKV tropism in human prostate cells using three contemporary ZIKV clinical isolates (FLA, FLR, and HN16). Currently, we are investigating the cytokine profiles produced by ZIKV infection of human prostate stromal mesenchymal stem cells (MSCs) and prostate epithelial cells with three ZIKV isolates as compared to dengue virus (DENV). The concentrations of various cytokines were determined by using multiplex immunoassays. Our results show that each ZIKV isolate elicits distinct cytokine profiles in infected MSCs and these are also different from the DENV cytokine profile. Furthermore, the data show an increase in pro-inflammatory cytokines, such as IL-1a, RANTES and MCP-3 in ZIKV-infected MSCs, as well as increased type II interferon and related proteins (IFNg and IP-10). These are consistent with published data showing upregulation of type II interferons promoting ZIKV replication in glioblastoma and placental cells. The results point to an upregulation of specific cytokines and IFNs associated with viral persistence in the urogenital tract, possibly contributing to the long-term presence of ZIKV in semen. Future studies will address the mechanism by which ZIKV upregulates specific cytokines in human prostate cells

and what contributes to the differential cytokine profiles among isolates. Assessing ZIKV urogenital tract tropism and elucidating viral persistence strategies will help develop appropriate treatments or prophylactics to prevent sexual transmission.

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A MULTI-COUNTRY EVALUATION OF DIAGNOSTIC ASSAYS FOR ZIKA INFECTIONS AND ACUTE FEBRILE ILLNESS

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Acute febrile illness (AFI) can be caused by a wide variety of viral, bacterial, or parasitic infections and is an important cause of morbidity and mortality worldwide. The frequency and underlying etiology of AFI varies geographically as well as seasonally and can be influenced by a number of factors including the use of vaccines, environmental or land use changes, urbanization and economic development. We initiated a study combining enhanced case finding, Zika virus testing, and evaluation of multiple diagnostic assays through surveillance platforms in nine countries. The information collected is being used to evaluate the utility of a TaqMan Array Card (TAC) designed to detect 31 viral, bacterial and parasitic pathogens associated with AFI. Individual targets on the TAC assay are being compared with either, an individual Zika PCR assay, or the CDC Trioplex (Zika, dengue, chikungunya) PCR assay as the standard of practice. In addition, a ChemBio Dual Path Platform (DPP) Zika/chikungunya/dengue IgM/IgG lateral flow assay is being evaluated against several ELISA assays designed to identify Zika virus infections. This study is being conducted in Guatemala, Haiti, Peru, Egypt, Kenya, China, India, and Thailand. Each country will enroll 700 patients with some countries also enrolling asymptomatic controls. Preliminary data from 5 countries indicate that the most frequently detected pathogens by TAC are dengue virus, *Plasmodium* spp., *Orientia tustsugamushi*, *Coxiella burnettii*, *Rickettsia* spp., *Salmonella* spp., and *Brucella* spp., in descending order. In addition, the lateral flow serologic assay data suggest considerable IgG cross-reactivity between Zika and dengue antigens. However, this cross-reactivity is lower with the IgM assay and may indicate an increased predictive value. This study will identify priority pathogens associated with AFI in regions of the Americas, Africa, and Asia and will provide insight regarding the utility of diagnostics for the detection of Zika virus and other etiologies of AFI.

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ZIKA VIRUS IN LONG-TAILED MACAQUES, PENINSULAR MALAYSIA

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Zika virus (ZIKV) has reemerged from Asia to cause recent unprecedented outbreaks in the Pacific and the Americas. Non-human primates (NHPs) are suspected to be animal reservoirs of ZIKV in Africa, but little is known of their role in maintaining a sylvatic cycle in Asia. We evaluated ZIKV prevalence in long-tailed macaques (*Macaca fascicularis*), the most common macaque species in Peninsular Malaysia. Serum samples were collected from 234 long-tailed macaques trapped at > 30 sites in the states of Selangor, Negeri Sembilan, Perak, Pahang, Penang and Johor. These comprised 145 samples collected in October - November 2009 and October 2010, and 89 collected in March and August 2016, which coincided with the ZIKV global epidemics, giving a total of 234 samples. None of the samples were positive for ZIKV RNA. Samples were tested for ZIKV neutralizing antibodies by plaque reduction neutralization test (PRNT) on Vero cells. Those with screening ZIKV $PRNT_{50}$ titers ≥20 were confirmed with Zika focus reduction neutralization test (FRNT), and also tested for potentially cross-reacting dengue virus serotype 1 and 2 antibodies by FRNT. A total of 6/234 (2.6%) had ZIKV neutralizing antibodies, and 3 of these had no detectable dengue antibodies, giving a final ZIKV seroprevalence of at least 3/234 (1.3%). Wild long-tailed macaques are exposed to ZIKV, but the low prevalence suggests that they are unlikely to be significant animal reservoirs in Malaysia, with the caveat that the long-term dynamics of ZIKV antibodies and infection in macaques is not known. This reinforces the need for study of other NHPs and mammals as reservoirs of ZIKV in Malaysia, to understand its transmission and mitigate future emergence.

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GENETIC DIVERSITY OF THE YOKOSE VIRUS, XYBX1332, ISOLATED FROM BATS (*MYOTIS DAUBENTONII*) IN CHINA

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A virus (XYBX1332) was isolated from serum specimens of *Myotis daubentonii* (order Chiroptera, family Vespertilionidae) collected in a cave located in the south of China. The virus was shown to have cytopathic effects in mammalian cells (BHK-21 and Vero E6). Genome sequencing indicated that it has a single open reading frame (ORF), with a genome of 10,785 nucleotides in total. Phylogenetic analysis of the viral genome suggests that XYBX1332 is a Yokose virus (YOKV) of the genus *Flavivirus*. Nucleotide and amino acid homology levels of the ORF of XYBX1332 and Oita-36, the original strain of YOKV, were 72% and 82%, respectively. The ORFs of XYBX1332 and Oita-36 encode 3422 and 3425 amino acids, respectively. In addition, the non-coding regions (5′- and 3′-untranslated regions [UTRs]) of these two strains differ in length and the homology of the 5′- and 3′-UTRs was 81.5% and 78.3%, respectively. Serological detection revealed that XYBX1332 exhibits a neutralizing antibody cross-reaction with Japanese encephalitis virus (P3), but not with yellow fever virus (YFV-17D). The isolation of YOKV (XYBX1332) from inland China thousands of kilometers from Yokosuka, Japan, suggests that the geographical distribution of YOKV is not limited to the Pacific islands and that it can also exist in the inland areas of Asia. However, there are large differences between the Chinese and Japanese YOKV strains, in both viral genome and antigenicity.

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MAPPING ZOONOTIC SPILLOVER AND URBAN TRANSMISSION TO ESTIMATE YELLOW FEVER VACCINATION IMPACT

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Although there is an effective vaccine for yellow fever (YF), there is growing concern about the potential for large urban outbreaks due to increased urban growth in close proximity to zoonotic transmission and continued expansion of the *Aedes aegypti* mosquito that vectors YF virus (YFV). Efforts to inform YF vaccination through transmission mapping have not distinguished between transmission with zoonotic versus urban origins. We developed a probabilistic framework for disentangling the contributions of these distinct transmission cycles to YF incidence, fitting a combined model of zoonotic spillover and urban transmission to 1,134 YF cases from South America in 2000-2014. Calibrating our model under the assumption that 26.4% (95% CI: 23.0-30.1) of these cases were urban based on their occupations, we estimated rates of zoonotic spillover that, compared to a baseline with no allowance for urban transmission, were lower in areas estimated to have high potential for urban transmission. Disentangling zoonotic and urban components of transmission enabled us to calculate location-specific probabilities of a large urban outbreak, which vaccination reduced by 72.33% (95% CI: 20.86-99.97) on average under three different scenarios about vaccination coverage.

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THE ZIKA VIRUS INFECTION IN PREGNANT WOMEN IN HONDURAS (ZIPH) COHORT STUDY

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The full spectrum of long-term complications of Zika virus (ZIKV) infection has not been thoroughly described, especially among children without clinical evidence of congenital Zika syndrome at birth, and among children born to ZIKV infected but asymptomatic mothers. We are currently conducting a prospective pregnancy cohort study in Honduras that will allow us to study ZIKV infection in a different context than South American countries. Enrollment in the cohort is ongoing in one health center. Women are enrolled at their first prenatal visit. Data on socio-demographics, ZIKV symptoms during pregnancy, and information to locate women for additional follow-up are collected through interview at enrollment. Maternal blood is collected immediately after enrollment and tested for ZIKV immunoglobulin M (IgM) by enzyme-linked immunosorbent assay (InBios, Seattle, WA). We plan to conduct ZIKV and dengue virus polymerase chain reaction testing. We expect more than 90% of the women to deliver in one of two public hospitals in Tegucigalpa. Longitudinal follow-up will be conducted for children of women with positive ZIKV IgM and a comparison group of children born to women with no evidence of ZIKV infection at enrollment. Neurodevelopment will be assessed with Bayley Scales of Infant and Toddler Development, 3rd edition. From July 2016 to February 2018, we have enrolled 2,143 women at their first prenatal visit. Gestational age at enrollment was <14 weeks for 56.9% of the cohort, 14-28 weeks for 25.2%, and > 28 weeks for 17.9%. Thirty-seven women (1.7%) were symptomatic at enrollment. About half of the enrolled participants have already delivered. Analyses of birth outcome data are ongoing. This study will allow us to better understand the longer-term outcomes of children exposed to ZIKV during pregnancy.

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GLOBAL RISK ASSESSMENT OF TRAVEL-RELATED YELLOW FEVER SPREAD: A SYSTEMATIC REVIEW

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Yellow fever (YF) virus is a flavivirus endemic to sub-Saharan Africa and tropical South America. However, due to a rapid increase in trade and travel volume globally, there is an increased risk of importation of YF to non-endemic areas. We systematically review data from travel-associated YF to help estimate the international risk of YF spread into non-endemic areas. PubMed, ProMed, and online repositories from the European Centre for Disease Prevention and Control and the World Health Organization databases were searched from inception to March 2018. We included all reports describing travel-related YF acquired in endemic countries. Variables of interest were extracted and plotted in Tableau software. We identified 497 unique publications and included 25. Between 1924 and 2018, 38 travel-associated YF cases have been reported. European and non-European-travelers accounted for 16 (42.1%) and 22 (57.9%) of the subjects, respectively. The countries that reported the most cases of imported YF cases were China (28.9%), USA (13.2%) and France (13.2%). Forty-two percent of the importation countries had competent YF vectors, but autochthonous YF transmission in the non-endemic area did not occur. Travelers acquired the disease in South America (56.8%) and Africa (42.2%). Brazil (38.9%), Angola (30.6%), Peru (8.3%), and Senegal (5.6%) were the countries most visited among travelers where YF was acquired. The traveler's mean age was 39.4 ± 12.4 years, and males predominated (76.7%). All except two (5.3%) were unvaccinated. YF was diagnosed with molecular assays (48.1%), a combination of assays (37%), and serology (7.4%). Hospitalization data was available for 32 (84.2%) cases, and of those 31 (96.9%) were admitted and 7 (58.3%) went to ICU care. Fifteen (48.4%) patients died from their illness. In conclusion, non-immunized travelers are at-risk of YF complications and capable of seeding local transmission in their countries. There is an urgent need to reinforce the implementation of YF vaccination in travelers visiting high-risk areas.

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AEDES AEGYPTI SALIVA PROTEIN INTERACTIONS WITH ZIKA VIRUS AND THEIR ROLE IN MAMMALIAN INFECTION

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Aedes aegypti is the primary vector of several flaviviruses including Zika virus. Zika virus-mediated vertical transmission and its association with nervous system disorders like microcephaly in infants prompted the World Health Organization to declare this correlation and potential complication a public health emergency. Zika virus is usually transmitted to human beings in saliva injected by the female *A. aegypti* during blood feeding. The saliva of mosquitoes represents a repertoire of factors including proteins, miRNAs and other biomolecules that are critical in establishing infection of arboviruses along with modulation of host homeostasis and immune response. In this study, we have collected the saliva form sugar and blood fed mosquitoes, performed binding assays with Zika virus and used liquid chromatography with mass spectrometry (LC+MS/MS) to identify interacting partners. Subsequent analysis and experimentation were done to elucidate the role of these mosquito saliva proteins during transmission and infection of Zika virus to mammalian cells. In addition, though studies have been carried out to highlight importance of mosquito salivary proteins, the limitation in most of those studies is the use of salivary gland extracts (SGE) for analysis rather than saliva. Our analysis allows for identification of unique peptides that belong to different functional groups in mosquito saliva. Furthermore, we have fractionated the saliva using reverse phase liquid chromatography and checked the effects of different saliva fractions on Zika virus infection and replication in mammalian cells. The outcome of this study informs the field on *A. aegypti* secretory proteins and an insight into their role in the infection of Zika virus.

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NEURODEVELOPMENTAL DELAYS ARISING FROM *IN UTERO* EXPOSURE TO ZIKA VIRUS IN SALVADOR, BRAZIL

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Since 2015, Brazil has experienced an unprecedented Zika virus outbreak. A devastating consequence of this viral infection is congenital Zika infection (CZI), which is transmitted from pregnant women to newborns. Most descriptions and publications regarding CZI focus on the clinical presentation of newborns and infants with microcephaly. Scarce information is available concerning children without microcephaly born from infected mothers. During 2016, a cross-sectional study enrolled 147 pregnant women who reported an exanthematous disease during the pregnancy. Of these, 101 (68.7%) presented anti-Zika IgG antibodies at the time of delivery. A total of 25 (17%) newborns were diagnosed with microcephaly, while and 122 (83%) were classified as newborns without microcephaly, of these, 91 had positive Zika serology or Zika RT-PCR. In June 2017, we began a prospective follow-up of these infants without microcephaly by evaluating neurodevelopment delays, performing neurological examinations and applying the Bayley Scales of Infant Development III (BSID-III). Auditory evaluations were performed with by Otoacustic emissions (OAE) and Brainstem Auditory Evoked Potential (BAEP). To date, we have evaluated 26 infants. Of these, 53.8% are male and the mean age is 1.7 years. Anti-Zika IgG serology was positive in 77% and five (19.2%) presented positivity for Zika by PCR on samples within 24h of birth. Based on head circumference (HC) at the time of birth, all were classified as normal by the Intergrowth scale and currently fall within normal HC percentiles. Cognitive delay was identified in 8 (31%) infants, language delay in 11 (42.3%) and motor delay in three (11.5%). Our preliminary results indicate that *in utero* exposure to Zika virus could be associated with neurodevelopmental delay, even in children born without microcephaly. Currently, only microcephalic newborns are referred to specialized care, while normocephalic children are maintained in primary health care. We believe that all newborns exposed to Zika *in utero* should be referred to specialized centers for the early detection of neurodevelopmental delays and timely intervention.

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SERIAL PASSAGING OF ZIKA VIRUS RESULTS IN ENHANCED REPLICATION IN *CULEX* CELLS

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Zika virus (ZIKV) is a flavivirus that is mainly transmitted by *Aedes aegypti* mosquitoes, which are largely anthropophilic. However, the recent ZIKV outbreak in the Americas has stimulated discussion about the role of other mosquito species in transmission. Other Aedes sp. mosquitoes, such as *Ae. albopictus* and *Ae. vexans* are also competent vectors. The role of *Culex* spp. mosquitoes in ZIKV transmission, however, remains controversial. We previously found that the Puerto Rican ZIKV isolate (PRVABC59) does not efficiently infect colonized *Cx. quinquefasciatus*, *Cx. pipiens* or *Cx. tarsalis* mosquitoes, yet other studies have suggested that *Cx.* spp. mosquitoes may be competent vectors. ZIKV infection of *Culex* mosquitoes may thus depend on the specific mosquito population, the virus isolate or other complex environmental factors. In this study, we aimed to understand whether specific adaptive mutations allow ZIKV to infect and replicate to higher levels in *Cx. tarsalis* cells. We co-cultured susceptible *Ae. aegypti* cells (Aag2) and somewhat refractory *Cx. tarsalis* cells (Chao Ball) at a ratio of 9:1 and infected the triplicate co-cultures with ZIKV (PRVABC59) at MOI 1. After 6 days, we collected supernatant and used 500µl to infect new co-cultures (retaining triplicate passage lineages). We passaged the virus 18 times on slowly increasing proportions of Chao Ball cells, up to a ratio of 1:9 (Aag2:Chao Ball). We also passaged ZIKV three times on Chao Ball cells without co-culture. We then established growth of the passaged viruses on Chao Ball cells and Aag2 cells. We found that passaging enabled ZIKV to replicate to higher levels in Chao Ball cells compared to our input stock. We sequenced the virus passages and identified mutations associated with passaging. In addition, we propagated the passaged ZIKV on Vero cells and determined vector competence of *Culex* spp. mosquitoes to this stock. In conclusion, we have characterized mutations in ZIKV associated with passaging in *Culex* cells and how they impact infectivity of *Culex* mosquitoes.

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HUMAN-ANIMAL INTERFACE AND VECTOR SURVEILLANCE IN ZIKA VIRUS PRONE TRANSMISSION AREAS IN BRAZIL

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Zika virus (ZIKV) was first discovered in 1947 in Uganda, but was not considered a public health threat until 2007 when it was found to be the cause of outbreaks in Asia. ZIKV spread to Brazil in 2014, and continues to spread in tropical and subtropical regions of the Americas, where *Aedes aegypti* mosquitoes are abundant. Despite being a zoonotic disease, there is little information about potential sylvatic vectors and animal amplifying hosts nor their role in virus maintenance and transmission. The role of other mosquito species as ZIKV vector, is also unclear. Recent evidence also suggests sylvatic non-human primates have also been exposed to ZIKV in the Americas. We conducted active surveillance for ZIKV among native primates, other non-human vertebrates, and mosquitoes in established field sites, in Mato Grosso (MT) and Mato Grosso do Sul (MS) states in the West-Central region of Brazil, where recent or active ZIKV transmission in humans has been documented. We collected samples during 4 periods from March 2017 to March 2018. Trapping efforts focused on wildlife, mosquitoes, and domestic animals from urban parks and surroundings areas of Cuiabá (MT) and Campo Grande (MS). We collected samples from more than 2000 vertebrates and 12.000 mosquitoes. Whole blood and mosquito samples are being screened for ZIKV RNA, by generic flavivirus real time RT-PCR, and will be confirmed by a ZIKV specific RT-PCR. Plasma samples are being screened and will be confirmed for presence of anti-ZIKV antibodies by neutralization tests. Preliminary serological testing results show that from 759 animal plasma tested, 68 (9%) neutralized ZIKV (ES2916/2015) isolated from human in Espírito Santo state, Brazil. The results of this study will lead to a better understanding of ZIKV's ability to establish a sylvatic cycle outside of human transmission, thus providing essential information for future surveillance and public health intervention efforts.

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GENETIC ANALYSIS OF ZIKA VIRUS INFECTIONS FROM AUTOCHTHONOUS TRANSMISSION IN THE PHILIPPINES, 2016-2017

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Zika virus is an emerging arbovirus known to circulate in Africa and South East Asia. In 2015, it was detected in Brazil causing serious birth defects in infants born to infected mothers. This prompted the World Health Organization to declare Zika virus disease as a global public health emergency. In the Philippines, genetic analysis of Zika virus was limited only in the isolated Zika case in 2012. This report presents a comprehensive phylogenetic analysis of Philippine strains after the outbreak in Brazil. A surveillance was initiated in 2016. Serum and urine samples from patients with fever and/or rash were sent to the Research Institute for Tropical Medicine to detect Zika by Real-time RT-PCR and antibody testing. The envelope (1,512nt) gene was amplified by two-step RT-PCR, followed by direct Sanger sequencing. Maximum likelihood phylogenetic analysis was done in MEGA 6 using the General Time Reversible +G model with 1,000 bootstrap replicates. A total of 3,365 samples were tested with Zika RNA detected in 60 (1.78%) samples, of which 21 samples (35%) were sequenced. All Philippine isolates were clustered into the Asian genotype with high similarity to the 2007 Micronesia strain. Likewise, the current Philippine strains have high homology with the reported Zika case in 2012. Analysis of the E gene revealed presence of F279S substitution that might cause virus resistance to neutralizing antibody. Phylogenetic analysis suggests that Zika virus may have been circulating in the country but remained underreported despite the abundance of competent vectors. The data from our study strengthen the hypothesis that Zika virus infections in the country result from autochthonous transmission. Sequencing analysis of Philippine isolates with reference sequence and 4 Brazilian sequences showed that both strains have same F279S substitution. Our findings improved the knowledge on Zika virus strain in the country. The high homology of Philippine sequences with the Micronesia strain that caused outbreaks in the past and the detection of F279S substitution warrant the need for intensifying surveillance and control measures to prevent virus transmission.

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OPTIMIZATION OF QUANTITATIVE RT-PCR PROTOCOL FOR ZIKA VIRUS DETECTION IN LOW VIRAL SAMPLE CONCENTRATIONS

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The 2015 Zika virus (ZIKV) outbreak in Brazil generated increased demand for sensitive molecular diagnostics to detect the presence of ZIKV in various patients. Most of the ZIKV diagnostics to date have been carried out using two qRT-PCR assays developed by Faye et al. and by Lanciotti et al. specifically for this purpose. These protocols use primer sets that target different regions of the ZIKV genome with enough specificity to use them as diagnostic tools. However, the sensitivity of both assays is lower than would be ideal, and in many cases ZIKV cannot be correctly diagnosed if present at low levels (CT values of up to 30). We have developed an improved protocol using optimized primer sets that can detect ZIKV at previously "undiagnosable" low levels (CT values as high as 34). Our efforts focused on the optimization of important primer parameters like primer length, annealing temperature, and GC content. Primers were tested using ZIKV samples of various concentrations and we show that samples that were previously considered false-negatives can give a positive result using our optimized conditions. This assay should address the issue of increased false-negative results during ZIKV diagnostics, and will be a useful tool for healthcare providers interested in a fast, rapid, and sensitive assay for clinical evaluation and diagnostics of ZIKV in samples of low viral concentrations.

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THE EFFECTS OF MOSQUITO MICROBIOTA ON ZIKV INFECTION AND TRANSMISSION IN *AEDES AEGYPTI*

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The microbiome of mosquitoes is known to impact their susceptibility to infection and their ability to transmit viral pathogens. *Aedes aegypti* mosquitoes are competent vectors of several viruses including dengue virus and Zika virus (ZIKV). Previous work has shown that specific bacteria isolated from mosquito vectors have the potential to reduce their susceptibility to infection by dengue virus through activation of host immune genes. Our studies aim to determine whether the resident microbiome of *A. aegypti* mosquitoes also impacted transmissibility of ZIKV, a medically important virus that has been linked to severe congenital syndromes including microcephaly in newborns and Guillain-Barre syndrome in adults. To test our hypothesis, we cleared the microbiome of lab reared *A. aegypti* mosquitoes collected from the Rio Grande Valley with oral antibiotic treatments and challenged with an American ZIKV strain (PRV-ABC059) after confirming the reduction of resident microbiome. We then compared viral infectivity, dissemination and transmissibility rates with untreated cohorts. Culture dependent and independent approaches were used to quantify the microbial load in antibiotic treated and untreated mosquitoes. In addition we screened a panel of immune-regulatory genes

by qRT-PCR to assess differences in the immune activation status between both groups. Vector competence for emerging and re-emerging viruses is shaped in part by a combination of environmental, genetic and microbial factors. Characterizing the influence of microbiota on ZIKV infection and transmission by its primary mosquito vector and understanding its ability to influence disease dynamics in the America's is critical for determining whether microbes may be harnessed to control the disease's potential spread.

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CAN THE HOST DENSITY RESCUE THE VIRAL TRANSMISSION? EFFECTS OF THE AVIAN-MOSQUITO COMMUNITIES ON THE ST. LOUIS ENCEPHALITIS VIRUS ACTIVITY

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Arbovirus represents an ecological clustered of arthropod borne viruses. They depend of the interaction among vectors and hosts, and those might be driven by the variations at community, population and individuals vector-host level. Dilution effects predicts an scenario, where the transmission risk, on multi-host systems, is reduced as host diversity increases. Here, we focused on the effects of the structure and composition of the avian-mosquito community on the activity of St. Louis encephalitis virus (SLEV, Flavivirus). Avian and mosquito communities and the frequency of SLEV infection in *Culex* mosquitoes were prospected during three transmission seasons across the periurban-urban landscape in Cordoba city, central area of Argentina. Hypothesis related at community structure and density avian-mosquito level were represented and analyzed by general linear mixed models, when environmental and temporal viral activity heterogeneity were explicitly considered. Then, models performance were ranked following the Information Theory framework. The presence and the transmission of SLEV were not explained by neither of the diversity measures explored. Although, Eared Dove and mosquito density were related with at least two-folds increases in the number of SLEV infected *Culex.* Dilution effect exerted by biodiversity might not be driving the SLEV activity in the Cordoba city, meanwhile viral trait as the generalist host use could provide resilience to transmission when the composition of host and vector change. Finally, the results support an amplification effect by the density of particular community members on the transmission and amplification of SLEV such as Eared Dove.

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IN VITRO ACTIVITY OF A 2'-C-METHYLURIDINE PROTIDE AGAINST THE ZIKA VIRUS

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The Zika virus (ZIKV) has been demonstrated to cause the development of microcephaly in newborns and has been linked to Guillain-Barré syndrome in adults; ZIKV remains a significant arboviral threat to world health, as there are no approved treatments for the infection and a new outbreak is likely to happen in the future. Recent reports have shown that sofosbuvir, an FDA-approved nucleoside analogue ProTide used in the treatment of Hepatitis C virus (HCV) infections, has antiviral activity against ZIKV. In this work, we show that the related ProTide of 2'-C-methyluridine has stronger activity (4.2 to 8.5 fold) than sofosbuvir in both neural stem cell and glioblastoma stem cell models of ZIKV infection using phenotypic and cell-viability assays, with selectivity indexes ranging from >4 to 24 for the ProTide of 2'-C-methyluridine for the different assays. Cell imaging reveals that the 2'-C-methyluridine ProTide strongly protects against the virus-induced cytopathic effect (CPE). Using biochemical gel-based assays, we demonstrate that while both the triphosphate form of sofosbuvir and that of 2'-C-methyluridine can act as substrates for the ZIKV NS5 RNA-dependent RNA polymerase, 2'-C-methyluridine triphosphate is better incorporated by the enzyme into RNA under identical conditions. These data suggest that ProTides of 2'-C-methyluridine may represent a new avenue for the development of potent anti-ZIKV compounds.

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DEVELOPMENT OF A LIVE-ATTENUATED DENV-2-BASED CHIMERIC VACCINE AGAINST ZIKA VIRUS

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The development of a safe and effective Zika virus (ZIKV) vaccine has become a high priority since the widespread epidemic in 2015-16. Here we report the development of a live-attenuated vaccine candidate that invokes a robust immune response and provides full protection against lethal ZIKV challenge in mice following a single immunization. Utilizing the same Dengue virus 2 (DENV-2) PDK-53 vaccine platform that has been used to develop a tetravalent dengue vaccine candidate that is currently in phase 3 trials, we introduced the PrM-E genes of ZIKV to generate chimeric D2/ZK vaccine candidates. In addition, we identified and introduced multiple mutations in the chimeric constructs to improve the fitness and stability of the viruses for efficient growth in Vero cells. We systematically incorporated different combinations of the mutations and have recovered virus from 7 versions of the constructs. Each version was also made in the wild type DENV-2 16681 (parental virus for the PDK-53) backbone for phenotypic comparison. Based on previously established attenuation phenotypes of the DENV-2 PDK-53 vaccine, these chimeric D2/ZK vaccine candidates will be evaluated for plaque phenotype, temperature sensitivity, replication in C6/36 cells, neurovirulence in neonatal mice, and infection/dissemination of mosquitoes. Currently, we have identified multiple chimeric D2/ZK vaccine candidates that were fully attenuated in neonatal mice by direct intracranial inoculation. AG129 mice immunized with one or two doses of the candidates produced high levels of neutralizing antibodies to ZIKV, and were fully protected against lethal ZIKV challenge without any detectable post challenge viremia. In addition, neither a second vaccination nor the wt ZIKV challenge resulted in any further increase in antibody titers acquired after primary immunization, suggesting a single dose was sufficient to induce sterilizing immunity against a lethal ZIKV challenge in AG129 mice.

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OVERVIEW OF ZIKA EN EMBARAZADAS Y NIÑOS EN COLOMBIA (ZEN): A PROSPECTIVE COHORT STUDY EXAMINING ZIKA VIRUS INFECTION DURING PREGNANCY AND RISK OF ADVERSE PREGNANCY, BIRTH, AND INFANT OUTCOMES

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Since its first case of Zika virus (ZIKV) infection in October 2015, Colombia has reported over 106,000 suspected ZIKV infection cases, including 20,000 pregnant women. ZIKV infection during pregnancy can cause

serious fetal harm, including microcephaly and related brain anomalies. Knowledge gaps remain about the risk associated with ZIKV infection for adverse pregnancy, birth, and infant outcomes. The Colombian INS and U.S. CDC are implementing *Zika en Embarazadas y Niños en Colombia* (ZEN), a prospective cohort study of 1,500 pregnant women, male partners, and their infants in multiple Colombian cities. Pregnant women were enrolled in the first trimester of pregnancy and followed until infants are 6 months old; male partners will be followed through the end of their partner's second trimester. ZEN objectives are: 1) describe sociodemographic and clinical characteristics of the population, 2) identify risk factors for ZIKV infection in pregnant women and their infants, 3) assess the risk for adverse maternal, fetal, and infant outcomes associated with ZIKV infection, and 4) assess modifiers of the risk for adverse outcomes among pregnant women and infants following ZIKV infection. To identify critical windows of infection for adverse outcomes and monitor persistence of ZIKV, sequential ZIKV testing of serum or urine with reverse transcription-polymerase chain reaction (rRT-PCR) will be performed for pregnant women (biweekly until 34 weeks gestation, and monthly until pregnancy end), male partners (monthly until their partner's 27th week of gestation) and infants (biweekly until 6 months of age). Participants are interviewed using structured questionnaires about risk factors for ZIKV infection, symptoms of ZIKV infection, and risk factors for adverse outcomes. Clinical information will be abstracted from medical records of pregnant women and their infants. ZEN enrollment occurred between February 9, 2017 and January 31, 2018. ZEN results will guide recommendations for preventing ZIKV infection, improve counselling of patients about the risks of ZIKV, and help agencies to provide services to affected children and their families.

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SURVEILLANCE OF MICROCEPHALY AND OTHER CONGENITAL CENTRAL NERVOUS SYSTEM MALFORMATIONS: THE COLOMBIAN EXPERIENCE DURING THE 2015-2016 ZIKA VIRUS EPIDEMIC

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The Colombian Instituto Nacional de Salud (INS) has conducted national surveillance of birth defects as part of its SIVIGILA surveillance system. During the 2015-2016 Zika virus (ZIKV) outbreak, INS established an enhanced surveillance protocol to monitor potential increases in microcephaly and other congenital central nervous system (CNS) malformations, to investigate causes, and to direct appropriate follow-up care. SIVIGILA received notification from physicians if microcephaly (head circumference below the 3rd percentile expected for sex and gestational age) or other CNS malformations were diagnosed prenatally. The surveillance protocol required an inpatient neonatal evaluation soon after delivery, including a complete physical and neurological exam, and a head ultrasound. If microcephaly or other CNS malformations were noted, further evaluation was recommended by the protocol. Cases were classified into etiological categories: congenital ZIKV infection, other infectious agents, genetic, multifactorial, additive, and unknown; this classification was based on laboratory evidence and extensive review of the clinical findings from medical records by qualified clinicians. Data reported here are from the implementation of the enhanced protocol to births in 2016. During 2016, SIVIGILA was notified of over 900 cases of microcephaly and other CNS malformations. Based on preliminary analysis, these numbers represent about a 40% increase compared to 2015. Case classification is ongoing. Work is ongoing to review all cases of microcephaly and other congenital CNS malformations reported for 2017. This enhanced surveillance protocol, built on an already existing surveillance platform, serves as a model of a comprehensive approach to investigating complex congenital conditions of unknown etiology and building capacity to detect emerging health public health threats. Adequate identification, notification and classification is essential in order to quantify the number of Zika-associated birth defects, and follow-up care is essential to fully characterize the impact of congenital ZIKV infection during pregnancy.

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A COMMUNITY INVESTIGATION OF THE YELLOW FEVER OUTBREAK IN SOUTH OMO ZONE, ETHIOPIA, 2012-2014

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Yellow Fever (YF) remains an important public health problem across Africa and South America due to its high case-fatality rate. A YF outbreak occurred in South Omo Region, Ethiopia in 2012-2014. This study aimed to analyse historical outbreak data and identify the local *Aedes* vector species, to assess the risk for future YF transmission. From October 2012 to March 2014, 165 cases and 62 deaths were reported, principally in the rural areas of South Ari region (83.6%), south-west Ethiopia. The overall male to female case ratio was 1.4:1. The majority of patients were 15-44 years old (74.5%) and most case deaths were males (76%). In 2014, the Ministry of Health organised a reactive vaccination campaign with a reported coverage of 89%. Between June and August 2017, 688 containers were sampled from across 177 households to identify the key breeding sites for *Aedes* mosquitoes. *Ensete ventricosum* ("false banana") was identified as the primary natural breeding site, and clay pots outside the home as the most productive artificial breeding site. Entomological risk indices from the majority of sites were classified as "high risk" for future outbreaks under current WHO criteria. Of the five villages surveyed, Shepi village had the highest traditional indices with House Index (HI) – 79; Container Index (CI) – 57.9; and Breteau Index (BI) – 237.2. Adult trapping resulted in the identification of members of the *Aedes simpsoni* complex. Molecular arbovirus testing of adult vector specimens found no active presence of YF or other arboviruses. In addition, a Knowledge, Attitude and Practices (KAP) survey was conducted to understand local knowledge levels following this re-emergence of YF. 87.8% of 177 participants had heard of YF, however many participants easily confused transmission and symptoms of YF with malaria, which is also endemic in the area. Significant predictors for KAP score included household location and prior vaccination history. Study results emphasis the need to strengthen local disease surveillance systems and in-country laboratory capacity to facilitate rapid responses to future outbreaks.

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LOW ZIKA VIRUS SEROPREVALENCE IN KUALA LUMPUR, MALAYSIA

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Zika virus (ZIKV) is an arbovirus that recently caused large epidemics in the Pacific and the Americas. ZIKV was first isolated outside Africa in Malaysian *Aedes aegypti* in 1966. It is unclear why only sporadic cases and a single outbreak (Singapore, 2016) have been reported in Southeast Asia, despite likely endemicity of ZIKV, abundance of *Ae. aegypti*, and recent heightened awareness. We examined if undetected endemic transmission results in high levels of population immunity. We used a Zika NS1 blockade-of-binding (BOB) assay to test 1,086 serum samples

collected in a hospital in Kuala Lumpur, Malaysia, comprising 727 residual diagnostic samples from inpatients and 359 samples from healthy blood donors. Samples were collected in 2012 (before the Pacific outbreaks), August 2014-March 2015 (early stages of the Americas epidemic), and 2017 (after the outbreak in Singapore). Approximately 30 samples were collected for each 10-year age group in each time period from the two populations studied (total n=91-154 per age group). A total of 76/1,086 (7.0%) samples had detectable anti-ZIKV antibodies following duplicate testing. No significant difference in seropositivity between diagnostic and blood bank samples was found; thus, the two populations were combined for further analysis. Seropositivity was not associated with gender or year of collection but was positively correlated with age (odds ratio 1.21 (95% CI 1.08-1.32) for each decade of age, p=0.001). Study limitations include the possibility of cross-reactivity due to other flaviviral infections (although this would lower the ZIKV seroprevalence rate below the 7.0% observed) and possible waning of antibody levels over time, though other studies support stable levels of anti-ZIKV antibodies detected by the Zika NS1 BOB assay over several years. Neutralization assays for DENV1-4 and ZIKV are being performed to confirm the seropositive samples. We conclude that there is low ZIKV seroprevalence in Kuala Lumpur, which thus does not explain the low incidence of Zika cases and outbreaks. Other factors, such as the possible protective effects of prior dengue virus infection, should be explored.

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FACTORS AFFECTING YIELD DURING THE PRODUCTION OF PURIFIED ZIKA VIRUS

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Optimization of virus purification methods is essential for obtaining good virus recovery and ensuring the removal of impurities that may interfere with downstream applications such as vaccine production, diagnostic assay standardization, sequencing, and electron microscopy. While it is well known that loss in virus titer takes place in any purification method, the total virus yield after purification is rarely described, and few studies evaluate the individual steps involved in purification for their capacity to retain infectious virus particles. The multi-step process needed to produce purified virus preparations makes it imperative to examine where virus loss could occur at each step. Here, we describe a method for improving the recovery of infectious Zika virus by determining the optimal conditions for harvest, clarification, precipitation, and purification. For this study, Zika virus was propagated in Vero cells (ATCC® CRL-1586™) under established optimal growth conditions to prevent the formation of defective interfering particles. For harvest, mechanical scraping and pooling the infected monolayer and culture supernatant was compared to freeze-thaw of infected cell pellets, and filter clarification and spin clarification methods were assessed. Finally, precipitation with 10% PEG 6000 or PEG 8000 was evaluated using various treatment methods prior to purification over a sucrose cushion. Sample aliquots were retained after each step in the process and were titrated to quantify the yield of infectious virus particles. The results from these experiments demonstrated that the most important step affecting virus titer was the harvest method. The resulting purified Zika virus from this study is available from BEI Resources as catalog number NR-50684.

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DENGUE VIRUS CROSS-NEUTRALIZING ANTIBODIES AGAINST ZIKA VIRUS INFECTION

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Dengue virus (DENV) and zika virus (ZIKV) are members of the mosquito-borne flaviviridae family that co-circulate in the same regions and impact public health worldwide. The closely related structural proteins, up to 40-50% of amino acid sequence homology between DENV and ZIKV, allow cross-neutralization and infectionenhancement. In this study, the cross-neutralization of DENV antibody against ZIKV infection has been investigated. DENV confirmed cases by serotype (DENV-1 to -4) and immune status (primary and secondary) have been selected (n=5 per group). The cross-neutralization of DENV antibodies against ZIKV infection was done by a flow cytometry-based neutralization assay using human monocyte-derived U937 cells expressDC-SIGN. Cross-neutralization by DENV antibodies was observed in 100% of all secondary DENV and primary DENV-4 infections and 80% for other DENV serotype primary infections. We found that secondary DENV antibodies from secondary infections had significantly (p-value <0.05) higher titer of cross-neutralizing antibodies against ZIKV infection than antibodies from primary DENV infections. We further observed the neutralizing role of DENV IgM and IgG antibodies. It was found that IgM acts in a complimentary manner (p-value >0.05) with IgG against ZIKV infection. We noted that DENV IgG antibodies is the main cross-reactivity antibody for both primary and secondary DENV infections (p-value < 0.05). However, ZIKV neutralizing antibody titers were lower than the homologous DENV serotypes. The results from this study demonstrate the cross-neutralization of DENV antibodies using a flow cytometry-neutralization assay against ZIKV and highlight the need for advanced diagnostics to differentiate between the two different infections.

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THE CHANGING TREND OF JAPANESE ENCEPHALITIS VIRUS EPIDEMICS IN CHINA

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Background: Japanese encephalitis (JE) is very prevalent in China. Methods/ Principal Findings: Data on JE in mainland China were collected between 2004 and 2014. We conducted spatial and temporal analyses on data from different age groups. Generally, children aged 0-15 years still represent the major population of JE cases in China, despite the gradual decrease in incidence over years. However, the incidence of JE among adults in several provinces is not ably higher than the national average, especially during the epidemic waves in 2006, 2009 and 2013. Our results indicate that the total number of JE cases in 2013 was significantly higher compared with those in 2011 and 2012. The population aged over 40 years was associated with the greatest increase of JE. JE incidence in the adult group in September and October is significantly greater compared to the other groups. Further analysis using Local Indicators of Spatial Association reveals that the distribution of adult JE cases in the six provinces north of the Yangtze River, between north 30-35°latitude and east 110-130° longitude, is a hotspot for adult JE cases. By the new developed TaqMan Real-time RT-PCR detection system, a total of 3937 batches of mosquitoes collected in nineteen provinces of China between 2004-2016 were detected for Japanese Encephalitis Virus (JEV) and then differentiated for genotype. Two hundred and seventy-five batches were identified as genotype 1 and only one batch were genotype 5. Conclusions/Significance: Unlike the JE epidemics primarily in children below 15 years old in southern China,

a significant outbreak of JE occurred in northern China in 2013, with the older age groups being the primary population affected. Molecular epidemiology of JEV in mosquitoes in China during 2004-2014 discovered that genotype 1 were the predominated strain circulating in China. The increasing incidence of JE in adults has become an important public health issue and poses a new challenge to the successful prevention and control of JE in China as well as other countries in East Asia.

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A WORLDWIDE SURVEY OF *AEDES AEGYPTI* SUSCEPTIBILITY TO ZIKA VIRUS SHEDS LIGHT ON THE AFRICAN EXCEPTION TO ZIKA EMERGENCE

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Zika virus (ZIKV) is a mosquito-borne flavivirus mainly transmitted among humans through the bite of infected *Aedes aegypti*. After it was first isolated in Uganda in 1947, ZIKV was shown to circulate in enzootic sylvatic cycles in Africa and Asia but human infections remained sporadic for half a century. The first reported human epidemic caused by ZIKV occurred in 2007 on the Pacific island of Yap in the Federated States of Micronesia. Subsequently, larger ZIKV outbreaks were recorded in French Polynesia and other Southern Pacific islands in 2013-2014. In 2015, ZIKV reached Brazil from where it rapidly spread across South and Central America, infecting millions of people. The emergence of ZIKV caused significant public health concern because of the associated birth defects and neurological complications that have been observed since 2013. Until now, the factors that have fueled the explosiveness and magnitude of ZIKV emergence in the Pacific and the Americas are still largely unknown. Another unresolved question is the lack of major human epidemic of ZIKV in Africa despite seemingly favorable conditions. In order to evaluate the potential role of vector population diversity in the recent patterns of ZIKV emergence, we conducted the first worldwide survey of *Aedes aegypti* susceptibility for ZIKV in natural populations. We established dose-response curves for 8 field-derived mosquito populations spanning the entire geographical distribution of the species, following experimental exposure to 6 ZIKV strains representing the current extent of viral genetic diversity. Our data show that susceptibility to ZIKV infection varies substantially across mosquito populations, ZIKV strains, and their specific pairings. Most importantly, our results reveal that African *Ae. aegypti* are significantly less susceptible than non-African *Ae. aegypti* across all ZIKV strains tested. Thus, low susceptibility of vector populations may have contributed to prevent large-scale human transmission of ZIKV in Africa.

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DEVELOPMENT OF A LATERAL FLOW ASSAY (LFA) FOR SIMULTANEOUS DETECTION OF NOROVIRUS FROM GENOGROUPS I, II AND IV

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Infection with Norovirus (NoV) is a leading cause of acute gastroenteritis frequently associated with large outbreaks in nursing homes, cruise ships and other institutional facilities. On average 20 million cases of NoV infection are identified in the United States each year. NoV is highly contagious - transmission occurs by the fecal-oral route, mostly person to person, but NoV outbreaks may also be associated with contaminated food or water. The symptoms of nausea, vomiting, acute diarrhea and fever usually resolve within 2-3 days, with a median duration for the illness of 4-6 days. Currently the most sensitive NoV diagnostic tests are based on RT-PCR methodology. However, RT-PCR testing is expensive, requires specialized equipment, and its clinical specificity is often poor. There is thus an urgent need for an inexpensive, rapid, sensitive and specific test for NoV that can be applied in the field to immediately identify NoV as the cause of an outbreak allowing implementation of remedial steps at an early stage. NoV is classified into five genogroups, with most human infections caused by genogroups I, II and, to a lesser extent, IV. These genogroups are subdivided into more than 30 genotypes with genotype GII.4 responsible for the majority of norovirus infections globally. Our objective is to develop a sensitive and specific immunoassay-based LFA to simultaneously detect NoV from genogroups I, II and IV. Test lines were sprayed using a combination of genotype-specific monoclonal antibodies (mAbs) which sandwich NoV Virus Like Particles (VLPs) when used in conjunction with a second set of NoV-specific mAbs conjugated to colloidal gold. Human fecal samples are collected and diluted with running buffer for immediate analysis, or placed in a long-term storage buffer for off-site analysis. When fecal samples are spiked with NoV VLPs of genotypes I, II or IV and spotted onto the test strip positive identification of infection can be determined within 30-minutes with a sensitivity down to 5ng/mL NoV VLP. This is the only NoV LFA test available capable of simultaneous early detection of all three genotypes of NoV that infect humans.

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THE 2017 MONKEYPOX OUTBREAK IN NIGERIA -REPORT OF OUTBREAK EXPERIENCE AND RESPONSE IN THE NIGER DELTA UNIVERSITY TEACHING HOSPITAL, BAYELSA STATE, NIGERIA

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After 30 years, Nigeria experienced the first outbreak of human monkeypox beginning in September 2017. We hereby present the outbreak experience and response in the Niger Delta University Teaching Hospital (NDUTH), a tertiary hospital situated in South-South Nigeria where the first case and majority of cases of human monkeypox were treated. This was a mixed model study undertaken between 22nd September and 31st December 2017. We report the plans and activities of the monkeypox response team, the opinions and behaviour of staff during the outbreak and the management of suspected cases and false alarms at the hospital. On 22nd September 2017, an 11-year-old boy was admitted at the NDUTH with generalised papulopustular rashes suggestive of monkeypox. Hospital, state and national authorities were immediately notified. With the support and partnership of the Bayelsa State Ministry of Health and

the Nigerian Centre of Disease Control (NCDC), the hospital provided isolation facilities and resources, organised sensitization and training of staff and designated clinical teams for case management. At the onset of the outbreak, fear of being infected and worries concerning the level of outbreak preparedness of the hospital were reported among staff. There were reported absenteeism, avoidance of isolation facilities and reluctance to participate in case management. However, after repeated sensitization and training, the hospital was able to quickly mobilise a multi-disciplinary team for surveillance and case management. There were more than 18 false alarms and 26 suspected monkeypox cases (18 laboratory confirmed cases) seen at the hospital during the study period. Apart from a case of suicide, all patients made good recovery and were discharged. Our results suggest that outbreak response in hospitals is negatively influenced by fear and limited epidemic preparedness. It however emphasizes the place of proactive leadership, partnership, expertise, training and team work in containing infectious disease outbreaks.

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ZIKA VIRUS AND THE WORLD HEALTH ORGANIZATION CRITERIA FOR DETERMINING RECENT INFECTION USING PLAQUE REDUCTION NEUTRALIZATION TESTING

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The recent Zika virus (ZIKV) epidemic swept across Latin America and the Caribbean, where dengue virus (DENV) is endemic. The antigenic similarities of these closely related flaviviruses left researchers and clinicians with challenges to interpret serological tests. Thirty-six women attending a prenatal clinic in Honduras and with positive DENV IgM ELISAs were screened with a ZIKV IgM ELISA, RT-PCR for ZIKV and DENV 1 - 4, and plaque reduction neutralization tests (PRNT) for ZIKV and DENV-2. PRNT results were interpreted using the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) criteria. Using WHO criteria of a PRNT90 titer ≥ 20 and a four-fold difference between ZIKV and DENV titers, we determined that 83.3% of samples had a recent ZIKV infection, compared to 5.6% using CDC criteria. The interpretation of ZIKV PRNTs in a DENV endemic region is highly dependent on the choice of interpretation criteria.

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TOTIVIRUSES, PARASITES, AND EVERYTHING ELSE

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Totiviridae are unsegmented, icosahedral dsRNA viruses which display a fascinating diversity of hosts, a disparity of host effects, and a divergence of transmission strategies. Hosts include human parasites like Giardia, plant parasitic oomycetes, fungi and yeasts, red macroalgae (seaweed), terrestrial crustaceans like woodlice, insects like flies, mosquitoes, ants and wasps, marine crustaceans like shrimp, but also fish, fresh water snails that are intermediate hosts to parasites, and plants like papaya, notoginseng, maize, and wild petunias. In Leishmania and Trichomonas, the viruses increase the virulence of the parasites (hypervirulence), while in Victoria blight of oats it reduces the virulence of the fungus (hypovirulence). In salmon, smelt, and shrimp, it causes myocarditis and myonecrosis, in golden shiners it is asymptomatic. In Leishmania and many fungi and some plants, it is non-infectious and vertically transmitted, while in Giardia, fish, shrimps, and papaya, it is horizontally transmitted. Using PCR with degenerate primer sets, we are trying to explore the taxonomic boundaries of the vertically transmitted viruses in parasites to estimate the evolutionary age of first infection, the virulence in Giardia, and the evolutionary origin of dsRNA viruses in arthropods, especially sand flies, which are vectors of Leishmania. In a new development, Leishmania species not infected with totiviruses show an infection with Narnaviruses. Both viruses are vertically transmitted. The original concept that the Leishmania clade has only been infected once and subsequently lost the infection in several lineages has been challenged with these new discoveries.

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EXTENSIVE SEROLOGICAL SURVEY FOR MULTIPLE SPECIES OF EBOLA VIRUSES IN A WIDE DIVERSITY OF BAT SPECIES IN GUINEA, CAMEROON AND THE DEMOCRATIC REPUBLIC OF CONGO

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The animal reservoir(s) and ecology of Ebola viruses (EBV) remains largely unknown, but previous detection of viral RNA and anti-EBV antibodies in bats suggest that they may play a role in zoonotic transmission. Bats were captured in Guinea, Cameroon and the Democratic Republic of Congo (DRC) between November 2015 and August 2017. Samples were screened for cross-reactive EBV antibodies with a high throughput Luminex-based assay to different antigens from four different Ebola viruses; Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Reston (RESTV). Cut-off values were calculated using four different more or less stringent methods and samples were considered positive when simultaneous presence of antibodies to NP and GP was observed. 4,022 bats were studied, from at least ten frugivorous (n=1,736) and 27 insectivorous (n=2,286) species. Between 0.05 and 0.92 % (2 to 37) bats have cross-reactive antibodies to EBOV and 0.0 to 0.75 % (0 to 30) to SUDV. Higher reactivity was seen in frugivorous bats. With stringent and less stringent cut-off values, EBOV and SUDV cross-reactive samples were seen in one insectivorous genus (*Mops*) and three frugivorous species (*Eidelon helvum*, *Hypsignatus monstruosus*, *Rousettus aegyptiacus*). With less stringent methods only, cross-reactive antibodies were also seen in three additional frugivorous species, EBOV in *Lissonycteris angolensis* and *Epomophorus sp.* and SUDV in *Micropteropus pusillus.* EBOV and SUDV cross-reactive samples were observed in Cameroun and Guinea, and in DRC only SUDV. All samples from *Epomops franqueti* and *Myonycteris torquata* were negative. We confirmed the presence of cross-reactive EBOV antibodies in three to five frugivorous bat species and one insectivorous genus previously reported seropositive, and one new frugivorous species (*L.angolensis*). EBOV and SUDV apparently co-circulate in certain species and could be widespread across Africa. More studies are needed on significance of EBV antibodies and viral persistance to clarify the role of bats in EBV ecology.

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MARBURG VIRUS ANGOLA STRAIN: MORE VIRULENT THAN MUSOKE IN CYNOMOLGUS MONKEYS *MACACA FASCICULARIS*?

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From 2004 to 2005, an outbreak of Marburg virus, a filovirus, in Angola led to a case-fatality rate of 90 percent. However, little information is available regarding the virulence of the Angola strain from this outbreak compared to the virulence of other strains. Therefore, we sought to assess time to selected outcomes in non-human primates (NHPs) experimentally infected with either Angola or Musoke Marburg strains. Between 2014 and 2017, eight therapeutic trials at the U.S. Army Medical Research Institute of Infectious Diseases were conducted in *Macaca fascicularis* monkeys challenged with 1000 plaque forming units of Marburg virus administered intramuscularly. The current study population was comprised of 47 control NHPs, of which, 18 were administered Angola strain in three separate trials and 29 with Musoke strain in five trials. Clinical responses including development of rash and oral intake were collected following infection. The primary outcome of interest was time to death or euthanasia post-inoculation comparing strains using Cox proportional hazards regression. Secondary endpoints included time to development of a petechial rash and time to decreased appetite. Following Marburg challenge, all NHPs died and most NHPs experienced decreased food consumption (96%), and petechial rash (93%). Irrespective of strain, petechial rash was preceded by decreased food consumption by 1.1 days (SD 1.9) on average. The median time to death for Angola-infected NHPs was 8.1 days (25th, 75th percentiles: 7.91, 8.90), whereas Musoke-infected NHPs survived for a median of 10.0 days (25th, 75th percentiles: 9.00, 10.88). Angola strain was associated with a statistically significant increased risk of death (HR= 22.10; 95% CI: 7.08, 68.93), development of petechiae (HR= 22.10; 95% CI: 7.08, 68.93) and loss of appetite (HR= 5.87; 95% CI: 2.76, 12.51). In conclusion, this was the first study to compare clinical characteristics in NHPs between these strains. Our data support increased virulence of Angola strain compared to Musoke strain. Pathophysiological mechanisms involved in increased virulence require further study.

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ADAPTIVE PHENOTYPIC PLASTICITY IN *AEDES* MOSQUITO POPULATIONS AND SPECIES IN RESPONSE TO VARYING TEMPERATURE REGIMENS

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The mosquitoes *Aedes aegypti* and *Aedes albopictus* are vectors of several arboviruses of global public health importance (dengue, yellow fever, chikungunya and Zika). The recent past has witnessed an unprecedented geographical range expansion of *Aedes* mosquitoes which will be exacerbated further by climate change in the future. To understand the fitness effect of temperature on *Aedes* mosquitoes, this study compares and describes the impact of varying temperature regimens on the immature and mature development stages of two populations and species [*Ae. aegypti* (Miami, Florida and Poza Rica, Mexico) and *Ae. albopictus* (Long Island, New York population)]. Mosquito were hatched and reared at high (day 32°C/ night 28°C), moderate (day 30°C/ night 26°C) and low (day 28°C/ night 24°C) temperature regimens to simulate current and predicted future conditions during peak transmission. Variation in the hatch rate was observed at both the population and species level (Chi square 342.3, df 5) P<0.0001. Adults of *Ae. aegypti* populations reared at the highest temperature regimen were the first to emerge and the last were *Ae. albopictus* species reared at the lowest temperature regimen. *Ae. aegypti* populations reared at the highest temperature regimen demonstrated an expedited development rate. Increased temperatures did not significantly affect blood feeding frequency (Kruskal-Wallis statistic = 3.484 P (0.6258) or adult longevity [Log rank (Mantel-Cox) test Chi-square 3.593, df 5 P (0.6094)]. Skip oviposition behavior was evident in *Ae. aegypti* Miami and *Ae. albopictus* populations. Adaptive phenotypic plasticity due to temperature variation is evident in the *Ae. aegypti* Miami population in this study. There exists inter-population and species-specific differences in life history traits. In order to more completely characterize the relationship between temperature and virus transmission vector competence of Zika virus was also assessed. Together, these studies provide a comprehensive assessment of the effect of temperature and population on vectorial capacity of *Aedes* mosquitoes in the Americas.

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NEED FOR REAL TIME ARBOVIRAL SURVEILLANCE DATA TO PREVENT OUTBREAKS. A RETROSPECTIVE STUDY OF ZIKA, CHIKUNGUNYA, AND DENGUE OUTBREAKS IN CALI COLOMBIA 2014 TO 2016

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Arboviruses are responsible for a large burden of disease globally. Although current surveillance systems around the world and in Cali, Colombia are insufficient to predict and control future arboviral outbreaks, we hypothesize that spatial and temporal patterns exist which could be used to enable better prediction and planning at the policy level. We queried The National System of Vigilance in Public Health (SIVIGILA) for chikungunya (CHIKV), dengue (DENV), and Zika (ZIKV) viral cases between October 2014 and April 2016 in the urban area of Cali, Colombia. Determination of infection included laboratory or syndromic surveillance. After one case was confirmed by RT-PCR in a municipality, patients not classified as risk groups could have been diagnosed clinically. Laboratory confirmation was based on IgM and IgG detection, NS1 detection, virus isolation, or RT-PCR. All cases were georeferenced using Municipal Secretary of Health software. Spatial and temporal clusters were identified using SatScan in R. The query resulted in 33,443 records. 26,985 were georeferenced: 2,636 CHIKV cases, 3,139 ZIKV cases, and 21,210 DENV cases were included in the final analysis. Of 21,210 DENV cases, 14% were lab-confirmed (0% ZIKV and 0% CHIKV). Acute testing vs clinical diagnosis was 56.5% sensitive, whereas severe dengue had higher sensitivity (85.7%) compared to non-severe dengue (56.1%) and dengue death (57.1%). We observed spatial clustering of acute testing in the north-west of the city (RR= 1.38 and p<0.001), suggests inequality in access to care and diagnostics. Sensitivity of lab vs clinical diagnosis was also clustered spatially, suggesting heterogeneity in clinical training. Patterns in incident cases emerged over time with clear outbreak seasons and concurrent outbreaks and over space in high risk neighborhoods. Despite gaps in the current surveillance system, we identified patterns over time and space. If these data were available prospectively, the public health

system could predict outbreaks, more efficiently allocate limited resources, reduce burden on the public health system, and prevent morbidity and mortality due to arboviruses.

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SURVEILLANCE OF NOROVIRUS AMONG CHILDREN WITH DIARRHEA IN FOUR MAJOR HOSPITALS IN BHUTAN: REPLACEMENT OF GII.21 BY GII.3 AS A DOMINANT GENOTYPE

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Diarrhea is a major cause of morbidity and mortality among Bhutanese children. Norovirus (NoV) is an important cause of diarrhea worldwide and in Bhutan. In 2010-2012, GII.21 was found the major genotype circulating in Bhutanese children in contrast to globally prevalent GII.4 genotype responsible for NoV infections. The study was conducted in 2013-2014 to update and describe the transformation and distribution of the NoV genotype among Bhutanese children. Total 623 diarrheal stool samples were collected at three-referral hospital and one general hospital from June 2013 through May 2014 from children under 5 years of age. NoV was detected by reverse transcription-polymerase chain reaction (RT-PCR) by amplifying the capsid gene. The RT-PCR results were confirmed by nucleotide sequencing of the amplicons. The proportion of NoV-positive stool samples was 23.6% (147/623), of which 76.9% was NoV GII. The median age of infected children was 15.5 months. NoV GII was most prevalent in the colder months (late November-mid April). The most common genotypes prevalent were GII.3 (42.6%), GII.4 Sydney 2012 (15.8%), and GII.4 unassigned (11.9%). No GII.21 was found in any child in the present study. NoV remains an important cause of diarrhea among Bhutanese children. Genotype GII.3 from a single ancestor strain has spread, replacing the previously circulating GII.21. To develop a NoV infection control policy, further studies are needed.

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INTERSECTORAL COLLABORATION EFFORTS AND LESSONS LEARNED IN EBOLA VIRUS DISEASE RESPONSE IN NIGERIA-2014

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The 2014 Zaire species, Ebola Virus Disease (EVD) outbreak was first reported in Guinea and spread to countries including Nigeria which reported the first confirmed case 20th July 2014, had 20 cases and 8 deaths (CFR 40%) and was declared Ebola-free by WHO 20th October 2014. We reviewed the response/collaborative efforts in EVD control in Nigeria We conducted a desk review of the Ebola response activities and collaborative efforts at the National level of administration following the outbreak of EVD in Nigeria between July and September, 2014. Of 20 cases and 8 deaths, healthcare workers (HCWs) who were primary contacts of index case constituted 9(45%) cases 4(50%) deaths; 2 (10%) cases and 1(12.5%) death were secondary contacts; one nosocomial infection also occurred. EVD response efforts involved collaboration of Government, Non-governmental organizations (NGOs) and private sector. Government provided EVD-specific financial resources and was responsible for response coordination (through an Ebola Emergency Operation Center), NGOs/ partners deployed experts/ staff and donated funds/ resources. Private sector donated funds, vehicles, ambulances, protective equipment, call centers, mobile phones (including complimentary airtime). Response activities included trainings of all cadres of HCWs at public and private HFs, sensitization, public enlightenment and community mobilization on EVD, EVD surveillance (active case search and contact tracing (891 of 892 (99.9%) contacts completed follow up), outbreaks and rumour investigation and response, laboratory investigation (62 specimens tested), case management, safe burial, psychosocial support, decontamination, operational research and ports of entry activities involving average of over 10,000 daily passenger screenings. A gap in hospital infection control aided the spread of EVD outbreak in Nigeria; restricted movement of primary contacts may have stemmed the spread. The collaboration of various sectors helped in adequate response activities to control the outbreak of EVD in Nigeria. Improved hospital infection control practices are recommended.

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PLUG AND DISPLAY VIRUS-LIKE-PARTICLE VACCINES FOR OUTBREAK PATHOGENS

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A fast and effective response to emerging and outbreak diseases requires a vaccine platform that is adaptable, highly immunogenic, rapidly produced and has a previously demonstrated safety profile. The SpyCatcher/SpyTag biochemical superglue technology enables the rapid and efficient development of highly immunogenic virus like particle (VLP) vaccines against target pathogens. The Spy (from Streptococcus pyogenes) protein was divided into a peptide, SpyTag, and a protein partner, SpyCatcher. Once combined, the two react to form a spontaneous irreversible isopeptide bond. VLPs are a platform technology that is used to produce vaccines against many different diseases. These technologies are particularly suited for the induction of strong antibody responses, which are achieved through presentation of an ordered antigen array to the immune system. The SpyCatcher/SpyTag technology has been used to establish a novel chimeric VLP vaccine platform technology for decorating VLPs simply by mixing with protein antigen in a "Plug-and-Display" manner. This approach overcomes the well-described challenges of producing VLP carriers with complex antigens genetically-fused on their surface, or low conjugation efficiency often reported when using chemical conjugation. The SpyTag-SpyCatcher technology has been used previously to enhance humoral immunogenicity by displaying malaria antigens on VLPs. The application of this technology for the development of vaccines against Ebola and Zika viruses will be discussed. Immunogenicity and neutralisation data from mice immunised with glycoproteins from Ebola and Zika viruses will be presented which demonstrate the versatility and applicability of the platform to generate vaccines against new epidemics and emerging infections.

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RESPIRATORY PATHOGENS IN CHILDREN ONE MONTH TO FIVE YEARS OF AGE PRESENTING WITH UNDIFFERENTIATED ACUTE RESPIRATORY DISTRESS IN TWO DISTRICT-LEVEL HOSPITALS IN GHANA

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A majority of the one million deaths from pneumonia in children under five each year occur in low- and middle-income countries where advanced

laboratory diagnostics are often lacking. This has led to a lack of accurate epidemiological data. We report diagnostic results of a cohort of children in Ghana presenting with acute respiratory distress that were tested for common respiratory pathogens. This study describes the epidemiology of respiratory pathogens in children one month to five years of age presenting with undifferentiated respiratory distress in two district-level hospitals in Ghana. Nasopharyngeal swabs were obtained at time of presentation and tested by the BioFire FilmArray PCR assay for 17 viral and 3 bacterial pathogens. 2176 children were tested and 1276 (59%) were found to be positive for one or more organisms. Most (83%) were single infections with rhinovirus/enterovirus (36%) being the most common organism detected, followed by respiratory syncytial virus (11%) and parainfluenza (7%). Influenza was detected in less than 3% of children in the cohort. Both respiratory syncytial virus and human metapneumovirus were more frequently detected during the rainy season. 64% of children with respiratory pathogens detected also tested positive for malaria. In conclusion, detection of a respiratory pathogen in children presenting with undifferentiated acute respiratory distress in a lower-middle-income country was common, with rhinovirus/enterovirus most commonly identified. Influenza was infrequently detected, and both respiratory syncytial virus and human metapneumovirus were detected more frequently during the rainy season as compared to the dry season. Malaria was a common co-infection.

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EVIDENCE OF CIRCULATION OF OTHOBUNYAVIRUSES IN MOSQUITOES FROM VANGA ISLAND, KWALE COUNTY, KENYA

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A new site, Vanga island in Kwale County was recently added to the on-going arbovirus surveillance program in Kenya. Vanga island is on the southern tip of Kenya, bordering Tanzania. The area has mangrove plantations and fishing is the main economic activity. No survey has been carried out in this particular geographical location. Approximately 9544 mosquitoes were collected using CO_2-baited CDC light traps and BG-Sentinel traps, identified to species and pooled ≤ 25 mosquitoes per pool. The 649 mosquito pools obtained were homogenized using Minimum Essential Medium supplemented with Foetal Bovine Serum, L-Glutamine and antibiotics. Homogenates were clarified by centrifugation and the resultant supernatants inoculated in monolayers of VERO cells in 24 well plates. The cultures were incubated at 37°C and monitored for cytopathic effects (CPE) daily for 14 days. Cultures showing CPE were harvested and viruses identified by RT-PCR and sequencing. Ten Orthobunyavirus isolates were obtained from pools of *Aedes pembaensis* (5), *Ae. tricholabis* (1), *Culex pipens* (3) and *Cx. zombaensis* (1). The preliminary sequencing results indicate that the isolates have 99% sequence homology to Bunyamwera and Ngari viruses. Specific PCR will be done using the S, L and M segments primers of Bunyamwera and the M segment primers for Batai virus to characterize the isolates further. These findings are important because reassortment and recombination occurs frequently in Orthobunyaviruses and may potentially cause the emergence of new viruses that may be of public health importance in the region. The site borders Tanzania and data from this study will also be able to assist in mapping out risk zones.

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ASKING THE RIGHT QUESTIONS: A COMMUNITY CONSULTATION APPROACH TO PROMOTING RABIES AWARENESS IN CHILDREN AND YOUNG PEOPLE IN RURAL TANZANIA

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Rabies, transmitted largely by dog bites, kills many young people in rural Africa. Solutions are available, but difficult to implement, and awareness-raising often focuses on schools only. Moreover, standard solutions may be limited in village communities by specific socio-economic factors. The aim of the study is to test the efficacy of preventing rabies in young people by involving key community stakeholders in formulating and implementing local action plans and building local support networks. A mixed qualitative and quantitative approach was used. In six randomly selected villages in Kilosa, Tanzania, teachers delivered standard rabies prevention information in schools. In three of the villages, key-informant interviews were also held with health and veterinary staff to help identify key village and professional stakeholders for interactive workshops, where low-resource strategies were discussed and agreed for overcoming limitations to prevention. Extensive KAP surveys were conducted in all six villages before and after the intervention, and rabies bite data will help assess the longer-term impact of the methodology. Findings show that a good understanding of prevention was reported in schools, but there was no mechanism for taking this forward. The interviews and workshops, however, revealed a wide range of local socio-economic obstacles to preventing rabies, and low-cost solutions which emerged from workshops were formulated into action plans to be implemented by each group. The process developed a cross-sector support network for all involved. Disease prevention involves a range of socio-economic factors specific to each community. Delivering standard prevention information is often insufficient to sustain the good practice, and communities need to share ownership of solutions, messages, and strategies. Identification and discussion of issues by a wide range of local stakeholders should, therefore, be an essential first step in interventions. Community-generated action plans can help governments and international bodies develop more integrated and sustainable prevention processes.

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IMMUNE RESPONSE PROFILING OF SUSPECTED LASSA FEVER CASES AT IRRUA SPECIALIST TEACHING HOSPITAL DURING 2018 LASSA FEVER OUTBREAK

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Lassa fever (LF) is a severe, often fatal, viral hemorrhagic fever endemic in several West African countries. Beginning in January 2018, Nigeria experienced an unusual surge of LF cases and as of March 18th the Nigerian Center for Disease Control reported a total of 1495 suspected cases with 376 laboratory confirmed, 9 probable LF, and 119 deaths for a case fatality rate (CFR) equal to 25%. During this period, ReLASV®

Pan-Lassa rapid tests and ELISA immunoassays (Zalgen Labs, Maryland, USA) were field tested in the Lassa Surveillance Program laboratory at Irrua Special Teaching Hospital (ISTH), Edo State, Nigeria. The ReLASV® Pan-Lassa *in vitro* diagnostics (IVD) utilized a mixture of recombinant Lassa virus (LASV) nucleoprotein (NP) antigens representing the known circulating strains of LASV in West Africa, including LASV lineage II and III in Nigeria. The Pan-Lassa Antigen Rapid Test (RDT) and Antigen ELISA Test are capable of detecting LASV NP antigenemia in LF patient samples. The Pan-Lassa IgG/IgM ELISA Test is capable of detecting NP-specific IgG or IgM in patient samples from the immune response to LASV viremia. During the field trial, suspected LF cases (n=762) were screened at admission by LASV qRT-PCR, 363 cases were positive without utilization of cycle cut-offs. 78% (50/64) of Pan-Lassa RDT positives were confirmed by qRT-PCR. 100% (48/48) Antigen ELISA positives were confirmed by qRT-PCR. Pan-Lassa IgG/IgM ELISA identified 155 of 345 samples as IgG/IgM sero-positive, 94.2% (146/155) were reactive by qRT-PCR. The ReLASV tests were capable of identifying active LF cases as demonstrated by increased CFR for LASV NP antigen positive samples 42.6% (29/68), reduced CFR for IgG/IgM positive samples 12.6% (14/111), and CFR only 3.2% (4/124) for immunoassay negative samples. The ReLASV® Pan-Lassa RDT and ELISA Test have demonstrated capacity to accurately detect active LF cases during point-of-care screening procedures, including testing of LASV antigenemia and IgG/IgM immune response. LF immunoassay screening, combined with qRT-PCR, is an important addition to medical countermeasures for LF surveillance in West Africa.

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SILENT POLIO OUTBREAK IN RAHAT, ISRAEL: EPIDEMIOLOGIC FINDINGS BASED ON MODELING OF ENVIRONMENTAL SURVEILLANCE DATA

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Israel experienced an outbreak of wild poliovirus type 1 (WPV1) in 2013-14, detected through environmental surveillance of the sewage system. No cases of acute flaccid paralysis were reported, and the epidemic subsided after a bivalent oral polio vaccination (bOPV) campaign. As we approach global eradication, polio will increasingly be detected only through environmental surveillance. We developed a framework to convert quantitative polymerase chain reaction (qPCR) cycle threshold data into scaled WPV1 and OPV1 concentrations for inference within a deterministic, compartmental infectious disease transmission model. We used this approach to estimate the epidemic curve and transmission dynamics, as well as assess alternate vaccination scenarios. Our analysis suggests that the outbreak peaked approximately one month earlier than previous estimates derived from analysis of stool samples, and we estimate the basic reproduction number was 1.36 (95% CI 1.22-1.48). Model estimates indicate that 37% of susceptible individuals (primarily children under 10) were infected with WPV1, mostly prior to vaccination campaign onset, and that the vaccination campaign averted 24% of WPV1 infections. As we approach global polio eradication, environmental monitoring with quantitative PCR can be used as a highly sensitive method to enhance disease surveillance. Our analytic approach brings public health relevance to environmental data that, if systematically collected, can guide eradication efforts.

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VIROLOGICAL INVESTIGATION OF ACUTE FLACCID PARALYSIS CASES IN ETHIOPIA; 2007-2017 RETROSPECTIVE STUDY ON DECADES OF VIROLOGICAL FINDINGS

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Polioviruses are highly infectious human entero-viruses mainly responsible for the clinical syndrome Acute Flaccid Paralysis (AFP). Despite remarkable worldwide success, emergence of potentially neuro-virulent strain of vaccine derived polioviruses (VDPVs) due to prolonged shedding and replication in immuno-compromised individuals (iVDPV) or following persistent circulation in populations with immunity gaps (cVDPV) and transmission from epidemiological blocks which are reservoirs of poliovirus constitute major concerns for the global polio eradication endgame strategy. This study aims to provide critical information on epidemiology and viral etiology of AFP cases reported over the last decade by the Ethiopian AFP surveillance system. Stool specimens collected from AFP patients were transported to the Ethiopian Public Health Institute. Virus isolation was done by inoculating stool suspension into RD and L20B cells lines and observing for any cytopathic effect. Virus isolates were serotyped using Real Time PCR technique. Data entry and analysis was done using Epi-Info software. A total of 25710 specimens were investigated from 2007-2017 and 2808 enteroviruses isolated. Out of these, 1031(36.7%) were polioviruses (PVs) and 1777(63.3%) were non-polio enteroviruses (NPEV). 14 of PV isolates were wild-type1 four of which isolated in 2008, nine in 2013 due to importations form South Sudan and Somalia respectively. The last case of wild-type 1 PV isolated in Ethiopia was in 2014 from Somali region. 15 of the PV isolates were VDPVs (type 2 &3) with genome variation in the VP1 region from corresponding OPV strain. The remaining 1002 virus isolates were vaccine related Sabin strains. Our findings indicate NPEVs being predominant viruses frequently isolated from AFP cases investigated suggesting paramount importance of routine entero-virus surveillance system, study of their transmission dynamics and circulating genotypes so as to determine their role as non polio AFP etiologies. Continued virus tracking and uninterrupted immunization to maintain high herd immunity remain crucial in polio eradication era and post-polio world.

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OROV ISOLATION IN A NORTHERN REGION OF PERU: FIRST MOLECULAR IDENTIFICATION AND CLINICAL CHARACTERISTICS

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Oropouche virus (OROV) is an emerging infectious disease that is under-reported due to the lack of molecular diagnostic tools and the clinical similarities with other endemic arboviral diseases. The circulation of OROV in Peru has been previously evidenced in regions such as Cuzco, Cajamarca, and Madre de Dios. In this study, we report the first outbreak

of OROV in the northern region of Piura in Peru that occurred during April to August of 2016. A total of 496 patients were enrolled from a local acute febrile illness surveillance program and their serum samples were analyzed by RT-PCR, for the presence of OROV. On-site health care professionals surveyed the clinical presentation of each enrolled patient and a follow-up was scheduled to detect disease progression, recurrence or deaths. Positive identification for OROV via RT-PCR, was found in 26,4% (n=131) of all samples. Among these, 55% (n=33) were males and the patients that were most affected had 18-39 years (25.2%, n=33). The most common symptoms reported by infected patients were headache with 82.4% (n=108), myalgias with 77.9% (n=102), arthralgias with 69.5% (n=91) and retro-ocular pain with 52.7% (n=69). During this outbreak, some patients had a clinical presentation suggestive of severe OROV infection including 3.1% (n=4) had intense abdominal pain, 0.8% (n=1) had low platelet count, 1.5% (n=2) had melena, and 0.8% (n=1) had gynecological bleeding. No recurrence or deaths were reported during follow-ups. In conclusion, this study reports an outbreak of OROV in a non-endemic northern region of Peru, where positive cases were identified for the first time. The disease caused by OROV remains an emerging and underdiagnosed infection that may be under-reported due to its clinical similarity with other arboviral diseases and the lack of on-site diagnostic methods suitable for endemic areas. This study demonstrates that the inclusion of OROV in the Peruvian national surveillance program is of paramount importance and that further research is required to determine the virulence, pathogenesis, host range and vectors involved in OROV transmission cycles.

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RETINOID PROFILES IN PATIENTS WITH SEVERE MALARIA: CASE CONTROL STUDY IN COLOMBIA

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The malaria parasites *Plasmodium falciparum* and *P. vivax* selectively absorbs vitamin A from host tissues and appears to depend on it for its metabolism. While essential in low concentration for many biological functions, vitamin A can be toxic in higher concentration. Such observations led to the hypothesis that the pathogenesis of malaria involves an endogenous form of hypervitaminosis A in which merozoite-stage parasites use the vitamin A accumulated in the liver as a cell membrane destabilizer to invade erythrocytes, which then circulate throught the body, inducing signs and symptoms of malaria as an endogenous form of hypervitaminosis A (Mawson, Pathogens and Global Health, 2013; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4003589/pdf/pgh-107-03-122.pdf). This study was undertaken to investigate the retinoid toxicity hypothesis of malaria in Colombia a country with high rate of infections from malaria in South America To test the hypotheses that acute malaria infection is associated with a retinoid (vitamin A) profile suggesting an endogenous form of hypervitaminosis A. With colleagues at the Universidad de la Sabana in Bogotá and Universidad de Cordoba in Monteria A case-control study will be carried out in Cordoba, in major endemic region of malaria in Colombia, to determine retinoid concentrations and RAR expression in patients with malaria and controls, subject to approval by the Institutional Review Boards of Jackson State University and the Universidad de la Sabana. to obtaining positive results, the research could lead to the development of retinoid profiling for diagnosing malaria in the early stages of infection, as well as potential new treatments aimed at blocking retinoid expression or inhibiting its metabolism.

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OVERATTRIBUTION OF *PLASMODIUM VIVAX* MALARIAL RECURRENCES TO HYPNOZOITE ACTIVATION

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The hypnozoite theory of relapse is obfuscating the probability that many homologous *Plasmodium vivax* malarial recurrences are not relapses (hypnozoite origin), but recrudescences (merozoite origin). What is new about this post-2011 argument is that attention will now be drawn, via the conference presentation, to the implications of relatively obscure information which shows that both short-term and long-term recurrences can follow blood-stage-induced primate malaria (hypnozoites do not occur in such infections). It follows that recurrences having the same non-hypnozoite parasite source or sources in the body, whatever it or they may be, most likely take place in mosquito-transmitted (i.e. sporozoite-initiated) *P. vivax* malaria as well. Thus, an unknown percentage of homologous *P. vivax* recurrences are no doubt erroneously being ascribed to activation of hypnozoites. The matter of how and where quiescent plasmodial parasites persist is not merely of academic interest, but of fundamental importance in relation to malaria eradication.

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THE *PLASMODIUM KNOWLESI* MAHRP2 ORTHOLOG LOCALIZES TO STRUCTURES CONNECTING SINTON MULLIGAN'S CLEFTS IN THE INFECTED ERYTHROCYTE

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During development within the host erythrocyte malaria parasites generate nascent membranous structures which serve as a pathway for parasite protein transport to modify the host cell. The molecular basis of such membranous structures is not well understood, particularly for malaria parasites other than *Plasmodium falciparum*. To characterize the structural basis of protein trafficking in the *Plasmodium knowlesi*-infected erythrocyte, we identified a *P. knowlesi* ortholog of MAHRP2, a marker of the tether structure that connects membranous structures in the *P. falciparum*-infected erythrocyte. We show that PkMAHRP2 localizes on amorphous structures that connect Sinton Mulligan's clefts (SMC) to each other and to the erythrocyte membrane. Three dimensional reconstruction of the *P. knowlesi*-infected erythrocyte revealed that the SMC is a plate-like structure with swollen ends, reminiscent of the morphology of the Golgi apparatus. The PkMAHRP2-localized amorphous structures are possibly functionally equivalent to *P. falciparum* tether structures. These findings suggest a conservation in the ultrastructure of protein trafficking between *P. falciparum* and *P. knowlesi*.

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PFEMP1 PROTEINS BINDING NON-IMMUNE IGM AND A2-MACROGLOBULIN ARE FREQUENT IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum malaria is particularly dangerous because the infected erythrocytes (IEs) can adhere to the vascular endothelium and to uninfected erythrocytes, causing organ-specific complications. This ability is related to the expression of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) proteins on the IE surface. Recent evidence obtained by our group support the hypothesis that *P. falciparum* can exploit human serum factors such as non-immune IgM and α_2-macroglobulin (α_2M) to increase PfEMP1 avidity for host receptors and to evade host immune responses. However, only few IgM- and α_2M-binding PfEMP1 proteins have been described. In this study, we aimed to identify and characterize such PfEMP1 proteins in the three parasite lines, NF54-G6, HB3, and IT4/FCR3. We used pVBH-transfected parasites to obtain starting populations with highly heterogeneous *var* gene transcription. IgM- and α_2M-binding IEs were single-cell sorted by FACS, and the *var* gene transcription profile was analyzed by qPCR. The ability of candidate PfEMP1 proteins to bind IgM and α_2M was assessed by flow cytometry (IEs, native PfEMP1) and ELISA (recombinant single- and multi-domain proteins). Several PfEMP1 variants containing DBLε, DBLζ, or DBLδ domains bound IgM and/or α_2M. The data indicate that IgM- and α_2M-binding are more frequent than previously reported and that the domain types involved are conserved. This provides novel insight to how *P. falciparum* exploits human host proteins for its own benefits.

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DEGRADATION OF ENDOTHELIAL GLYCOCALYX IN *PLASMODIUM FALCIPARUM* MALARIA IN TANZANIAN CHILDREN

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Work by our group has shown that patients with *P. falciparum* malaria have endothelial dysfunction, whichplays a major role in malaria pathogenesis. Adherence of parasitized RBC (pRBC) to endothelial cells induces endothelial activation and results in pRBC sequestration, impaired perfusion, tissue hypoxia, and organ dysfunction. The endothelial glycocalyx (eGC) is a gel-like layer that covers the luminal surface of the vessel. This eGC functions to maintain vascular homeostasis by regulating permeability, modulating blood flow-induced shear stress signals including formation of nitric oxide (NO), and inhibiting endothelialadherence of leukocytes and platelets. We hypothesized that breakdown of the eGC occurs in malaria infection leading to endothelial activation and microvascular dysfunction. Ourobjectivewas to compare levels of glycocalyx breakdown products [glycosaminoglycans (GAG)] in urine of children with *P. falciparum* malaria and healthy control (HC) subjects. The study was done at Hubert Kairuki Medical Center in Dar es Salaam, Tanzania. HC children and children with severe (SM) and moderately severe malaria (MSM) age 1 to 10 years were studied from2016 to 2018.The diagnosis of *P. falciparum* was established by microscopy and RDT.Total urinary GAG levels were measured by dimethylmethylene blue (DMMB) assay and plasma angiopoietin-2 by ELISA. The study thus far included41 HC,24 MSM, and 26 SM.Total urine GAG levels were significantly higher in SM (mean±SD 13.7±6.4g/molcreatinine)andMSM (11.7±6.4) thaninHC subjects (3.6±3.1); p<0.0001.Levels of GAG in malaria patients correlated significantly with malaria severity as judged by statistically significant correlation withperipheral parasitemia, hemoglobin, platelet count, and angiopoietin-2 levels.In conclusion, Tanzanian children with falciparum malaria have evidence of glycocalyx degradation based on significantly increased urinary GAG levels. It is likely that this glycocalyx damage contributes to the pathogenesis of the vascular dysfunction. Agents that protect against glycocalyx damage may be useful as adjunctive treatments for malaria.

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VISUAL IMAGING OF THE ENDOTHELIAL GLYCOCALYX IN TANZANIAN CHILDREN WITH FALCIPARUM MALARIA

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The endothelial glycocalyx (eGC) is composed of membrane-bound proteoglycans and glycoproteins interacting with plasma components, which contribute to eGC stability. The eGC acts as a protective barrier for the endothelium. We earlier demonstrated microvascular dysfunction in patients with *Plasmodium falciparum* malaria, and this dysfunction plays a major role in malaria pathogenesis. eGC can be imaged with sidestream dark-field (SDF) microscopy to measure its structure and integrity. We hypothesized that children with falciparum malaria have damaged eGC that can be quantitatively imaged by SDF. We used a "GlycoCheck" camera to study healthy children and those with moderately severe or severe falciparum malaria at the Hubert Kairuki Medical Center in Dar es Salaam, Tanzania. Healthy control (HC) children and children with malaria were studied from 2016 to 2018. Sublingual imaging in many of the children was not possible, so we imaged via skin of the axilla and pinna, with analysis of (*a*) RBC column width (RCW); (*b*) perfused boundary region (PBR); (*c*) total microvascular density (TMD); (*d*) valid microvascular density (VMD); and (*e*) vessel RBC filling (VRF). Of these, the PBR most accurately reflects the eGC measurement. Total urinary glycosaminoglycan (GAG) levels measured by the dimethylmethylene blue assay served as a chemical marker of glycocalyx degradation. We imaged 30 HC, 12 moderately severe (MSM), and 16 severe malaria (SM) subjects. We used microscopy and RDT to establish malaria diagnosis. Our separate unpublished results show eGC damage in malaria based on increased urinary GAG in MSM and SM compared to that in HC children. Pinna imaging revealed no significant differences in the RCW, PBR, or VRF, but TMD and VMD were significantly increased in malaria (TMD mean±SEM 580±57 μm/mm^2 in HC vs. 787±74 in malaria; and VMD 333±39 HC vs. 428±27 malaria; p = 0.039). In conclusion, elevated urinary GAG levels in the malaria group indicate eGC damage, but the PBR as a measure of glycocalyx integrity and depth was not increased. The increased microvascular density may be a reflection of recruitment of vessels into these tissues in response to hypoxia.

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INFECTIVITY OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS TO *ANOPHELES GAMBIAE* IN SENEGAL

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The transmission of malaria from an infected human host to a susceptible mosquito is mediated by a specialized sexual stage of the *Plasmodium* species commonly known as the gametocyte. Although crucial for malaria transmission, the presence of gametocyte in the blood does not mean infection in humans. After ingestion of gametocytes by the mosquito, the probability of having an infection requires a combination of factors such as maturation and density of male and female gametocytes but also the immune system of both hosts. Thus, the objectives to determine parasite density, evaluate the infectivity of mosquitoes and to assess antibody profile against sexual stage parasite in two areas with different malaria transmission setting in Senegal. A cross sectional study was enrolled at the end of the transmission season. Was included, 1120 asymptomatic volunteers aged 5 years and over. Approximately one teaspoon [5 ml] of blood was collected for each patient for RTDs, microscopy, plasma

collection. For those with RTDs positives, some blood was used for membrane feeding *assay* for mosquito infectivity. From 560 slides in each area, 24.6 % and 2.1 % were positives for the south and the center respectively. 15 individuals were tested for infectivity against a *P. falciparum*-sensitive *Anopheles gambiae* strain in two different localities. In the center of Senegal, 20% of tested individuals were mosquitoes infected and 5% of tested mosquitoes were oocysts infected. But in the south, 30% patients were mosquitoes infected and 9% of mosquitoes tested were oocyst infected. In conclusion, both studies sites, malaria transmission is well maintained by some individuals of both populations hosting the sexual stage of *P falciparum*.

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BLOOD DONOR VARIABILITY AS A MODULATORY FACTOR IN *PLASMODIUM FALCIPARUM* PHENOTYPING ASSAYS

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Plasmodium falciparum uses multiple ligand-receptor interactions to invade red blood cells (RBCs). Blood stage malaria vaccines mainly target *P. falciparum* antigens involved in RBC invasion. Thus, unraveling the nature of ligand-receptor interactions involved in invasion is crucial in malaria vaccine development. Conducting large scale *P. falciparum* phenotyping studies inevitably involves the use of blood from different blood donors, which could affect the outcome of *in vitro* invasion inhibitory assays. However, the effect of blood donor variability in characterizing *P. falciparum* phenotypic diversity remains unaddressed. Therefore, we are currently investigating variations in invasion efficiency/phenotype observed using different donor RBCs. RBCs were treated with different enzymes and labeled with a fluorescent dye. *P. falciparum* clinical isolates and laboratory lines were maintained *in vitro* in a gassed atmosphere and further used to conduct standard invasion assays. The percentage of invasion was determined by flow cytometry and for all assays, the parasite's invasion phenotype was adjudged by comparing invasion in untreated and enzyme-treated acceptor RBCs. Our data show that invasion efficiency of both *P. falciparum* clinical isolates and laboratory adapted strains is affected by the nature (e.g. blood group or hemoglobin genotype) of the acceptor RBCs. Data with regard to the effect of blood group and sickle cell trait will be presented at the conference. Additionally, our data also show that RBCs from different donors show different levels of sensitivity to enzyme treatments and this sensitivity seemed to be driven by the receptor density on the RBC surface. This suggest that, like the parasite's genetic make-up, the intrinseque properties of the target RBCs may also play a role in the observed invasion phenotype. In conclusion, the data show that blood donor variability is a modulatory factor influencing invasion efficiency. Altogether, the study demonstrates that blood donor variability in *P. falciparum* should be an important consideration in invasion phenotyping assays.

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DECIPHERING THE ASSOCIATION OF A NOVEL *PLASMODIUM FALCIPARUM* EXPORTED PROTEIN WITH PARASITE-INDUCED STRUCTURES

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Plasmodium falciparum survives within host erythrocytes by devising strategies for successful export of several proteins beyond the parasitophorous vacuole. This has fostered interest in some of the membranous structures established by the parasite during protein export. We have identified a novel *P. falciparum* exported protein that harbors a canonical PEXEL variant. To functionally characterize the protein, three peptides (P1, P2, and P3) were chemically synthesized based on B-cell epitope mapping and screening for coiled-coil signatures. Stage-specific expression analysis by immunofluorescence assay (IFA) showed that the protein is expressed in late ring, trophozoite and schizont stages. Antibodies were generated against the three peptides but only anti-P3 antibody detected the full-length native parasite protein (50 kDa) in detergent-treated schizont lysates, while anti-P2 antibody detected a truncated fragment (~22 kDa) of the native protein close to the PEXEL motif and anti-P1 antibody did not detect the native protein. We also showed that the export of the protein is Brefeldin A-sensitive by comparative IFA analysis of trophozoite-infected erythrocyte ghosts, selective permeabilization of infected erythrocytes and immunoblotting. Since protein export precedes gametocytogenesis, we have shown the localization of the protein in different stages of gametocytes. Cellular fractionation experiments revealed the differences in the subcellular localization of the protein which was attributed to changes in solubility states of the protein within the parasite-host compartment. Further work on protein-protein interaction experiments is ongoing to substantiate an initial evidence suggesting that the novel *P. falciparum* exported protein (50 kDa) forms a 200 kDa multi-protein complex in trophozoite lysates. It is expected that this study may shed more insights on the association of this protein with parasite-induced membranous structures.

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EVIDENCE OF ALTERED LIVER FUNCTION AND CYTOKINE RESPONSE PROFILES IN PREGNANT WOMEN WITH MALARIA AND CHRONIC HEPATITIS B

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The overlap of malaria and chronic hepatitis B (CHB) is common in endemic regions, however, the impact of this co-infection on liver function and immune responses is unknown. This study sought to investigate these interactions in pregnant women reporting to an antenatal clinic in Ghana. Levels of malaria parasitemia, hepatitis B viremia, liver biochemical parameters and inflammatory cytokines were assayed and compared across four categories of pregnant women: uninfected, infected with *Plasmodium falciparum* alone *(Malaria group)*, infected with hepatitis B virus alone (*CHB group*) and co-infected with *P. falciparum* and hepatitis B virus (*Malaria+CHB group*). Relative to the *CHB group*, the *Malaria+CHB group* had lower viremia levels. However, levels of malaria parasitemia were similar in women in the *Malaria* and *Malaria+CHB groups*. Furthermore, levels of markers for liver injury/damage, including alanine aminotransferase, aspartate aminotransferase and total bilirubin were elevated in women in the *Malaria+CHB group* relative to those in the other groups. Similarly, pro-inflammatory cytokines, including tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6 were higher in women with *Malaria+CHB* compared to those in the other categories. In addition, pro-inflammatory cytokine levels had a significant positive correlation with viremia, and negative correlation with parasitemia. For anti-inflammatory cytokines, including IL-10 and IL-4, the pattern was exactly the opposite of that for the pro-inflammatory cytokines. Our findings showed that malaria/CHB co-infection in pregnancy appeared to exacerbate the release of biomarkers for liver damage and inflammatory mediators while reducing immune-modulatory mediators. The exacerbated inflammatory response appears to help control malarial parasitemia in co-infected women.

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FUNCTIONAL INSIGHTS ON THE ROLE OF *PLASMODIUM FALCIPARUM* CLAUDIN-LIKE APICOMPLEXAN MICRONEME PROTEIN (PFCLAMP), AN ESSENTIAL GENE

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Malaria is still a public health burden. With the recent reports of artemisinin resistance in *Plasmodium falciparum* coupled with the low efficacy of the available commercially approved vaccine, there is the need to continue developing new targets by functionally characterizing some of the ~60% of the parasite's genes with unknown functions.. This will foster the identification of viable vaccine and possible drug targets for the development of interventions against the parasite. To this, we have studied *Plasmodium falciparum* Claudin-Like Apicomplexan Microneme Protein (PfCLAMP) (3D7_1030200) and its role during the parasite development. It has been shown to be highly conserved in apicomplexans, with its orthologue in *P. falciparum* essential for parasite growth and invasion. We have confirmed the localization of CLAMP at the apical portion of merozoites using specific antibodies raised against the extracellular domain of the protein. We have also demonstrated that CLAMP is differentially expressed across the different asexual stages of the parasite, with the dominant expression being in the late trophozoite and schizont stages. We have shown and validated that some clinical isolates habour multiple copies of the *CLAMP* gene. In addition, CLAMP forms complexes with other proteins, and in this study we have characterized its interacting partners. Altogether, our data demonstrates that CLAMP provides a potentially attractive target for further investigation for drug and vaccine development.

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INVOLVEMENT OF EPHA2 IN INVASION OF *PLASMODIUM VIVAX* EXO-ERYTHROCYTIC STAGE

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Malaria is a serious health problem. It is widely distributed in the tropical and subtropical regions of the world. It was estimated to cause approximately 0.4 million deaths annually. The initial step of human malaria involves infection of hepatocytes by the parasite. Proteins on the surface of the host hepatocytes are believed to be important for parasite invasion. This has been demonstrated for human malaria parasite *Plasmodium falciparum* and rodent malaria parasites. However, it is not clear whether these proteins are likewise important for *Plasmodium vivax*, the most common malaria parasites that cause recurring infections. This study aims to investigate the role of host cell receptor EphA2 in hepatocyte invasion by *P. vivax*. Our specific aim is to determine whether the abundance of surface EphA2 enhances parasite infection. To achieve this goal, we first stained hepatoma cell line (HC-04) with fluorescent anti-EphA2 antibodies and used fluorescence activated cell sorting (FACS) to harvest cells that had high EphA2 surface expression ($EphA2^{High}$). *P. vivax* sporozoites, freshly harvested from the salivary glands of infected *Anopheles* dirus, were then allowed to infect the sorted $EphA2^{High}$ cells as well as the unsorted cells. After 4 days, we fixed cell cultures with paraformaldehyde and used immunofluorescence staining (IFA) to compared the infection rates of the $EphA2^{High}$ and unsorted HC-04. We found that the infection rates (N = 16) of $EphA2^{High}$ were around 2-4 folds higher than that of the unsorted HC-04. To further demonstrate involvement of EphA2 in *P. vivax* invasion, we performed invasion inhibition assays by pre-incubating the host cells with anti-EphA2 antibodies before sporozoite addition. We found that the antibody reduced sporozoites infection in a dose dependent manner. Overall, our data provide strong experimental support for a role of EphA2 in *P. vivax* infection of hepatocytes.

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EXPOSURE TO DIFFERENT COMMUNITIES OF VENDOR-ASSOCIATED FECAL MICROBES DETERMINES MALARIA INFECTION SEVERITY AND PREGNANCY OUTCOME IN AN OUTBRED MOUSE MODEL FOR MALARIA IN PREGNANCY

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Placental malaria, a severe clinical manifestation of *Plasmodium falciparum* infection, is a major cause of pregnancy loss, neonatal mortality, and severe maternal illness. We have developed a novel mouse model for pregnancy maintenance during maternal malaria infection utilizing outbred Swiss Webster mice. When infected with *Plasmodium chabaudi* in early gestation, several inbred mouse strains will abort their pregnancies at mid-gestation. However, outbred Swiss Webster mice infected with *P. chabaudi* in early gestation carry their pregnancies to term, allowing us to explore the immunological balance between parasite clearance and pregnancy success. The composition of the gut microbiota may alter this balance. As described by Villarino *et al.*, C57BL/6 mice sourced from different vendors display gut microbiota-dependent differences in *Plasmodium* infection severity resulting in a susceptible or resistant phenotype. Similarly, *P. chabaudi chabaudi* AS-infected pregnant Swiss Webster mice exposed to susceptibility-conferring fecal microbes develop higher parasite burdens than mice exposed to resistance-conferring fecal microbes following the disruption of the native gut microbiota by broad-spectrum antibiotic treatment. The microbiota-mediated reduction of parasite burden is associated with reduced maternal morbidity and improved fetal outcomes. Specifically, fetal viability and weight at gestational term are increased in infected dams exposed to resistance-conferring fecal microbes relative to infected dams exposed to susceptibility-conferring fecal microbes. To assess post-natal growth, pups from infected and uninfected dams exposed to resistance- and susceptibility-associated fecal microbes were delivered by caesarean section and placed with a foster dam. Although pup growth prior to weaning is not significantly influenced by either infection status or fecal microbe exposure, pups produced by highly susceptible dams displayed a statistically significant reduction in survival in the first days of life, suggesting that these pups may be less fit than pups born to dams with lower parasite burdens.

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TARGETING SPOROZOITE-HEPATOCYTE INTERACTIONS WITH A PHAGE DISPLAY LIBRARY

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After inoculation by the bite of an infected mosquito, the *Plasmodium* sporozoite enters the blood stream and infects the liver with unique specificity. Prior to liver infection, sporozoites must leave the circulation by traversing the liver sinusoid lining, preferentially trough liver macrophages (Kupffer cells). Using a phage display library we previously selected peptides which structurally mimic (mimotope) a sporozoite ligand (GAPDH) for Kupffer cell recognition and the Kupffer cell receptor (CD68). Importantly we showed that a mimotope peptide of sporozoite GAPDH serves as a vaccine candidate to generate sterile protection. Encouraged by these positive results, we screened the same phage display library to target sporozoite-hepatocyte interactions. The selected hepatocyte-binding peptides competitively inhibit sporozoite invasion of hepatocytes *in vitro*. Antibodies against the selected peptides recognize a ~50 kDa sporozoite surface protein. Significantly, immunization with one of the selected peptides inhibited *Plasmodium* liver invasion. We hypothesize that this peptide is a mimotope (mimics the structure) of a sporozoite surface ligand for hepatocyte invasion. This sporozoite ligand constitutes a potential vaccine antigen targeting malaria liver invasion.

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SELECTION OF SEVEN-MUTATION *PFCRT-PFMDR1* GENOTYPE AFTER SCALING SEASONAL MALARIA CHEMOPREVENTION WITH SULPHADOXINE-PYRIMETHAMINE AND AMODIAQUINE IN MALI

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WHO recommended in 2012 the seasonal malaria chemoprevention (SMC) in the Sahel countries in Africa to reduce Malaria among children less than 5 years of age with sulfadoxine-pyrimethamine and amodiaquine (SP+AQ). This strategy is scaled up in Mali since 2012. The use of millions of doses of SP+AQ could generate potential *Plasmodium falciparum* resistance mutant parasites. The aim of this study was to monitor the prevalence of *Pfdhfr+Pfdhps+pfcrt+pfmdr1* mutations in the target population of the health district of Koutiala. Two cross-sectional surveys were conducted before (August 2012, n=662) and after (June 2014, n=670) a pilot implementation of SMC in Koutiala. Children aged 3-59 months received 3 and 4 rounds of curative dose of SP+AQ over two malaria seasons in 2012 and 2013, respectively. Genotypes of *P. falciparum Pfdhfr* codons 51, 59 and 108; *Pfdhps* codons 437 and 540, *Pfcrt* codon 76 and *Pfmdr1* codon 86 were analysed by PCR on DNA from SMC population samples (after and before) and non-SMC population aged 7 years or above (November 2014, n=500). In SMC population 191 and 85 children before and after SMC implementation respectively were included in the molecular analysis. In the non-SMC population 220 were successfully PCR analysed. In the SMC population, the prevalence of the six-mutation *Pfcrt* [*Pfdhfr-dhps* quintuple + *Pfcrt*-76T] genotype increased significantly after SMC implementation, from 0.0% before to 7.1% (*p=0.0008*). The post-intervention prevalence of six-mutation *Pfmdr1* [*Pfdhfr-dhps* quintuple + *Pfmdr1*-86Y] and seven-mutation *Pfcrt+Pfmdr1* [*Pfdhfr-dhps* quintuple + *Pfmdr1*-86Y + *Pfcrt*-76T] genotypes were both the 1.2% among SMC population. No six-mutation and seven-mutation genotypes were observed neither among SMC population at baseline nor in the non-SMC population *(p=0.30)*. In conclusion, SMC increased the prevalence of the six-mutation *Pfcrt* genotype of *P. falciparum* that can lead to resistance in a population exposed to SMC with SP+AQ. However, there was no significant of six-mutation *Pfmdr1* and seven-mutation *Pfcrt-Pfmdr1* genotypes resistance in general parasite population.

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PREVALENCE SURVEY OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL DRUG RESISTANCE MARKERS IN CHINA

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China has launched national malaria elimination program (NMEP) with an ultimate goal to interrupt the local malaria transmission by 2020. But the increasing imported cases with extensive distribution and dozens of deaths has be as one of the most challenges for national malaria elimination. Over the past 50 years, *Plasmodium falciparum* has developed resistance to all antimalarial drugs that have been used including chloroquine, amodiaquine, sulfadoxine-pyrimethamine, quinine, piperaquine and mefloquine. More recently, the emergence and spread of multidrug resistance including artemisinin and partner drug resistance of *P. falciparum* in Southeast Asia poses a significant risk to malaria control and eradication goals in the world. Therefore, it is urgent to monitor antimalarial drug resistance and track the emergence and spread of drug resistance in China. This was a cross-sectional, observational study using dried blood samples collected from *P. falciparum*-infected individuals at the time of malaria diagnosis from nine provinces which were selected based on the number of reported malaria cases in the past five years. The prevalence of known and candidate molecular markers of resistance to artemisinin, ACT partner drugs and other antimalarials would be measured by PCR and sequencing. Also, this study evaluated the population structure and gene flow of *P. falciparum* either between or within geographic regions using clustering algorithms such as those employed by programs like STRUCTURE or by principal components analysis (PCA). This study will provide evidence of the antimalarial drug resistance to guide the national malaria elimination programme.

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OPTIMIZATION OF EMETINE DIHYDROCHLORIDE AS AN EFFECTIVE OPTION FOR MALARIA TREATMENT USING NANOPARTICLE DELIVERY SYSTEM

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The field of drug development experiences very low success rates concerning drugs that enter the market. These are due to toxicity of the therapeutic compounds and poor solubility leading to lowered bioavailability. The major disadvantages of conventional antimalarial drugs are the development of multiple drug resistance and nonspecific drug targeting, resulting in the need for high dose administration and subsequent intolerable side effects that ultimately lead to patient non-compliance. To counteract these trends, research has been done in nanotechnology for the development of new biocompatible systems capable of incorporating drugs, lowering the resistance progress, control and treatment of malaria by target delivery. Targeting drugs specifically to their site of action would indeed enable optimal concentration in parasite-infected RBC. Various materials have been used in the formulation of nanoparticles for drug delivery research. Among these, nanoparticles prepared with albumin are biocompatible, biodegradable, non-antigenic, and relatively easy to prepare. We will present here preliminary data investigating the potential of human serum albumin nanoparticles in enhancing the treatment efficacy of antimalarials. Our preliminary validation data on artemether-loaded HSA nanoparticles show a 50% reduction in the IC_{50} values against *Plasmodium falciparum* K1 in comparison to drug only controls. Therefore, the current study was undertaken to define the efficacy of human serum albumin (HSA) as a nano-carrier strategy to improve drug delivery, enhance treatment efficacy and reduce non-target side effects for emetine dihydrochloride hydrate a drug the discovered through repositioning method at University of Salford. Our *in vitro* results indicated that emetine-loaded HSA nanoparticle permitted ~70% dose reduction compared to emetine only controls. It is expected that this study will eventually lead to a better understanding of nanotechnology delivery system and provide insights into new strategies for developing smart, well-tolerated, and efficacious therapeutic that could be a future ultimate way to cure this disease.

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EX VIVO SUSCEPTIBILITY AND GENOTYPING OF *PLASMODIUM FALCIPARUM* ISOLATES FROM PIKINE, SENEGAL

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The monitoring of *Plasmodium falciparum* sensitivity to anti-malarial drugs is a necessity for effective case management of malaria. This species is characterized by a strong resistance to anti-malarial drugs. In Senegal, the first cases of chloroquine resistance were reported in the Dakar region in 1988 with nearly 7% population prevalence, reaching 47% by 1990. It is in this context that sulfadoxine–pyrimethamine temporarily replaced chloroquine as first line treatment in 2003, pending the introduction of artemisinin-based combination therapy in 2006. The purpose of this study is to assess the *ex vivo* sensitivity to different anti-malarial drugs of the *P. falciparum* population from Pikine. Fifty-four samples were collected from patients with non-complicated malaria and aged between 2 and 20 years in the Deggo health centre in Pikine in 2014. An assay in which parasites are stained with 4′, 6-di-amidino2-phenylindole (DAPI), was used to study the *ex vivo* sensitivity of isolates to chloroquine, amodiaquine, piperaquine, pyrimethamine, and dihydroartemisinin. High resolution melting was used for genotyping of pfdhps, pfdhfr, pfmdr1, and pfcrt genes. The mean IC50s of chloroquine, amodiaquine, piperaquine, dihydroartemisinin, and pyrimethamine were, respectively, 39.44, 54.02, 15.28, 2.23, and 64.70 nM. Resistance mutations in pfdhfr gene, in codon 437 of pfdhps gene, and an absence of mutation at position 540 of pfdhps were observed. Mutations in codons K76T of pfcrt and N86Y of pfmdr1 were observed at 51 and 11% population prevalence, respectively. A relationship was found between the K76T and N86Y mutations and *ex vivo* resistance to chloroquine. In conclusion, an increase in sensitivity of isolates to chloroquine was observed. A high sensitivity to dihydroartemisinin was observed; whereas, a decrease in sensitivity to pyrimethamine was observed in the parasite population from Pikine.

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A BARCODE OF MULTILOCUS NUCLEAR DNA IDENTIFIES GENETIC RELATEDNESS IN PRE- AND POST-ARTEMETHER-LUMEFANTRINE TREATED *PLASMODIUM FALCIPARUM* IN NIGERIA

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Molecular surveillance for drug resistance in malaria-endemic regions is vital to detect the emergence and spread of mutant strains. We observed 89 malaria patients for efficacy of artemether-lumefantrine treatment of uncomplicated *Plasmodium falciparum* infections in Lagos, Nigeria and determined the prevalence of drug resistance in the population. Parasite clearance rates were assessed using *var* gene acidic terminal sequence (*var*ATS) polymerase chain reaction for patients' samples on days 0, 1, 3, 7, 14, 21 and 28 after commencement of treatment. The genomic finger print of pre- and post-treatment parasites were determined using 24 nuclear single nucleotide polymorphisms (SNP) barcode for *P. falciparum*. Drug resistance associated alleles in chloroquine resistance transporter gene *(crt*-76), multidrug resistance genes *(mdr*1-86 and *mdr*1-184), dihydropteroate synthase (*dhps*-540), dihydrofolate reductase (*dhfr*-108) and kelch domain *(K-13* 580) were genotyped by high resolution melt analysis. Twelve (18.5%) of the participants had detectable parasites three days after treatment, while eight (12.3%) individuals presented with day 28 parasitaemia. Barcode (ϖ) pairwise comparisons showed genetic relatedness of day 0 and day 28 parasite isolates in three (37.5%) of the eight re-appearing infections. No mutation in the *K-13* 580 was observed. Evidence of genetic relatedness between pre- and post-treatment infections despite the absence of K-13 580 warrants genomic investigation in a larger population for signs of reduced ACT efficacy in Nigeria.

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CHANGES IN THE PREVALENCE OF DRUG RESISTANCE MEDIATING *PLASMODIUM FALCIPARUM* POLYMORPHISMS ACROSS UGANDA OVER TIME

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Antimalarial drug resistance, mediated in part by known *Plasmodium falciparum* genetic polymorphisms, is worrying. Chloroquine plus sulfadoxine-pyrimethamine (SP) was replaced as the Ugandan treatment regimen by artemether/lumefantrine (AL) in 2006. SP is used to prevent malaria in pregnant women. Recent reports show changing prevalence of key parasite polymorphisms. We conducted surveillance to assess recent trends. Fifty samples were collected in each of 3 surveys every 6 months in 2016-17 from children (6 mo-10 y) diagnosed with malaria by microscopy or rapid test at each of 10 sites across Uganda. Polymorphisms were characterized by PCR and ligase detection reaction fluorescent microsphere assays (transporter and antifolate mutations), copy number (CN) variation was assessed by qPCR, and the K13 propeller domain was sequenced. Prevalences of polymorphisms varied across the country, but temporal trends were similar. For transporters, increasing prevalence of *pfcrt* K76 (65.9-100% at different sites in the most recent survey), *pfmdr1* N86 (all 100%), and *pfmdr1* D1246 (84.6-100%) wild type alleles, all associated with increased susceptibility to aminoquinolines and decreased susceptibility to lumefantrine, were seen. Polymorphisms in antifolate enzymes associated with resistance to SP (*pfdhfr* 51I, 59R, 108T and *pfdhps* 437G and 540E) were very common, and additional polymorphisms associated with higher-level resistance (*pfdhps* 581G 0-44.4% and *pfdhfr* 164I 0-11.4%) were seen, especially in SW Uganda. Other polymorphisms associated with altered sensitivity to lumefantrine (increased *pfmdr1* CN), piperaquine (increased *plasmepsin* CN), and artemisinins (K13 mutations) were uncommon. We identified gradual reversion to wild type transporter alleles with increasing use of AL to treat malaria, persistent prevalence of 5 common antifolate mutations despite decreased use of SP, additional antifolate mutations that predict high level SP resistance, and limited prevalence of additional polymorphisms now mediating ACT resistance in SE Asia. Continued surveillance for drug resistance markers is an important priority.

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SYNTHESIS, CHEMICAL AND BIOLOGICAL VALIDATION OF ARTEMISININ-BASED PROBES FOR ARTEMISININ'S DERIVATIVES ANTIPARASITIC MECHANISM OF ACTION STUDY IN *PLASMODIUM FALCIPARUM*

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Over 3 billion of people are exposed to malaria, caused by *Plasmodium* genus and responsible for about 416.000 deaths in 2016 worldwide. Artemisinin derivatives (ART) are the first-line treatment in most malaria-endemic countries for nearly two decades. The emergence of As reported previously, *Plasmodium falciparum* resistant parasites to ART in Southeast Asia and the fear of their spread to endemic areas with high transmission rate are threatening this treatment. Although progresses on elucidation of ART's mechanisms of action, its exact targets and detailed mechanism of action have not been fully understood yet. ART is thought to be activated in parasite food vacuole by iron (Fe^{2+} or haem). Once activated, it generates carbon-centered radicals via endoperoxide cleavage which in turn alkylate parasite macromolecules, leading to the parasite death, as reported previously. Mechanistic study of ART, based on its behavior in terms of stability, chemical proteomics and imaging approaches are needed to determine its location in the parasite and to identify its targets. To achieve these objectives, three fluorescent dihydroartemisinin-based probes were successfully synthesized and characterized. Furthermore, their stability was assessed by Fluorescent-High performance/Mass spectrometry in different conditions and their biological activity assessed in ART sensitive and resistant strains (*3D7*, *NF54K13* which are sensitives and *NF54K13C580Y* which carries a C580Y mutation on *PfK13* gene responsible for artemisinin resistance). The best probe has proven to be stable overtime (2hours) and very effective on strains with IC_{50} value 22.52 nM, 11.16 nM and 9.55 nM respectively, compared to some major ART-based probes, as reported previously. To this date, there is no other stability study of ART-based probes in those conditions. Abstract ASTMH-2018 Sissoko Abdoulaye Those results allowed us to validate biologically our chemical tools, making them suitable for proteomics studies which are ART's targets tracking by Fluo-PAGE (ongoing promising experiments).

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SURVEILLANCE OF ANTIMALARIAL DRUG EFFICACY IN MANGALORE, KARNATAKA, SOUTH INDIA

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Malaria continues to be a devastating disease in India and Mangalore is endemic to malaria. The aim was to assess antimalarial drug efficacy in Mangalore, South India by clinical and parasitological evaluation. This study was conducted at the Urban health centre and District hospital Mangalore. Clinical history and oral temperature were recorded in patients with microscopically confirmed malaria. Peripheral blood smear was done on day 0, 3, 7, 28 and parasite load was calculated. As per the WHO guidelines clinical response was classified as (i) Early Treatment Failure, (ii) Late Clinical Failure (LCF), (iii) Late Parasitological Failure (LPF) and (iv) Adequate clinical and parasitological response. Therapeutic Failure was considered if any of the above criteria was present. Eight hundred thirty two *Plasmodium vivax, P. falciparum* or mixed infection were identified and included. Six hundred fifty nine patients completed the 28 day follow up (79.2%) while one hundred and seventy three were lost to follow up. As per the policy of the National Vector Borne Disease Control Programme (NVBDCP) of India, *P.vivax* was treated with Chloroquine and Primaquine regimen, while *P. falciparum* and mixed infections were treated with ACT Artesunate+Sulphadoxine+Pyrimethamine and Primaquine regimen. There were a total of 6 cases of LCF out of which 3 cases of *P.vivax* infection later developed *P. falciparum* infection before day 28 with clinical symptoms suggesting LCF of *P. falciparum* to chloroquine. There was 1 case of *P.vivax* infection who later developed *P.vivax* infection again on day 15 suggesting LCF of *P. vivax* to chloroquine. There were 2 cases of *P. falciparum* infection which later developed *P. falciparum* infection again before day 28 suggesting LCF of *P. falciparum* to Artesunate+Sulphadoxine-Pyrimethamine. There were 14 cases of LPF out of which 11 were *P. vivax* infection with persistence of parasite on day 7; 3 of *P. vivax* infection who later developed *P. falciparum* infection on day 28 with no clinical symptoms suggesting LPF of *P. falciparum* to chloroquine. This study demonstrates an estimate of around 3% treatment failure to antimalarials in Mangalore.

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HIGH THROUGHPUT RESISTANCE PROFILING OF *PLASMODIUM FALCIPARUM* INFECTION IN TWO ECOLOGICAL REGIONS OF GHANA USING MOLECULAR INVERSION PROBES AND NEXT GENERATION SEQUENCING

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A key drawback to monitoring the emergence and spread of antimalarial drug resistance in sub-Saharan Africa is early detection and containment. Next-generation sequencing (NGS) methods offers the resolution, sensitivity and scale required to fill this gap during surveillance of molecular markers of drug resistance. We applied the targeted sequencing approach using Molecular Inversion Probes (MIP) for targeting and MISEQ for high throughput sequencing of five *Plasmodium falciparum* genes (*Pfcrt*, *Pfdhfr*, *Pfdhps*, *Pfmdr1* and *Pfk13*) implicated in anti-malarial resistance to chloroquine (CQ), sulphadoxine-pyrimethamine (SP) and artemisinin, A total of 800 dried blood spots on filter paper were collected from symptomatic children, aged between 6 months and 14 years, infected with *Plasmodium falciparum* mono-infections in the coastal savanna (Cape-Coast) and the forest zones of Ghana (Begoro) from 2014 to 2017. DNA was extracted and sequenced on the MISEQ platform after targeting the drug resistance genes using MIPs and then amplification. Molecular markers associated with CQ resistance (*Pfcrt* and *Pfmdr1*) significantly reduced over the four years of study (χ^2=40.57 P<0.0001 for 76T and χ^2=8.76 p = 0.003 for 86Y) except *Pfmdr1*184F which has remained high over the years (68% to 83%). *Pfcrt* 76T has reverted to wild type K76 in the forest ecological zones in Ghana after 13 years of drug removal. The individual markers that make up quintuplet haplotype (IRNGE) which is associated SP efficacy are almost at fixation except E540 which is very low in Ghana; thus making the IRNGE haplotype non-existent in Ghana. In addition to the *Pfdhps* 437G and 540E mutations, this study identified other mutations 436A (41% - 66%) and 613S (6% - 18%) in Ghana. There was no evidence of markers implicated in artemisinin resistance in Ghana. There was a steady progression towards the re-expansion of the chloroquine sensitive parasite K76 although the progress is faster in the forest region than on the coastal belt. This study also identified other mutations associated with pyrithiamine resistance that forms other haplotype combinations that can exacerbate SP resistance in Ghana.

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PLASMODIUM FALCIPARUM IN VIVO CLEARANCE TIME AFTER ARTESUNATE TREATMENT IN MALI

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The molecular mechanisms used by the plasmodium to survive the drug exposure time and expand afterward are unclear. Several studies describe the dormancy phenomenon as a possible way for the parasite to slow down or stop its metabolism in order to over cross the threatening time of drug exposure resulting in post treatment recurrent parasitemia or prolonged parasite clearance time (PCT). In Africa, the few cases of treatment failures with ACTs and delayed PCT are not attributable to the South-East Asian K13 resistance associated mutations. A pilot study in Mali showed that despite high artesunate efficacy and the absence of PfK13 mutations, a significant difference exists between patient's parasite clearance times in two villages. There is the need to investigate parasite and host factor's role in the differential post treatment clearance time. A study is currently being conducted in the Malian village of Faladjé on volunteers over 6 months of age presenting with uncomplicated malaria and treated with artesunate in monotherapy during 7 days. We plan to i)investigate the proportion of viable vs dormant parasite using flow cytometry, ii) assess the relationship between dormancy and parasite clearance time and the occurrence of recurrent parasitemia, and iii) analyze differential parasite transcriptomes under drug pressure. During the last malaria transmission season venus blood and plasma samples were collected for each patient at H0, H8 and H16 Plasma samples were collected prior to treatment and processed on the ArrayCAM® 400-S Microarray Imaging System to assess the role of the host immunity level in the clearance time. To date, 68 patients were included RNA collected and frozen. All the plasma samples were scanned for 250 antigens of *Plasmodium falciparum*. Data analysis is underway and results will be presented at the congress. Result from the study would help in understanding the multifactorial dimension of malaria parasite clearance as well as the molecular or biochemical mechanisms of artemisinin resistance in high transmission areas of sub-Saharan Africa.

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EMERGING SOUTHEAST ASIAN PFCRT MUTATIONS CONFER *PLASMODIUM FALCIPARUM* RESISTANCE TO THE FIRST-LINE ANTIMALARIAL PIPERAQUINE

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The widely-used antimalarial combination therapy dihydroartemisinin plus piperaquine (DHA+PPQ) has failed in Cambodia. Here, we report genomic analysis that reveals rapid proliferation of novel mutations in the *Plasmodium falciparum* chloroquine resistance transporter PfCRT in Cambodia following DHA+PPQ implementation. Using gene editing, we added these mutations into Dd2 parasites or removed them from contemporary Cambodian isolates. Results show that the PfCRT mutations H97Y, F145I, M343L, and G353V each confer resistance to PPQ, albeit at a steep fitness cost for all but M343L. These mutations sensitized Dd2 parasites to the antimalarial drugs chloroquine, amodiaquine, and quinine. Multicopy *plasmepsin 2*, previously reported as a molecular marker, was not necessary for PPQ resistance. Our studies also identified aberrant digestive vacuole morphologies in gene-edited PPQ-resistant Dd2 parasites, but not in the PPQ-resistant Cambodian isolates. Our findings provide compelling evidence that emerging mutations in PfCRT can serve as a molecular marker and mediate PPQ resistance.

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PREVALENCE OF *PFK13*-PROPELLER AND *PFCRT* MUTATIONS IN ISOLATES FROM CHILDREN WITH UNCOMPLICATED MALARIA IN SOUTHWEST NIGERIA

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The emergence of resistance in *Plasmodium falciparum* to commonly used antimalarial drugs, such as Artemisinin derivatives, is a major threat to malaria control and elimination worldwide. One of the surveillance strategies to monitor drug resistance is the analysis of single nucleotide polymorphisms (SNPs) of the associated genes. Monitoring of resistance in malaria-endemic areas is essential to identify strategies to prevent the spread of resistances in the population. *P. falciparum* isolates from children attending three outpatient clinics in southwestern Nigeria were collected on filter paper. Genomic DNA was extracted and the *PfK13*-propeller domain amplified by nested PCR and Sanger- sequenced. In addition, the *Pfcrt* gene at codon 76 was monitored using mutation-specific nested PCR and the restriction fragment length polymorphism (PCR-RFLP) method. The *Pf*K13 propeller domain was sequenced for 90 isolates. Five patients (6%) carried the following synonymous mutations, R528R, A627A, Y493Y, I587I and Y493Y. Mutations of the *Pfcrt* gene at codon 76 (K76T) were observed in 42% of 122 isolates. No significant difference between parasitaemia geometric means in wild type and mutant alleles of the isolates was noted. Of note, two (out of the five) isolates that showed synonymous mutations at the *K13* propeller domain simultaneously featured the *Pfcrt* K76T mutant allele. This cross-sectional study highlights the need for continuous molecular surveillance of antimalarial resistance in Nigeria to develop and adjust national antimalarial treatment guidance. Strikingly, emergence of artemisinin resistance has not occurred yet despite the widespread and lasting routine application of artemisinin-combination therapies, which are mostly produced by national drug companies. Our data support the notion of high standard antimalarial drugs in circulation in Nigeria.

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PREVALENCE OF *PFMDR1*, *PFK13* AND *PFCRT* POLYMORPHISMS DURING A THERAPEUTIC EFFICACY STUDY IN WESTERN KENYA

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WHO recommends that therapeutic efficacy studies (TES), currently considered the gold standard for monitoring parasite resistance to antimalarial drugs, be conducted routinely in endemic countries and that they incorporate surveillance for molecular markers of drug resistance

along with clinical and parasitological efficacy outcomes. As part of TES conducted in 2016-17 to monitor the efficacy of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) for treatment of uncomplicated *Plasmodium falciparum* malaria in western Kenya, we tested for drug resistance markers of *PfK13* propeller domain, *Pfmdr1* and *Pfcrt* genes by sequencing. Additionally, *Pfpm2* gene copy number was assessed by real-time PCR. All the available samples (325 day 0 and 110 recurrent infections) were tested. Sequencing of the *PfK13* gene was successful for 406 samples, of which 11 (2.7%) samples had non-synonymous mutations: 7 had A578S, 2 had S522C, 1 had E596D and 1 had C580F. However, none of these mutations have been associated with artemisinin resistance. For multidrug resistant marker *Pfmdr1*, 418 samples were successfully sequenced. Out of 323 day 0 samples, 1 had N86Y, 192 had Y184F and 30 had D1246Y mutations. Out of 95 samples with recurrent infections, 1 had N86Y, 59 had Y184F and 5 had D1246Y mutations. Overall, 152/418 (36.4%) samples were wild type (NYSND). For *Pfcrt*, 415 samples were successfully sequenced and none had a C72S mutation; 6 samples had M74I, 8 had N75D/E and 8 had K76T mutations. In total, 407/415 (98.1%) samples were wild type (CVMNK). All 12 recrudescent samples (8 from DP and 4 from AL arms) analysed for *Pfpm2* gene copy number had a single gene copy. In summary, we detected no *PfK13* mutations associated with artemisinin resistance in this Kenyan TES. Although a high frequency of *Pfmdr1* Y184F mutations was detected, the implications of the mutation remain unclear. The prevalence of chloroquine resistance markers was very low and there was no increase in *Pfpm2* copy numbers associated with DP resistance. Continued biennial monitoring for resistance mutations is needed for providing timely evidence-based malaria treatment policies.

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DECLINING RESPONSIVENESS OF CHILDHOOD *PLASMODIUM FALCIPARUM* INFECTIONS TO ARTEMISININ-BASED COMBINATION TREATMENTS TEN YEARS FOLLOWING DEPLOYMENT AS FIRST-LINE ANTIMALARIALS IN NIGERIA

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Development and spread of artemisinin-resistant falciparum malaria in Greater Mekong Subregion has created impetus for continuing global monitoring of efficacy of artemisinin-based combination therapies (ACTs). At 14 sentinel sites in six geographical areas of Nigeria, we evaluated treatment responses in 1341 children 6-59 months old and in additional 360 children 6-191 months old with uncomplicated falciparum malaria enrolled in randomised trials of artemether-lumefantrine *versus* artesunate-amodiaquine at 5 years interval in 2009-2010 and 2014-2015 (PACTR201510001189370 and PACTR201709002064150) and at 2 years interval in 2009-2010 and 2012-2015 (PACTR201508001191898 and PACTR201508001193368), respectively after deployment in 2005. Proportions of children with residual asexual parasitemia [asexual parasite positivity] 1 day post-treatment initiation (APPD1) rose from 54 to 62% and 2 days post-treatment initiation (APPD2) from 5 to 26% in 2009-2010 to 2014-2015 (P=0.002 and P<0.0001, respectively). Parasite clearance time increased significantly from 1.6 days (95%CI 1.55-1.64) to 1.9 days (95%CI 1.9-2) and parasite reduction ratio 2 days post-treatment initiation (PRRD2) decreased significantly from a geometric mean of 11000 to 4700 folds per cycle within the same time period (P<0.0001 for each). Parasitemia >75000μL^{-1}, hematocrit >27% 1 day post-treatment initiation, treatment with artemether-lumefantrine and enrolment in 2014-2015 independently predicted APPD1. In parallel, Kaplan-Meier estimated risk of recurrent infections by day 28 rose from 8% to 14% (P=0.005) and from 9% to 15% (P=0.02) with artemether-lumefantrine and artesunate-amodiaquine, respectively. Asexual parasitemia half-time increased significantly from a mean of 1.1 hour to a mean of 1.3 hour within 2 years (P<0.0001). Enrolment in 2012-2015 was significantly associated with 50% increase in mean asexual parasitemia half-time from the baseline. The declining parasitological responses through time to the two ACTs may be due to emergence of parasites with reduced susceptibility or decreased immunity to the infections in these children.

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PAIRWISE COMPETITIONS OF CLONAL *PLASMODIUM FALCIPARUM* ISOLATES CARRYING DIFFERENT *KELCH13* MUTATIONS QUANTIFY FITNESS RELATIONSHIPS

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Drug resistance confers a fitness advantage to parasites exposed to frequent drug pressure, but these mutations often impose a relative fitness cost in the absence of drug pressure. With the emergence of artemisinin (Art) resistance, it is essential to understand the relative competitive fitness of resistant *Plasmodium falciparum* clones in the presence and absence of drug pressure, especially in the context of diverse *kelch13* mutations, to assess their impact on competitive growth outcomes. *In vitro* competitive growth assays provide accurate estimates of relative growth rates of different parasite genotypes as well as a quantitative index of fitness disparities. These readouts can be used to infer relatively fast-growing parasites in monoclonal infections common in Southeast Asia and also provide a glimpse of possible in host dynamics in multi-clone infections. Pairwise competitive growth outcomes of genetically distinct Art-resistant and Art-sensitive clones recently isolated from the Thailand-Myanmar border were evaluated after short perturbations of dihydroartemisinin (DHA) at 700 nM. Our novel *in vitro* 96-well plate competitive growth assays and fluorescent labeled microsatellite markers were used to measure the relative growth densities of each parasite throughout the co-growth period of 80 days. Assessing fitness advantages and costs of Art-resistance *in vitro* could provide insights about how drug resistance will spread and evolve in a competitive environment with infrequent exposure to artemisinin drugs and inform strategies for targeted malaria therapy.

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RE-EXPANSION OF CHLOROQUINE SENSITIVE HAPLOTYPES IN THE *PLASMODIUM FALCIPARUM* RESERVOIR OF INFECTION IN BONGO DISTRICT, GHANA

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Widespread resistance to chloroquine (CQ) informed the switch to artemisinin combination therapy (ACT) for malaria treatment in the early 2000s. CQ sensitive *P. falciparum* have since re-expanded in parts of Africa including Ghana, after withdrawal of CQ use. In Ghana, the genomic signatures of clinical isolates suggest artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) exert differential selection at the two drug transporter genes, *Pfcrt* and *Pfmdr1*. What is happening to the spread of resistance genes in the large reservoir of asymptomatic infections compared to clinical cases has been poorly studied. Hence we investigated this question in Bongo District, Ghana at the end of the 2012 dry season. *P. falciparum* isolates were sequenced at *Pfcrt* (codons 72-76; N=170) and *Pfmdr1* (codons 86,184; N=198). The prevalence of the CQ sensitive haplotype, *Pfcrt* CVMNK was 88.2% and the resistant haplotype, CVIET, was 3.5%. For *Pfmdr1*, the prevalences of the wildtype (NY) and mutant (NF) were 17.7% and 69.7%, respectively. To investigate selection of these haplotypes, isolates with single genomes were further typed at microsatellite loci linked to *Pfcrt* (N=48) and *Pfmdr1* (N=46). The percentage of unique multilocus haplotypes was high for *Pfcrt* CVMNK (95.8%) and the diversity was also high (H_e=0.75), suggestive of soft selective sweeps. Our results indicate that the CQ sensitive parasites have re-expanded from a genetically diverse parasite reservoir that predates ACT introduction. For *Pfmdr1* NY and NF, all isolates had unique multilocus haplotypes, and there was no significant difference in the H_e (NY: 0.85, NF: 0.83), suggestive of equal selective advantage. ACTs have a role in diversifying *Pfmdr1*, which warrants further investigation to decipher the patterns of selection. We have shown that the asymptomatic reservoir, which has little exposure to antimalarial drugs at the end of the dry season, harboured highly diverse *Pfcrt* and *Pfmdr1* haplotypes. The treatment policy change has influenced this diversity and as such, it is important to prioritize this reservoir during monitoring programs to inform policy.

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PLASMODIUM FALCIPARUM TRIPLE MUTANT IN CAMBODIA: PHENOTYPIC CHARACTERIZATION OF RESISTANCE

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Despite a large reduction of malaria prevalence in part due to Artemisinin based combination therapies (ACTs) implementation, Cambodia is still the epicentre of *Plasmodium falciparum* multidrug resistance. The consecutive use of the artesunate-mefloquine (AS-MQ) and dihydroartemisinin-piperaquine (DHA-PPQ) combinations in this country between 2000 and 2016 has selected for both artemisinin and partner drug resistance. As a result, a high treatment failure rate was observed for these two combinations and ACTs resistance is now widespread in the Greater Mekong Subregion (GMS). The situation is made worst by the recent *P. falciparum* triple-mutant parasites emergence in Cambodia, exhibiting the three genotypes associated with both artemisinin, mefloquine and piperaquine resistance. However, the impact of this triple-genotype combination on treatment efficiency has not yet been studied, and the *in vitro* phenotype of these parasites still has to be characterized. We observed that the *P. falciparum* triple-mutant parasites found in Cambodia (presenting *Kelch13* mutation and amplification of *Pfmdr1* and *Pfplasmepsin2* genes) were not associated with treatment failure in patients. In addition, *in vitro* susceptibility assays (piperaquine survival assay and mefloquine IC_{50} determination) have shown that this triple-resistant genotype was not associated with a triple *in vitro* resistance to both artemisinin, mefloquine and piperaquine. An inverse correlation between piperaquine and mefloquine resistance was even observed *in vitro*, suggesting a too important cost for triple-mutant parasites fitness to express both *Pfmdr1* and *Pfplasmepsin2* genes. Altogether, our results allow a better characterization of triple-mutant *P. falciparum* parasites observed in Cambodia, and are reassuring concerning the dangerousness level of these parasites. Nonetheless, further efforts to strengthen the triple-mutant parasites monitoring remain crucial since the triple combination therapy (artemisinin, mefloquine and piperaquine) is considered by WHO as a possible treatment for uncomplicated *P. falciparum* Malaria.

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DIAGNOSING A RARE CAUSE OF MASSIVE SPLENOMEGALY IN RURAL RWANDA

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A 21-year-old female with a history of recurrent malaria presented in rural Rwanda with two weeks of hematemesis, malaise, and abdominal pain, in the setting of chronic abdominal distention. On exam, her abdomen was diffusely tender, distended, with grade V massive splenomegaly extending beyond the midline and umbilicus. Labs showed WBC 3,000/uL, Hb 9 g/dL, HCT 27%, PLT 40,000/uL with 6% reticulocytes. Thick smear was negative for malaria and peripheral blood smear showed microcytosis and macrocytosis without malignant cells. In this limited-resource area, anti-malarial antibodies could not be obtained. She was Hepatitis C antibody positive. Abdominal ultrasound showed massive splenomegaly (diameter 30 cm), portal hypertension, and ascites. She was diagnosed with Hyperreactive Malarial Splenomegaly (HMS) and started on three days of artemether/lumefantrine, doxycycline, and folic acid without response. Clinical improvement may take weeks. On day seven, she went into status epilepticus and vomited several times, was intubated, and passed away the next day. Urine culture grew Klebsiella pneumonia on day seven, although she was asymptomatic. It is plausible that a urinary tract infection led to her seizures and respiratory failure from aspiration led to her death. HMS is a diagnosis of exclusion based on Fakunle's major criteria: Gross splenomegaly, elevated IgM, high anti-malarial antibodies, clinical response to antimalarial therapy. In case studies from malaria-endemic areas, excluding other causes of splenomegaly was adequate in diagnosing HMS. Massive splenomegaly presents in certain conditions, such as leukemia, lymphoma, or thalassemia—deemed less probable—and HMS. Hematology was consulted and based on her history, massive splenomegaly, cytopenias, and peripheral blood smear without malignant features, a clinical diagnosis of HMS was made. HMS is a complication of recurrent malaria, in which chronic antigenic stimulation with repetitive Plasmodium species infections is postulated to lead to overproduction of IgM and splenic sequestration. Prompt recognition of HMS is vital, and if left untreated it is often fatal.

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CORRELATION BETWEEN THROMBOCYTOPENIA AND ANEMIA IN *PLASMODIUM FALCIPARUM* MALARIA AMONG PATIENTS IN KISUMU COUNTY-WESTERN KENYA

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Malaria is associated with haematological complications which may be avoided by early diagnosis and treatment. Microscopic diagnosis showing presence of malarial parasites remains the gold standard and is needed for

confirmation which requires technical expertise. The study was therefore carried out to determine the correlation between thrombocytopenia and anaemia in *Plasmodium falciparum* malaria. The study was conducted in Kisumu County-Kenya which is a highly endemic malaria region. Both thick and thin blood smears were used to determine *P. falciparum* infection status in a total of 228 patients comprising of 157 infected and 71 non-infected patients presenting with acute febrile illness. All participants' demographics and haematological parameters i.e. Haemoglobin level, platelet count, mean platelet volume and platelet distribution width were analysed. Haemoglobin and platelet count were significantly lower in the malaria infected group (p<0.001). Conversely, mean platelet volume and platelet distribution width were higher in comparison to non-infected group (p<0.001). Severe to moderate anaemia was present in 78 %(n=122) of malaria infected patients against 47 % (n=33) of the non-infected group (p<0.001) while thrombocytopenia was present in 87 %(n=137) of the infected patients against 10 % (n=7) of the non-infected group (p<0.001). There was a positive correlation between anaemia and thrombocytopenia in malaria (r= 0.26, p<0.001). In conclusion, low platelet count and Haemoglobin levels positively correlate and can be important predictors of *P. falciparum* malaria when used in combination with other clinical manifestations. This could help in avoiding unnecessary expensive tests to rule out other febrile conditions. The above findings also have therapeutic implications of avoiding unnecessary platelet infusion in malaria.

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RELAPSE ACCOUNTED FOR AN OVERWHELMING NUMBER OF VIVAX MALARIA CASES BOTH IN THE MAJOR AND MINOR TRANSMISSION SEASONS OF ETHIOPIA

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Plasmodium falciparum and *P. vivax* are co-endemic in Ethiopia and the latter accounts for about 40% of malaria cases. A single infection with *P. vivax* and *P. ovale* causes repeated bouts of illness weeks to months after the primary infection. Efforts to control and eventual elimination will be challenged in the absence of drugs able to cure both the blood stage and the liver stage infections, and thereby prevent both relapse and recrudescence. Indeed, the proportion of vivax malaria cases due to relapse in the wet and dry seasons remain unaddressed. Seasonal patterns in malaria cases were analysed using statistical models, identifying the peaks in cases, and the seasonally varying proportion of *P. vivax* cases attributable to relapses. A total of 3,161 febrile patients visited Adama malaria diagnostic center. Finger-prick blood samples were collected for thin and thick blood film preparation. The proportion of patients with malaria detectable by light microscopy was 36.1% (1141/3161) of which *P. vivax*, *P. falciparum*, and mixed infections accounted for 71.4, 25.8 and 2.8%, respectively. Of the febrile patients diagnosed, 2134 (67.5%) were males and 1919 (60.7%) were urban residents. The model identified a primary peak in *P. falciparum* and *P. vivax* cases from August to October, as well as a secondary peak of *P. vivax* cases from February to April attributable to cases arising from relapses. During the secondary peak of *P. vivax* cases approximately 77% (95% CrI 68, 84%) of cases are estimated to be attributable to relapses. During the primary peak from August to October, approximately 40% (95% CrI 29, 57%) of cases are estimated to be attributable to relapses. It is not possible to diagnose whether a *P. vivax* case has been caused by blood-stage infection from a mosquito bite or a relapse. However, differences in seasonal patterns of *P. falciparum* and *P. vivax* cases can be used to estimate the population-level proportion of *P. vivax* cases attributable to relapses. These observations have important implications for initiating therapy that is effective against both blood stages and relapses.

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STABILIZATION OF RDT TARGET ANTIGENS PRESENT IN DRIED *PLASMODIUM FALCIPARUM*-INFECTED SAMPLES FOR VALIDATING MALARIA RAPID DIAGNOSTIC TESTS AT THE POINT OF CARE

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Malaria rapid diagnostic tests (RDTs) are a great achievement in implementation of parasite-based diagnosis as recommended by World Health Organization. A major drawback of RDTs is the lack of positive controls to validate different batches at the point of care. Dried *Plasmodium falciparum*-infected samples with RDT target antigens have been suggested as a possible positive control but their utility in resource limited settings is hampered by rapid loss of activity over time. In this study we evaluated effectiveness of chemical additives to improve long term storage stability of RDT target antigens (HRP2, pLDH and aldolase) in dried *Plasmodium falciparum*-infected samples using parasitized whole blood and culture samples. Samples were treated with ten selected chemical additives mainly sucrose, trehalose, LDH stabilizer and their combinations. After baseline activity was established, the samples were air dried and stored at room temperatures (~25°C). Testing of the stabilized samples using SD Bioline, BinaxNOW, CareStart, and First Response was done at intervals for 53 weeks. Stability of HRP2 at ambient temperature was reported at 21 to 24 weeks while that of PAN antigens (pLDH and aldolase) was 2-18 weeks of storage at all parasite densities. The ten chemical additives increased the percentage stability of HRP2 and PAN antigens. Sucrose alone and its combinations with Alsever's solution or biostab significantly increased stability of HRP2 by 56% at 2000p/µL (p<0.001). Trehalose and its combinations with biostab, sucrose or glycerol significantly increased stability of HRP2 by 57% (p<0.001). Unlike sucrose, the stability of the HRP2 was significantly retained by trehalose at lower concentrations (500p/µL, and 200p/µL). Trehalose in combination biostab increased the percentage stability of PAN antigens by 42%, and 32% at 2000p/µL and 500p/µL respectively (p<0.01). This was also the best chemical combination with the shortest reconstitution time (~<20minutes). These findings confirm that stabilizing RDT target antigens in dried *Plasmodium falciparum*-infected samples provides field-stable positive controls for malaria RDTs.

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DETECTION OF SUBMICROSCOPIC MALARIAL PARASITES USING THE ILLUMIGENE MALARIA LAMP

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In the context of low transmission of malaria, the challenge is to be able to detect low parasite densities which could be missed when using routine microscopy. Yet, those submicroscopic infections may be the reservoirs of the parasites contributing to maintain the disease transmission and this could hamper the malaria elimination efforts. We selected 200 malaria suspected patients in three malaria hypoendemic sites of Senegal. We performed from each patient blood sample 1) a thick and thin smear slide for microscopy, 2) the illumigene Malaria LAMP test S-PREP (raw DNA extraction) and M-PREP (DNA extraction followed by chromatography purification). Slides were stained using 10% Giemsa and were read by WHO level 1 certified microscopists. The results of microscopy were

then compared to those of LAMP. Considering illumigene LAMP S-PREP as standard, we noted sensitivity and specificity of 90% and 100% respectively and an agreement of 91% between the two techniques. Microscopy sensitivity was even lesser (83.3%) when illumigene M-PREP was the standard; however, the specificity remained identical (100%). Thus, the prevalences of submicroscopic malaria infections were 10 % and 16.7% respectively using the illumigene LAMP S-PREP and the illumigene M-PREP protocols. The illumigene Malaria test may be used suitably in malaria low transmission in order to detect submicroscopic malarial parasites. This would help policies targeting parasites reservoirs to interrupt the transmission chain.

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AUTOMATED MALARIA PARASITE CLASSIFICATION ON THICK BLOOD SMEARS

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Malaria is a worldwide life-threatening disease. According to the 2017 World Health Organization malaria report, an estimated 216 million malaria cases were detected in 2016, causing approximately 445 000 deaths. Microscopy examination of stained thick and thin blood smears is the gold standard for malaria diagnosis. Thick blood smears contains more blood and are used to detect the presence of malaria parasites, while thin blood smears are used to differentiate parasite species. Microscopy examination is of low cost and is widely available, but is time-consuming. Moreover, the effectiveness of microscopy diagnosis depends on the parasitologists' expertise in the subject. Therefore, the development of a computerized system to aid in malaria diagnosis is an appealing research goal. Using deep learning for machine classification, this work proposes a convolutional neural network (CNN) model for parasite classification to automatically classify preselected malaria parasite candidates in digital images of thick blood smears. Our customized CNN model consists of 3 convolutional layers, two fully connection layers and a softmax classification layer. Following each convolutional layer, a batch normalization layer, an activation layer, and a max-pooling layer are introduced to select feature subsets. Experiments are performed on 1817 blood sample images from 150 infected patients, which were acquired via Mahidol-Oxford Tropical Medicine Research Unit in Bangkok, Thailand, and manually annotated by an experienced parasitologist. For training, a total number of 84,894 positive parasite patches are cropped from the images based on the experts' annotations, and an equal number of negative patches (distractors) are generated using an intensity-based greedy method. A patient-level five-fold cross-validation demonstrates the effectiveness of our customized CNN model in discriminating between positive and negative patches. . We compute the following performance indicators for our system: accuracy (98.78%±0.12%), AUC (99.88%±0.05%), sensitivity (98.58%±0.61%), specificity (98.99%±0.58%), and precision (98.99%±0.57%).

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EVALUATION OF THE PERFORMANCE OF SD BIOLINE *PF/PV* TESTS FOR THE DIAGNOSIS OF MALARIA IN A MALARIA ENDEMIC AREA OF ETHIOPIA

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Early and accurate diagnosis of malaria followed by prompt treatment reduces morbidity and mortality in endemic regions. Presumptive treatment of malaria is widely practiced where microscopy or rapid diagnostic tests are not readily available. Introduction of rapid diagnostic tests (RDTs) for the treatment of malaria in many low-resource settings need evaluation of their performance. This study evaluated the performance of RDT in two health centers in Adam and Amaya. This study aim to evaluate the diagnostic performance of SD BIOLINE malaria Ag *Pf/Pv* test relative to microscopy for the diagnosis of *Plasmodium falciparum* and *P. vivax* malaria in Ethiopia. The study was cross-sectional study from November to December 2015 on patients who had malaria symptoms and visited the two health facilities in Oromia Region, Ethiopia. Thin and thick blood smears were prepared from finger prick and stained by 10% Giemsa. Microscopic examination was done under 100x magnifications for *Plasmodium* species identification and determination of parasitemia. The RDT was performed as per the manufacturer instruction. From A total of 547 febrile patients were diagnosed, of which 127 were microscopy positive for *Pf* (n=38) and *Pv*(n=85). The sensitivity, specificity, positive and negative predictive value of SD BIOLINE malaria Ag *Pf/Pv* test were 92.1%, 99.1%, 95.9% and 98.2%, respectively. Therefore, we conclude the diagnostic performance of SD BIOLINE malaria Ag *Pf/Pv* test in this study shows good performance and further study is recommended in different study areas with different epidemiological status.

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DETECTION OF MALARIA THROUGH AUTOMATED DIGITAL MICROSCOPY WITH INTEGRATION OF ARTIFICIAL INTELLIGENCE

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Traditionally microscopy has been used as gold standard in malaria diagnosis. However, it has limitations to be applied to low resources settings because it needs well-equipped laboratory which is costly and skilful technicians to conduct complicated diagnostic experiments. Accuracy of the result is also limited depending on expertise of technicians and quality of experimental resources. Addressing these challenges, we have developed a fully automated digital microscopy platform. The platform enables diagnosis of the disease by simply dropping finger-pricked blood on a consumable chip (Lab on a Chip technology based), conducting automated blood smearing and staining inside the platform, capturing high-resolution blood images by internal digital microscopy, and analyzing them by artificial intelligence (AI). Its network-compatible platform also provides function for surveillance by delivering real-time data to any malaria control program, and instantly and simultaneously collecting and presenting epidemiological data with in-house developed software. We assessed the platform's performance in Cambodia and Malawi. It took one day to train technicians for their adept operation of the platform. Diagnosis time was 15 to 20 minutes per patient. Its detection level of parasitemia was 20p/μl. The AI's sensitivity was 96.21%, and specificity was 97.94%. Through its embedded 3G network, real-time diagnosis data was collected and shared with other networked systems from remote regions. We found that the digital microscopy platform with AI integrated has potential to overcome limitations of traditional microscopy which is operated by technicians in typical laboratory settings.

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USE OF CAPILLARY BLOOD LEADS TO HIGHER PARASITEMIA ESTIMATES AND HIGHER DIAGNOSTIC SENSITIVITY OF MICROSCOPIC AND MOLECULAR DIAGNOSTICS OF MALARIA THAN VENOUS BLOOD SAMPLING

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Simple and cost-efficient diagnostic methods are needed for the control of malaria, as it occurs mostly in resource-limited settings. Malaria is usually diagnosed from samples of peripheral blood. However, it is still unclear, whether venous or capillary blood samples provide equal diagnostic performance. This study investigated quantitative differences of parasitemia between capillary and venous blood and the diagnostic performance characteristics of the two blood sources. Recruitment took place between September 2015 and February 2016 at two clinical research centers in Gabon and 376 patients were included. Light microscopy and qPCR were used to quantify peripheral parasitemia of paired capillary and venous blood samples. Performance characteristics of capillary and venous samples using microscopy were evaluated against a qPCR gold-standard. Light microscopy revealed a higher parasitemia of +16.6% (95% CI: +0.7 to +35) in capillary samples compared to venous samples. Concordant evidence was produced in qPCR, showing that on average -0.278 (95% CI: -0.473 to -0.083) ct-cycles were required until signal detection in capillary samples. Sensitivity analyses revealed an 8% higher sensitivity of capillary blood samples (81.5%; 95% CI: 77.4 to 85.6) compared to venous blood samples (73.4%; 95% CI: 68.8 to 78.1). Capillary blood sampling improves diagnostic sensitivity and leads to higher results of parasitemia compared to venous blood sampling. Solely using capillary blood for the detection of malaria could constitute a simple and cost-efficient optimization of malaria diagnosis. This finding may have important implications for routine diagnostics of malaria, epidemiological and drug development studies and the assessment of novel diagnostic tools.

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COMPARING DETECTION OF MALARIA PARASITES BY POLYMERASE CHAIN REACTION, RAPID DIAGNOSTIC TEST, BLOOD SMEAR, HISTIDINE-RICH PROTEIN 2 (HRP2) ANTIGENEMIA, AND LONG AND SHORT TERM IGG ANTIBODIES

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Declining malaria prevalence in some previously high transmission zones has made measurement of sustained decrease in malaria transmission difficult, and has increased the need for cost-effective methods to detect low density infections. Multiplex detection of parasite antigens and antibodies to malaria antigens is a promising strategy for measuring population exposure to malaria and for detecting infections characterized by low-level parasitemia. Patients seeking care for fever were enrolled, and blood smear and dried blood spots on filter paper were collected at the time of the rapid diagnostic test (RDT) for diagnosis. Smears were read by expert microscopists. Samples were analyzed with PET-PCR, and multiplex assays were used to measure median fluorescence intensity (MFI) of HRP2 antigen and long (PfMSP1 and PfAMA1) and short (PfCSP, PfHRP2, LSA1, GlurpRO, StarpR, and SALSA) half-life IgG antibodies to *Plasmodium falciparum*. We compared the results of RDT, expert microscopy, PCR, HRP2 antigenemia, and IgG antibody markers. Using PET-PCR as the gold standard, blood smear had a sensitivity of 90% and a specificity of 100%, RDT had a sensitivity of 95% and a specificity of 94%, and HRP2 detection by multiplex assay had a sensitivity of 98% and a specificity of 48%. Adding short- and long-half-life antibodies to HRP2 antigenemia increased sensitivity to 100% and decreased specificity to 9%, using the PET-PCR assay as the gold standard for diagnosing active infection. No long- or short-half-life antibodies, either individually or in combination, approached the sensitivity or specificity of RDT, blood smear, or HRP2 multiplex assay, though logistic regression demonstrated a positive association of LSA1, StarpR, and GlurpRO antibodies with PCR positivity. Detection of HRP2 antigenemia by multiplex assay was more sensitive than RDT or smear for *P. falciparum* infection. Testing with a combination of HRP2 antigenemia detection and measurement of long- and short-half-life antibodies was a sensitive predictor of PCR positivity, and could be useful for screening RDT-negative samples from low-prevalence settings for further analysis.

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PERFORMANCE OUTCOMES FROM AFRICA-BASED MALARIA DIAGNOSTIC REFRESHER TRAINING COURSES

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Since 2010, the World Health Organization has recommended parasite-based diagnostic testing for all patients suspected of having malaria before a treatment regimen is started. Lack of funding and rigorous programs to support continuous training and monitoring of staff may contribute to poor malaria diagnosis resulting in improper management of illness. The MalariaCare project was tasked with implementing an external quality assurance scheme to support malaria diagnostics and case management across a spectrum of health facilities in participating African countries. A component of this program was a 5-day, competency-based, malaria diagnostic refresher training (MDRT) course for health facility laboratory staff conducting malaria microscopy. The MDRT course provided a method to quantify participant skill levels in microscopic examination of malaria across 3 major diagnosis areas: parasite detection, species identification, and parasite quantification. A total of 817 central, regional, and peripheral-level microscopists from 45 MDRT courses across 9 African countries were included in the analysis. Differences in mean scores with respect to daily marginal performance were positive and statistically significant ($p < 0.001$) for each challenge type when considering the entire cohort of MDRT participants. From pretest to assessment day 4, mean scores for parasite detection, species identification, and parasite quantification increased by 19.1, 34.9, and 38.2 percentage points, respectively. Additionally, sensitivity and specificity increased by 20.8 and 13.8 percentage points, respectively, by assessment day 4. Further, the ability of MDRT participants to accurately report *Plasmodium falciparum* species when present increased from 44.5% at pretest to 67.1% by assessment day 4. The MDRT course has shown to rapidly improve the microscopy performance of participants over a short period of time. Due to its rigor, the MDRT course could serve as a mechanism for measuring laboratory staff performance against prescribed minimum competency standards and could easily be adapted to serve as a national certification course.

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GENERATION OF ANTIBODIES SPECIFIC TO *PLASMODIUM FALCIPARUM* HISTIDINE RICH PROTEIN-3

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Rapid diagnostic test (RDT) based detection of malaria infections caused by *Plasmodium falciparum* relies to a large extent based on detecting *Plasmodium falciparum*-specific histidine-rich protein 2 (HRP2). HRP2-based tests comprise a majority of the over 300 million RDTs sold annually worldwide. However, malaria parasites that don't express the HRP2 antigen can lead to false negative test results with this diagnostic method. Histidine-rich protein 3 (HRP3), another soluble antigen specifically produced by *P. falciparum*, shares 85-90% sequence homology with HRP2, and anti-HRP2 monoclonal antibodies are known to cross-react with this protein. *P. falciparum* may lack one or both genes for HRP2 and HRP3 and the ability to distinguish specific reactivity to either protein is essential to surveillance efforts aimed at determining the continued utility of HRP2-based tests in different areas of the world. Here we describe the generation of IgG antibodies specific to *P. falciparum* HRP3 which do not cross-react with HRP2 as determined by ELISA and western blot immunoassays. A 28-amino acid long peptide corresponding to the non-repeat region of HRP3 was synthesized and utilized for rabbit immunizations. Purified IgG antibodies from immunized animals specifically detected native HRP3 antigen in *P. falciparum* Dd2 culture strain supernatants which only expressing HRP3 and not HRP2. Purified native HRP3 from Dd2 culture allowed estimation of the limit of ELISA detection of these antibodies for HRP3 at 250pg/mL. These anti-HRP3 antibodies failed to react to HB3 culture supernatants (only expressing HRP2) and did not bind to recombinant HRP2 protein. An assay that combined detection of HRP2 and HRP3 antigen would offer improve differentiation of parasites that lack HRP3 gene from HRP2 deleted parasites in field surveys and surveillance of the global deletion phenomenon.

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FIELD EVALUATION OF THE ILLUMIPRO-10 ILLUMIGENE MALARIA DIAGNOSTIC BY AMPLIFICATION OF *PLASMODIUM SPP* DNA: A VIABLE OPTION TO ACCELERATE MALARIA ELIMINATION

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Malaria is one of the major health problems in Zambia. Zambia has set a goal of eliminating malaria by 2021. By ensuring 100% suspected malaria cases receive parasitological diagnosis and 100% parasitologically confirmed malaria cases receive prompt, effective treatment. Currently, malaria diagnosis relies on parasite detection by microscopy or antigen-based rapid diagnostic tests (RDT). Although microscopy and RDT have been rolled out there are still limitations that compromise their effectiveness. This is particularly important to help identify and manage patients and ultimately elimination of malaria. There is a need to identify newer, more sensitive and specific but cost effective methods for malaria diagnosis. Performance of Illumigene Malaria LAMP, microscopy and RDT against Real Time PCR as the reference standard were evaluated. 282 study participants with signs and symptoms of *Plasmodium* infection were recruited from Nchelenge, Zambia. Whole blood specimens were used. Of the 282 samples RDT positive were 47.9%, microscopy 34%, Illumigene 66% and RT-PCR 64.5%. Sensitivity versus Specificity of RDT, microscopy and Illumigene compared with the gold standard RT-PCR for the diagnosis of Plasmodium species at 95% CI were 69.8% Vs 92%, 53.3%Vs 100% and 94.54% Vs 86% respectively. Performing tests for diagnostic accuracy, Illumigene showed the highest diagnostic sensitivity and strong agreement (k=0.812) with RT-PCR as the reference standard. Sensitivity for both RDT and microscopy were lower. Discordant results were observed across the four tests with low sensitivity and false negatives by RDT and microscopy. Overall, a total of 19.6% and 33.6% did not receive treatment based on RDT and microscopy respectively, creating a transmission reservoir. Though sensitivity of RDT is higher than microscopy, it is not comparable to molecular based methods. The field deployment of molecular testing, should address sensitivity challenges observed with RDT and microscopic tests in this study to achieve malaria elimination. A comparative study in a low malaria endemic region is warranted.

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DURATION OF MALARIA RAPID DIAGNOSTIC TEST (RDT) POSITIVITY FOLLOWING DEFINITIVE ANTIMALARIAL TREATMENT AMONG CHILDREN IN WESTERN KENYA

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For *Plasmodium falciparum* infections, Rapid Diagnostic Tests (RDTs) targeting the histidine-rich protein (HRP2) antigen are more sensitive than tests targeting the lactate dehydrogenase (pLDH) antigen. Interpretation of HRP2 RDTs in the weeks after effective treatment is a challenge due to antigen persistence. The Kenya Ministry of Health (KMoH) guidelines recommend that symptomatic patients diagnosed and treated for malaria in the last two weeks should be tested by microscopy. We performed a study to evaluate the duration of RDT positivity following definitive antimalarial treatment in children <5 years of age with confirmed

uncomplicated malaria in a high malaria transmission setting in western Kenya. From June 2016 to March 2017, 340 children with *P. falciparum* mono-infection were randomly assigned to treatment with artemether-lumefantrine or dihydroartemisinin-piperaquine. Blood samples collected at day 0, 2, 3 and weekly to day 42 post-treatment were tested by microscopy and three RDTs— CareStart (HRP2), First Response (HRP2/pLDH), and SD Bioline (HRP2/pLDH). The duration of RDT positivity was assessed by Kaplan-Meier analysis with data censored at reinfection or drop out. At 14 days, 80% of HRP2 RDTs remained positive. Median time to negative result was 35 days; 33% (95% CI 27-38%), 34% (95% CI 28-39%), and 34% (95% CI 28-39%) of tests stayed positive at day 42 for First Response, Carestart, and SD Bioline HRP2 tests, respectively. Median time to negative result was <1 day for pLDH RDTs; 9% (95% CI 5-12%), and 5% (95% CI 3-8%) of tests remained positive by day 3 for First Response and SD Bioline, respectively. Duration of positivity and baseline parasite density were positively correlated (<50,000/µl vs. ≥50,000/µl; p<0.035) for HRP2 RDTs only. All HRP2 RDTs had a similar duration of positivity, and the median duration was >2 times the timeframe that KMoH guidelines recommend for testing with microscopy after a previous malaria treatment. Further evaluations of the RDT positivity persistence after definitive antimalarial treatment should be done to inform policy on appropriate timeframes for the use of alternate diagnostics in settings where this is possible.

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LESSON LEARNED: DEVELOPMENT OF NATIONAL ARCHIVE OF MALARIA SLIDES (NAMS) IN THE DEMOCRATIC REPUBLIC OF CONGO (DRC)

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The accuracy of malaria diagnosis by microscopy has been a challenge in health facilities in the DRC due to inadequate training, quality assurance, and maintenance of microscopy skills. In order to improve quality assured malaria microscopy in the DRC, MalariaCare supported the Institut National de Recherche Biomédicale (INRB) and National Malaria Control Program to develop the National Archive of Malaria Slides (NAMS). This slide bank will be used for microscopy training, supervision and proficiency testing. 51 blood samples of *P. falciparum*, non-*falciparum*, mixed infections and negatives were collected in the DRC from May 2016 through June 2017. The NAMS protocol was developed by the INRB in collaboration with MalariaCare following the WHO protocol and approval by an ethics board. As there was no *P. vivax* present during blood collection, 200 validated *P. vivax* slides were purchased externally. All slides were developed at the INRB and barcode labels were applied to each slide. Two slides per donor were selected for slide validation by six WHO-accredited level 1 microscopists in the DRC and Mali. Each blood donor was validated by PCR to confirm malaria infection and species. The quality of each slide was validated. Microscopy detection of infection showed high sensitivity (98%) and specificity (100%) when compared to PCR, whereas microscopy species detection was 84% (range: 74% - 90%) concurrent with PCR. Among 51 blood donors, there were 3 discrepancies between the PCR and microscopy results and these were excluded from the slide bank. The remaining 48 donor samples were accepted and used to produce 18,369 fully validated malaria slides. The DRC NAMS will be used for national and regional malaria microscopy training and can be used throughout the country to perform proficiency testing and on-site supervision. The NAMS will be an important tool in the DRC's efforts to improve the quality of clinical care and also will assure the ability to develop new generations of laboratory specialists as the country moves towards improved case management and control of malaria.

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ANTIMALARIAL AND ANTISCHISTOSOMAL EFFICACY OF PYRIDOBENZIMIDAZOLE DERIVATIVES

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Tropical diseases, exemplified by malaria and schistosomiasis-the two most prevalent parasitic infections endemic in Africa, South East Asia and parts of South America-present daunting public health and socio-economic challenges. Besides geographical reasons, poor hygiene and sanitation encourage the transmission of these infections, sustaining a cycle of poverty in these regions. Public health approaches have been instrumental in the control of malaria and schistosomiasis leading to a decline in their prevalence during the last century. Chemotherapy, however, remains a fundamental component in the treatment of these infections with resistance constantly posing a threat to the gains made towards eradicating malaria. Conversely, over reliance of a single drug, praziquantel, for the treatment of schistosomiasis evokes concerns of resistance developing in future. Concerted efforts must, therefore, be sustained in the search for new drugs to curb these diseases of poverty. Phenotypic whole cell screening has continued to deliver new hits for drug discovery and development. Alternatively, drug repositioning, whereby a drug or lead compound provides a medicinal chemistry template to yield analogues for testing in a different disease other than the initial one, has become increasingly popular. Drug repositioning holds the potential to reduce drug development costs and timelines; an appealing substitute in drug discovery for diseases of poverty which have little, if any, commercial incentives to the pharmaceutical industry. In our work, a pyridobenzimidazole hit previously identified after a whole cell screening assay against human *Plasmodium falciparum* has been utilised to generate analogues, several of which have shown potent *in vitro* and *in vivo* antimalarial activities. In a drug repositioning context, exemplified by the antischistosomal efficacy of several known antimalarial agents, we evaluated the antischistosomal activity of these analogues. Further medicinal chemistry optimisation is ongoing to progress these compounds as prospective antimalarial and antischistosomal leads.

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IN SILICO STUDY OF PLASMODIUM 1-DEOXY-DXYLULOSE 5-PHOSPHATE REDUCTOISOMERASE (DXR) FOR IDENTIFICATION OF NOVEL INHIBITORS FROM SANCDB

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Malaria remains a major health concern with a complex parasite constantly developing resistance to the different antimalarials, threatening the efficacy of the current (Artemisinin Combination Therapy) ACT. Drugs with different mechanisms of action are ideal to decrease chances of resistance occurring. Blocking the methylerythritol phosphate pathway through 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) inhibition fits well this profile. Previously, a promising DXR inhibitor, fosmidomycin showed poor drug-like properties. In this study, we intended to find potential inhibitors with better drug likeness from the South African National Compound Database (SANCDB; https://sancdb.rubi.ru.ac.za) using structural bioinformatics tools. *Plasmodium spp* DXR structures in its open and closed conformations were modelled using MODELLER followed

by a High Throughput Virtual Screening (HTVS) with Autodock Vina of the SANCDB compounds to identify hits. Compounds' pharmacological properties using the QED (Quantitative estimate of druggability) scores from FAF-Drugs4 (Free ADME-Tox Filtering Tool) were included in the hit selection process. Finally, selected hits were submitted to 100 ns molecular dynamics (MD) simulations using GROMACS to assess the stability of the protein-ligand complexes. SANC00152 (-8.4 Kcal/mol), SANC00236 (-10.2 Kcal/mol), SANC00339 (-9.2 Kcal/mol), SANC00438 (-9.9 Kcal/mol) and SANC00570 (-8.1 Kcal/mol) were identified as hits from the docking showing good binding affinities with QED scores of 0.46, 0.68, 0.83, 0.78 and 0.68 respectively with one being the best score and zero the poorest. All compounds had different scaffolds from fosmidomycin. Finally, the different protein-hit complexes appeared to be stable during the MD simulations with Root Mean Square Deviation (RMSD) lower than 2.75 nm (maximum RMSD observed). Furthermore, these hits showed stable binding in the protein active site with ligands' RMSDs showing very low values (maximum being 0.15 nm), thus confirming their high binding affinities. Five natural compounds from SANCDB were thus identified as potential DXR inhibitors.

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DECIPHERING THE TARGETS OF RETROVIRAL PROTEASE INHIBITORS IN *PLASMODIUM BERGHEI*

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Retroviral protease inhibitors (RPIs) such as lopinavir (LP) and saquinavir (SQ) are active against *Plasmodium* parasites. However, the exact molecular target(s) for these RPIs in the *Plasmodium* parasites remains poorly understood. We hypothesised that LP and SQ suppress parasite growth through inhibition of aspartyl proteases. Using reverse genetics approach, we embarked on separately generating transgenic parasite lines lacking Plasmepsin 4 (PM4), PM7, PM8, or DNA damage-inducible protein 1 (Ddi1) in the rodent malaria parasite *Plasmodium berghei* ANKA. We then tested the suppressive profiles of the LP/Ritonavir (LP/RT) and SQ/RT as well as antimalarials; Amodiaquine (AQ) and Piperaquine (PQ) against the transgenic parasites in the standard 4-day suppressive test. The Ddi1 gene proved refractory to deletion suggesting that the gene is essential for the growth of the asexual blood stage parasites. Our results revealed that deletion of PM4 significantly reduces normal parasite growth rate phenotype ($P = 0.003$). Unlike PM4 knock out (KO) parasites which were less sensitive to LP and SQ ($P = 0.036$, $P = 0.030$), the suppressive profiles for PM7_KO and PM8_KO parasites were comparable to those for the WT parasites. This finding suggests a potential role of PM4 in the LP and SQ action. On further analysis, modeling and molecular docking studies revealed that both LP and SQ displayed high binding affinities (-6.3 kcal/mol to -10.3 kcal/mol) towards the *Plasmodium* aspartyl proteases. We concluded that PM4 plays a vital role in assuring asexual stage parasite fitness and might be mediating LP and SQ action. The essential nature of the newly identified Ddi1 gene warrants further studies to evaluate its role in the parasite asexual blood stage growth as well as a possible target for the RPIs.

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METABOLIC DEPENDENCY OF CHORISMATE IN *PLASMODIUM FALCIPARUM*

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Human malaria accounts for more than 400,000 deaths a year, with *Plasmodium falciparum* being the deadliest of the five species that infect humans. Artemisinin Combination Therapies (ATCs) are the frontline treatment for malaria; however, resistance to artemisinin has been confirmed and is increasing in prevalence. Therefore, there is an urgent need to identify novel inhibitors to overcome parasite resistance, especially inhibitors safe for use in pregnant women and children. Among the potential metabolic drug targets absent in humans is the shikimate pathway for chorismate biosynthesis, which has been postulated to be essential for *P. falciparum's* survival. Interestingly, two recent studies indicated that chorismate synthase is not essential in *P. berghei*. Chorismate is a branching metabolite used to synthesize *p*-aminobenzoic acid (pABA, an intermediate in folate biosynthesis), aromatic amino acids, and *p*-hydroxybenzoic acid (pHBA, an intermediate in ubiquinone biosynthesis). This study aimed to assess the fate of chorismate and its metabolic dependencies. We have identified an inhibitor that targets the shikimate pathway using reversal of growth inhibition, and it was used as a tool to assess the metabolic dependencies of chorismate in *P. falciparum*. The identified inhibitor is cytotoxic for the malaria parasite, specifically affecting the transition of trophozoite to schizont stage. Our results support that the molecular target is within the 5-step AROM multicomplex of the shikimate pathway inhibiting chorismate's production and that folate biosynthesis is the main metabolic fate of chorismate in *P. falciparum*. Inhibition of chorismate biosynthesis does not affect the synthesis of ubiquinone, indicating that the production of pHBA, the precursor of the benzoquinone ring, may not be derived from chorismate or that alternative sources may be used. Further studies will be needed to elucidate the origin of the ring of ubiquinone in *P. falciparum*.

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IN SILICO CHARACTERIZATION OF PLASMODIAL TRANSKETOLASES AS POTENTIAL MALARIA DRUG TARGETS

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Malaria continues to plague the world, especially sub-Saharan Africa, with high mortality and morbidity. The absence of an approved vaccine and the emergence of drug resistant *Plasmodium* parasite is a public health concern. As a result, calls for the identification of novel targets for more efficacious antimalarials are escalating. Transketolase (TK) is a vital enzyme of the pentose phosphate pathway involved in the production of NADPH and ribose-5-phosphate. The aim of this study was to characterize plasmodial TKs through *in silico* approaches and to identify potential inhibitors from South African Natural Compound Database (SANCBD; https://sancdb.rubi.ru.ac.za). *Plasmodium falciparum* 3D7 TK (XP_966097.1) was retrieved from National Center for Biology Information (NCBI) database. Sixteen homologs from other plasmodial species and one human TK protein sequence were obtained by BLAST. Multiple sequence alignment, phylogenetic tree calculations and motif identification approaches were used to assess important sequence, structural and evolutionary variations. High quality three-dimensional homology models were generated using MODELLER. AutoDock Vina was then used to dock 623 SANCDB compounds against eight TK proteins. Docking results were analyzed to identify potential hits selectively inhibiting plasmodial TKs. Druglikeness of identified hits were evaluated using Molinspiration and SCFBio software. We observed a sequence identity of plasmodial TKs ranged between 79% to 84% compared to *P. falciparum* TK. However, *H. sapiens* TK shared a low sequence identity of 28% to the plasmodial TKs. Sequence-based analysis revealed highly conserved thiamine diphosphate and "TK" regions in the plasmodial TKs sequences, however, *H. sapiens* showed slight differences in residue composition. Active site inhibitors

identified were SANCDB411 and SANCDB620. Identified hits interacted with the Pyrophosphate and Pyrimidine domain catalytic residues and bind selectively across all plasmodial TKs and had good Druglikeness scores.

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MANAGEMENT OF MALARIA IN NIGERIA WITH NATURAL COMPOUNDS IN RATIONAL DRUG DESIGN

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Nigeria have been reported to be country with greatest number of cases of tropical diseases such as malaria. Despite the availability of different therapeutic and vaccine, about 80% of the populations still rely on traditional medicine for their primary healthcare. These are recognized as an accessible, affordable and culturally acceptable form of health care. Some Nigerian researchers and scientists have screened several medicinal plants in order to understand and scientifically justify the tradition believes of their properties or activity. However, there is no new therapeutics that has been generated through their research. This is due to inadequate transfer of methodology, information on the mode of action and synergistic behavior of these medicinal plants. This has created gap between the research and commercialization. However, this gap can be fill with proper rational drug design and modification of natural therapeutics. Therefore, the use of structural biology and enzymology to inform and improve therapeutics developed from natural product is the solution to the problem.

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ANTIMALARIAL PANTOTHENAMIDE METABOLITES TARGET ACETYL-COA SYNTHESIS IN *PLASMODIUM FALCIPARUM*

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Malaria eradication is critically dependent on novel drugs that target resistant *Plasmodium* parasites and block transmission of the disease. Here we report the discovery of potent pantothenamide bioisosteres that are active against blood-stage *P. falciparum* and also block onward mosquito transmission. These compounds are resistant to degradation by serum pantetheinases, show favorable pharmacokinetic properties and clear parasites in a humanized rodent infection model. Metabolomics revealed that CoA biosynthetic enzymes convert pantothenamides into drug-conjugates that interfere with parasite acetyl-CoA anabolism. *In vitro* generated resistant parasites showed mutations in acetyl-CoA synthetase and acyl-CoA synthetase 11, confirming the key roles of these enzymes in the sensitivity to pantothenamides. These new pantothenamides provide a promising class of antimalarial drugs with a unique mode of action.

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PLASMODIUM VIVAX SPOROZOITE PLATFORM IN INDIA

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Plasmodium vivax is no more a benign malaria. In recent years, *Plasmodium vivax* is causing serious clinical problems in terms of morbidity and mortality, similar to falciparum. Currently, India is responsible for 81% of deaths from vivax malaria among four countries, with an additional challenge represented by relapse due to the dormant form (hypnozoites) of the parasite. Sporozoite is the only stage that develops in the liver. It is important to produce a huge number of sporozoites in laboratory reared mosquitoes. Here, we report successful establishment of fully temperature and humidity controlled insectarium where numerous fit and healthy *Anopheles stephensi* are grown following modified protocol. These mosquitoes are allowed to feed through membrane feeding cups on the patient blood infected with *P. vivax* malaria. On an average 40,000 to 50,000 sporozoites were harvested per mosquito. These sporozoites are used for vivax liver stage assays for screening new drugs against sporozoites/hypnozoites. This resource can be used for several studies to characterize sporozoites/hypnozoites so that successful elimination of malaria can be achieved.

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LONG-ACTING INJECTABLE ATOVAQUONE NANOMEDICINES FOR MALARIA PROPHYLAXIS

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Chemoprophylaxis is currently the best available prevention from malaria, but its efficacy is compromised by non-adherence to medication. Here we develop a long-acting injectable formulation of atovaquone solid drug nanoparticles that confers long-lived prophylaxis against *Plasmodium berghei* ANKA malaria in C57BL/6 mice. Protection is obtained at plasma concentrations above 200 ng ml^{-1} and is causal, attributable to drug activity against liver stage parasites. Parasites that appear after subtherapeutic doses remain atovaquone-sensitive. Pharmacokinetic-pharmacodynamic analysis indicates protection can translate to humans at clinically achievable and safe drug concentrations, potentially offering protection for at least 1 month after a single administration. These findings support the use of long-acting injectable formulations as a new approach for malaria prophylaxis in travellers and for malaria control in the field.

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CHARACTERIZATION OF THE MECHANISM OF RESISTANCE OF THE AMINOMETHYLPHENOL, JPC-3210 (MMV892646)

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The nonquinoline 2-aminomethylphenol, JPC-3210 (MMV892646), has recently been selected by Medicines for Malaria Venture (MMV) for

advanced preclinical development. JPC-3210 possesses low nanomolar *in vitro* antimalarial activity against *Plasmodium falciparum* multidrug resistant lines, low cytotoxicity and a low curative oral dose of 4 mg/kg/day in the mouse-*P. berghei* model (modified Thompson test) and at 5 mg/kg (single oral dose) it was curative against the *P. falciparum* FVO strain in *Aotus* monkeys. Pharmacokinetic studies revealed that JPC-3210 has a very lengthy blood elimination half-life in both mice and monkeys of about 5 and 21 days, respectively, which would suggest that if the compound is used, parasites are likely to encounter sub-therapeutic concentrations of JPC-3210 for a considerable time. Therefore, it is important to evaluate the potential for malaria parasites to develop resistance to JPC-3210. In the present study, we exposed 3 independent cultures of artemisinin and mefloquine-resistant *P. falciparum* MRA1240 parasites to escalating concentrations of the compound starting from 4 to 48 nM over 14 months. Following exposure, there was a 6-fold increase in IC_{50}s of JPC-3210 against the resistant lines, as well as 5-fold decrease in susceptibility to mefloquine, whereas no decrease in susceptibility was seen for chloroquine, dihydroartemisinin, piperaquine and pyronaridine. The decrease in susceptibility to mefloquine was consistent with an amplification of *Pfmdr1* gene from 3 to 5 copies in the resistant lines. In addition, whole genome sequencing of the resistant progeny revealed an amplification of the region on chromosome 12, containing a member of ABC transporter family, in one of the isolates and a number of SNPs identified in the resistant lines, which are currently being analysed. Our findings will provide more insights on the potential molecular markers responsible for parasite resistance to JPC-3210. The considerable effort required to induce the JPC-3210 resistant *P. falciparum* lines *in vitro* further supports the preclinical development of JPC-3210 for malaria treatment and prophylaxis.

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THE AMINOMETHYLPHENOL (JPC-3210; MMV892646) IS HIGHLY EFFECTIVE IN CURING HUMAN MALARIA IN *AOTUS* MONKEYS FOLLOWING SINGLE ORAL DOSE ADMINISTRATION

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The Medicines for Malaria Venture (MMV) is seeking to develop new antimalarial drugs that have the potential to treat malaria patients with a single oral dose, including those with malaria parasites resistant to current artemisinin based combination therapies. Recently, MMV in partnership with Jacobus Pharmaceutical Company (JPC) initiated advanced preclinical evaluation of a new aminomethylphenol (JPC-3210; MMV892646) that possesses high *in vitro* antimalarial activity against multidrug-resistant *Plasmodium falciparum* lines, low cytotoxicity against mammalian cell lines, and potent efficacy in the *P. berghei*-mouse model. In mouse studies, JPC-3210 is rapidly absorbed, has high oral bioavailability and low metabolism, and possesses a lengthy blood elimination half-life (5-6 days). This provides the potential for the compound to be part of a single dose combination providing a long chemosuppressive action in preventing the recurrence of malaria infections. In the present study, we assessed JPC-3210's potency in the traditional human malaria-*Aotus* monkey model. In descending single oral dose studies, JPC-3210 (20, 10 and 5 mg/kg) rapidly cleared established infections of the amodiaquine, chloroquine, and quinine-resistant FVO strain of *P. falciparum* in naive *Aotus* monkeys (groups of 3 monkeys), with no recrudescence over a 90-day follow-up period. We also showed that single oral doses of 10 and 20 mg/kg of JPC-3210 cured *Aotus* monkeys infected with the chloroquine and antifolate-resistant AMRU1 strain of *P. vivax* malaria. Sequential blood sampling of the treated monkeys revealed that JPC-3210 had a long elimination half-life of about 21 days. Parasitaemia clearance profiles and pharmacokinetics of JPC-3210 in the treated monkeys will be presented. The implication of JPC-3210 as a long acting partner compound for both single dose treatment and malaria elimination will also be discussed.

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MALARIA PREVENTION: DEVELOPMENT OF IMPLANTABLE MALARIA CHEMOPROPHYLAXIS

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Prevention of malaria is highly dependent on subject compliance with a prescribed daily chemoprophylaxis regimen. However, maintaining drug compliance by U.S. service members can be difficult due to a daily oral dosing requirement, side effects, organizational culture, command emphasis and the demands of the operational environment. The Experimental Therapeutics (ET) Branch at Walter Reed Army Institute of Research (WRAIR) is the U.S. Army's premier research program for the development of anti-malarial drugs. A current effort of ET, in collaboration with the Southwest Research Institute and Titan Pharmaceuticals, is to develop long-term release anti-malarial drug implants. These subdermal implants provide continuous drug release over an extended period and could potentially relieve deployed service members from adherence to a daily oral drug dosing schedule. EVA (ethylene-vinyl acetate) implants containing atovaquone and proquanil were tested in mice to characterize the pharmacokinetic (PK) profile and long-term prophylactic efficacy *in vivo.* The PK profile from this ongoing study exhibited drug release through 16 weeks, and maintained stable plasma levels of atovaquone. Furthermore, after 12 weeks of implantation, the atovaquone implants demonstrated complete protection from malaria infection in mice. The development of long-acting prophylactic implants with greater potency and safety is a novel approach that could greatly improve the compliance of deployed service members in malaria-endemic regions. Furthermore, the products targeted for this Industry collaboration will support the multi-domain battlefield operational concept by allowing ground combat forces to maneuver and perform in an uninterrupted manner in resource-constrained environments. Previously reported doxycycline implant data and these preliminary findings with atovaquone and proguanil allow us to pursue further formulation refinement and *in vivo* characterization in pursuit of a series of long-acting implants infuse with FDA-approved malarial prophylaxis drugs - either an atovaquone/proguanil combination or doxycycline.

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DISCOVERY OF GSK701, A NOVEL ORALLY EFFECTIVE PRECLINICAL DRUG CANDIDATE FOR THE TREATMENT OF MALARIA

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Malaria remains a major global health problem. In 2016 alone, 216 million cases of malaria were reported, and more than 400,000 deaths occurred. Since 2010, emerging resistance to current front-line ACTs (Artemisinin Combination Therapies) has been detected in endemic countries. Therefore, there is an urgent need to replace those drugs compromised by resistance, as well as identifying potential novel therapies that offer significant advantages over the current standard of care. During the past few years, the antimalarial community has focused their efforts on phenotypic screening as a pragmatic approach to identify new hits. Within

the TCAM set of phenotypic hits identified at GSK, a pyrrolidinamide chemical series was selected because its chemical novelty and putative new MoA, offering promising properties as antimalarial. Lead optimization efforts within the pyrrolidinamide series led to the identification of GSK701 as a new preclinical candidate that will offer opportunities linked to its killing profile and potency comparable to artemisinins as well as its new mode of action. The overall properties of GSK701, including the low predicted human dose, constitute a promising profile supporting further development to provide novel antimalarial therapeutic opportunities. A detailed description of the Medicinal Chemistry identification and development of GSK701 will be provided in this comunication.

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CHARACTERIZING THE RESISTANCE TO CLINICALLY-RELEVANT *PLASMODIUM FALCIPARUM* DIHYDROOROTATE DEHYDROGENASE INHIBITORS IN *IN VITRO* AND *IN VIVO* CONTEXTS

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As resistance to front-line antimalarials poses an ever-growing threat, the identification of novel drug targets will be crucial to the continued success of control and eradication efforts. Inhibitors of the dihydroorotate dehydrogenase (DHODH) target have advanced through the drug-development pipeline, including the triazolopyrimidine DSM265, which was recently assessed in Phase 2 clinical trials. We previously demonstrated that resistance to other DHODH inhibitors can be acquired through single point mutations in the *dhodh* locus, suggesting a potential drawback to the clinical use of such compounds. To investigate how resistance to DSM265 occurs, we performed *in vitro* selections with DSM265 and its close analog DSM267. To identify resistance pathways that may be more likely to occur in a clinical setting, we additionally developed a novel platform to select resistant parasites in a humanized mouse model. In both *in vitro* and *in vivo* systems, resistance arises rapidly. Sequencing of resistant parasites identified a variety of mutations in the *dhodh* locus, most of which line the species-selective inhibitor binding site. Interestingly, we identified similar mutations *in vitro* as *in vivo,* suggesting that *in vitro* selections are a relevant model of resistance evolution for this target. To explore the fitness consequences of these mutations, we performed *in vitro* competitive growth assays between wild-type and mutant clones. Previous studies have shown that DHODH:E182D mutant parasites are less fit than wild-type clones, suggesting they are unlikely to compete in natural populations. In contrast, we show that DHODH:C276Y, one of the most common mutations arising from our *in vitro* selections, does not confer a fitness cost. Taken together, these results predict that resistance to DSM265 could arise and spread readily in natural parasite populations. However, we also find that many DSM265-resistant clones exhibit increased sensitivity to other chemotypes of DHODH inhibitors (i.e. collateral sensitivity). This finding indicates the potential of a well-designed combination therapy approach to slow the emergence of resistance.

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INCORPORATING AN INNOVATIVE *IN VITRO PLASMODIUM CYNOMOLGI* ASSAY INTO THE EXPERIMENTAL THERAPEUTICS' DRUG SCREENING PARADIGM FOR THE DISCOVERY OF NOVEL COMPOUNDS AGAINST *PLASMODIUM* HYPNOZOITES

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Experimental Therapeutics at the Walter Reed Army Institute of Research has played a major role in the discovery and development of many successful antimalarials by utilizing a sophisticated gated-tier system for hit-to- lead discovery of active compounds against *Plasmodium* parasites. This deliberative drug screening paradigm searches for compounds that are active against several species and life cycle stages, with an emphasis on *P. f* and *P. v* malaria. To achieve global eradication, it is imperative to develop drugs that not only target blood stage parasites, but ones that also inhibit the development of dormant liver stage forms, as hypnozoites are the life cycle stage in certain malaria species that can cause relapsing infections. However, a critical gap in current methods for liver stage screening is the ability to screen for hypnozoite inhibition *in vitro* before advancing a compound to the low-throughput and costly non-human primate model. Therefore, in collaboration with the University of South Florida, WRAIR has adapted a method utilizing a 384-well *in vitro P. cynomolgi* culture system to produce viable dormant liver stage parasites in primary NHP hepatocytes and has begun incorporating this assay into the ET drug screening paradigm. Using USF's innovative *in vitro* approach, we have been able to successfully screen hundreds of experimental compounds in both causal prophylactic and radical cure mode and have identified several positive drug hits targeting hypnozoites. Currently our assay is run in 8-point replicates with 4-fold compound dilutions, and sporozoites are sourced from *Anopheles dirus* mosquitoes maintained by the Entomology group at AFRIMS in Bangkok, Thailand. AFRIMS Entomology can produce 1.5 billion *P. cynomolgi* sporozoites per month, giving us substantial access to highly viable parasites and allowing us to continue assay optimization and increase throughput. Here, we discuss how our *in vitro* results correlate with *in vivo* models for known malaria drugs, provide initial causal prophylactic and radical cure data on novel compounds, and present the progress of incorporating this assay into our drug screening paradigm.

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KINETIC DRIVERS OF ANTIBACTERIAL DRUGS AGAINST *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR CLINICAL DOSING

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Antibacterial drugs are an important component of the antimalarial armamentarium, and recommended dosing regimens for the two indications are similar. We examined the pharmacokinetic-pharmacodynamic relationships of chloramphenicol, tetracycline, ciprofloxacin and clindamycin against *Plasmodium falciparum* using our custom glass hollow fiber *in vitro* system that exposes parasites to dynamically changing drug concentrations. To identify the driver of antimalarial efficacy (concentration or time of exposure), the same total dose and AUC (area under the concentration-time curve) of drug was deployed against parasites as either a single short-lived high peak concentration (bolus) or as a constant low concentration (infusion). Parasites counts at 96 h were compared with those of untreated controls.

All four antibacterials were unambiguously time-driven against malaria parasites. For the same total dose and AUC, in ≥ triplicate determinations, efficacy of bolus *vs.* infusion regimens was 25 *vs.* 54% ($p = 0.04$) for chloramphenicol, 26 *vs.* 44% ($p = 0.01$) for tetracycline, 34 *vs.* 70% ($p = 0.001$) for ciprofloxacin, and 40 *vs.* 73% ($p = 0.01$) for clindamycin. On average, antimalarial activity doubled when drug was deployed to favor time. For ciprofloxacin in particular, this finding is in sharp contrast to its concentration-driven antibacterial action, and suggests that dosing regimens tailored for bacteria may be inappropriate against malaria. In keeping with this is the reportedly variable clinical efficacy of antibacterials against *falciparum* malaria, especially as monotherapy, which may reflect the inability of current dosing regimens to provide suitable PK. Finally, the finding that a given drug may have a kinetic driver that varies depending on the target organism, despite (apparently) having the same operative molecular mechanism of action, offers a possible window into the now totally empirical kinetic driver, suggesting that governance by concentration *vs.* time of exposure may be dictated by events downstream from the molecular mechanism of action. XX1850 words allowed; now 1779.

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EVALUATION OF ACTION OF ANTIMALARIAL DRUGS USING MICROCAPILLARY CYTOMETRY

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The increasing literature of *Plasmodium* resistance to several antimalarial drugs amplifies the need for discovery of more effective drugs along with *in vitro* methods and technologies to assess parasite susceptibility. Many of the existing assays today are challenging in either being manually laborious, or requiring complex instrumentation, expert operators, or in being less sensitive/reproducible. Hence there is a need for reproducible and reliable *in vitro* assays/platforms that can be used in many environments easily and provide information on parasite susceptibility/infectivity as well as status of key parasite proteins on the same system. We have recently demonstrated the capability of a simple, low cost, easy to use cytometry platform, the Muse® Cell Analyzer to provide parasitemia related information using a red blood cell assay as well as being able to confirm presence of key parasite proteins such as HRP2 and LDH with high sensitivity. In this study, we evaluated *in vitro* effects of Chloroquine (CQ), Mefloquine (MQ), Quinine (QN), Dihydroarteminisin (DHA) and Doxycycline (DOX) on *P. falciparum* cultures 3D7 and D10 at different times and dosage of treatment. Samples were analyzed for parasitemia levels by Muse® Parasitemia Assay. HRP2 and LDH proteins were characterized by Muse® *Pf/Pv* Assay. The relative effects of the drugs in this study were compared based on inhibitory concentrations required, time for action and capability to reduce recurrent parasitemia. The inhibitory concentration of tested drugs varied in the range of 6.25nM - 200nM for CQ, MQ, QN, DHA or 10μM - 50μM for DOX due to the differences in each drug's mechanism of action and potencies. Our studies will present combined information on parasitemia and key parasite protein detection levels with treatment. The availability of multiple methods to characterize *Plasmodium* cultures in a simple, easy to use microcapillary- based cytometer such as Muse® Cell Analyzer can greatly facilitate screening for conditions and drugs that modulate malarial parasite activity and infection levels.

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COMPARISON OF PROTEIN ENERGY MALNUTRITION AND *PLMODIUM FALCIPARUM* MALARIA LEVELS IN COMMUNITY BASED EDUCATION AND SERVICE IN TWO CATEGORIES OF CENTERS IN WESTERN KENYA

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Nutritional status and levels of P. falciparum malaria in children in two categories of COBES centres: Those with the programme on Academic Model Providing Access To Healthcare (AMPATH) and those that were non-AMPATH were determined. The overall objective was to ascertain if the presence of AMPATH had any effect in reduction of malnutrition and malaria in the centres. For the nutritional status, cross-sectional studies were carried out between March 2008 to May, 2013, in 16 COBES centres in Western Kenya. Cluster sampling technique was used with each health centre as the sampling unit. Anthropometric measurements were performed on all children aged 5-59 months within the households sampled. The parameters considered included Age (in months), Weight (Kgs) and the mid upper arm circumference (cms). The WHO recommended Z- score values as well as the Kenya Government Ministry of health recommended charts based on anthropometric measurements were used as standards. Analysis of nutritional data was carried out using Epi-info 2000 computer program to determine the Z- score values from anthropometric data. For Malaria, the health centre records were assessed for the prevalence during the period of study and compared for both the AMPATH and NON-AMPATH centres. A total of approximately 700 children were measured for anthropometry in the seven Health Centers: (Stunting- HAZ<-2, Wasting-WHZ <-2, underweight –WAZ<-2 and MUAC, < 12.5mm). Meteitei (non-AMPATH) had the highest malnutrition prevalence (53% HAZ, 15% WHZ, 27% WAZ and 18.1 MUAC) whereas Chulaimbo (AMPATH) showed the lowest prevalence (7% HAZ, 3% WAZ). Other centres showed mixed prevalence. Malaria was the leading cause of mortality and morbidity in all the COBES centres except for only two. AMPATH COBES centres showed improved nutrition status compared to non-AMPATH COBES centres. Presence of AMPATH had no effect on prevalence of malaria.

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THE EFFECT OF PREGNANCY-ASSOCIATED MALARIA ON INFANT GROWTH AND NEUROCOGNITIVE DEVELOPMENT

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Pregnancy-associated malaria is associated with adverse outcomes for children and may cause neurocognitive deficits through the influence of inflammatory mediators. We have shown that placental malaria infection is associated with an increased risk of malaria infection and disease during infancy. Here we aimed to determine the influence of maternal malaria on infant growth and neurocognitive development. We followed 307 mother-infant pairs from 20-26 weeks gestation through the first two years of life. All pregnancies were dated using ultrasound in the second trimester and women received malaria prevention interventions during pregnancy. Biometric information was collected quarterly and the Malawi Developmental Assessment Tool was administered at six, 12 and 24 months. We detected significant differences in growth and neurocognitive development between preterm and term infants. In our study, malaria

infection was not associated with low birthweight or preterm delivery. Among the mother-infant pairs, 230 had no malaria during pregnancy, 39 had peripheral malaria infection only and 39 had placental malaria (six had active infection at delivery). However, there were no significant differences in gross motor, fine motor, social or language skills based on maternal malaria status at six, 12 or 24 months. Less than five percent of children failed any developmental milestone at any visit. Malaria infection in the mother was not associated with differences in infant weight, length or head circumference at any of the quarterly visits. In sum, placental malaria and maternal peripheral malaria infection had not impact on infant growth or neurocognitive development. While it is possible that our power was limited due to low numbers of placental malaria cases and few cases of chronic infection, these reassuring data suggest that when malaria prevention measures are implemented as recommended, infants are protected from developmental and growth delays when exposed to malaria infection *in utero*.

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PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION AND ANTIMALARIAL RESISTANCE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN MONROVIA, LIBERIA

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Provision of malaria care in Liberia was severely disrupted during the 2014-16 Ebola outbreak. Once the epidemic ended, there was an urgent need to restore a functional malaria control strategy for the most vulnerable (i.e. pregnant women). National-level data on the burden of *Plasmodium falciparum* (*Pf*) infection in pregnant women has never been collected. In mid-2016, we conducted a cross-sectional study at the Saint Joseph's Catholic Hospital, a maternal referral hospital in Monrovia. The study aimed to determine *Pf* prevalence among pregnant women at the first antenatal care (ANC) visit before intermittent preventive treatment (IPTp) was given, and to assess molecular markers of drug resistance. 195 women aged 14 and older participated. Their mean age was 27.3 years, and 28% of them slept under an insecticide-treated net the night before. Twenty-four women (12%) were shown to be *Pf*-infected by real-time qPCR. Only three of them had fever, and ten had low-density infections (<10 parasites/µl). Mean hemoglobin levels were lower in *Pf*-infected than in non-infected women (10.8 vs 11.2 mg/dL). Infection was only significantly associated with younger age, fever and first gravidity. qPCR tests of *Pf* isolates showed absence of *pfdhps* K540E, the epidemiological marker of the quintuple mutation, and a >5% prevalence of polymorphisms associated with resistance to Sulfadoxine. This study shows that midwives in Liberia could expect at least one in eight women to be *Pf*-infected in their first ANC. The risk of being *Pf*-infected is higher among the young primigravidae. Drug resistance analyses showed no compromised efficacy of IPTp. However, most of the women had either submicroscopic or asymptomatic malaria, indicating that they -and their newborn- could develop ill health in the long term. To strengthen malaria control and further protect the most vulnerable, updated robust epidemiological data are crucial. In Liberia, where massive annual malaria surveys cannot be funded, ANC-based prevalence and drug resistance surveillance could be set up, capitalizing on the investment in molecular technologies that the country received during the Ebola crisis.

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SPATIO-TEMPORAL HETEROGENEITY OF MALARIA MORBIDITY IN GHANA: ANALYSIS OF ROUTINE HEALTH FACILITY DATA

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Malaria incidence is largely influenced by vector abundance. Among the many interconnected factors relating to malaria transmission, weather conditions such as rainfall and temperature are known to create suitable environmental conditions that sustain reproduction and propagation of anopheles mosquitoes and malaria parasites. In Ghana, climatic conditions vary across the country. Understanding the heterogeneity of malaria morbidity using data sourced from a recently setup data repository for routine health facility data could support planning. Monthly aggregated confirmed uncomplicated malaria cases from the District Health Information Management System and average monthly rainfall and temperature records obtained from the Ghana Meteorological Agency from 2008 to 2016 were analysed. Univariate time series models were fitted to the malaria, rainfall and temperature data series. After pre-whitening the morbidity data, cross correlation analyses were performed. Subsequently, transfer function models were developed for the relationship between malaria morbidity and rainfall and temperature. Malaria morbidity patterns vary across zones. In the Guinea savannah, morbidity peaks once in the year and twice in both the Transitional forest and Coastal savannah, following similar patterns of rainfall at the zonal level. While the effects of rainfall on malaria morbidity are delayed by a month in the Guinea savannah and Transitional Forest zones those of temperature are delayed by two months in the Transitional forest zone. In the Coastal savannah however, incidence of malaria is significantly associated with two months lead in rainfall and temperature. In conclusion, data captured on the District Health Information Management System has been used to demonstrate heterogeneity in the dynamics of malaria morbidity across the country. Timing of these variations could guide the deployment of interventions such as indoor residual spraying, Seasonal Malaria Chemoprevention or vaccines to optimise effectiveness on zonal basis.

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THE USE OF GPS DATA LOGGERS TO DESCRIBE SPATIO-TEMPORAL MOVEMENT PATTERNS AND THE IMPLICATIONS FOR MALARIA CONTROL IN THREE EPIDEMIOLOGIC SETTINGS IN SOUTHERN AFRICA

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Human mobility has been identified as a driver of malaria transmission at both large and small spatial scales. However, the contribution of individual movement patterns in different epidemiologic settings has not been comprehensively examined. Between 2013 and 2017, three population movement studies using global positioning systems (GPS) data loggers were conducted in rural southern Africa as part of the Southern and Central Africa International Centers of Excellence for Malaria Research. The three sites included were: Choma District, Southern Province, Zambia, a pre-elimination setting with declining malaria transmission; Nchelenge District, Luapula Province, Zambia, a high-transmission setting with ineffective malaria control; and Mutasa District, Manicaland Province,

Zimbabwe, a moderate-transmission setting with seasonal malaria. At each site, participants aged 13 years and older were invited to carry a GPS data logger for one month during all daily activity. GPS devices were motion-activated and recorded coordinates, date, and time every 2.5 minutes while in motion. At logger distribution and final collection, participants completed a survey and provided a finger prick blood sample for *hrp-2* RDT and *cytb* PCR testing. Data were uploaded into ArcGIS and R statistical programs to create movement tracks and density maps, and patterns in activity space were compared across participants. In Choma, Nchelenge, and Mutasa Districts, respectively, 69, 84, and 180 participants were enrolled. Distinct movement patterns were informative in characterizing malaria transmission at each site. Seasonal long-distance movement could explain parasite importation in Choma District. High local mobility in Nchelenge District was expected to reduce the effectiveness of targeted intervention strategies. Extensive cross-border movement into Mozambique from Mutasa District was linked to importation of cases and attenuation of malaria control activities. The different movement patterns characterized in these studies have implications for malaria control strategy across epidemiologic settings in southern Africa.

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EPIDEMIOLOGY OF *PLASMODIUM VIVAX* MALARIA INFECTION IN NEPAL

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Malaria is endemic in the southern plain of Nepal (the Terai) which shares a long and porous border with India. More than 80% cases of malaria in Nepal are caused by *Plasmodium vivax*. Nepal is currently at the malaria pre-elimination stage with a target of elimination by 2025. The main objective of this study was to review the changing epidemiology of *P. vivax* malaria infections as recorded by the national malaria control programme of Nepal between1963 and 2016. National malaria data was retrieved from the National Malaria programme in the Ministry of Health, Government of Nepal. The epidemiological trends and malariometric indicators were analyzed. The most recent malaria risks in known endemic districts (Kailali, Kanchanpur and Jhapa) were mapped by GIS Arc version 10.2. *Plasmodium vivax* malaria has predominated over *P. falciparum* in the past 53 years, with *P. vivax* malaria comprising 70-95% of the annual malaria infections. In 1985, a malaria epidemic occurred with 42,321 cases (82 % *P. vivax*, 17% *P. falciparum*). Nepal had experienced further outbreaks of malaria in 1991 and 2002. Although the total number of malaria cases was declining, *P. falciparum* cases increased from 2005 to 2010, but since then declined. Analyzing the overall trend between 2002 (12,786 cases) until 2016 (1,009 cases), shows a case reduction by 92%. In contrast imported malaria cases showed an increasing proportion from 18% of cases in 2001 to 50% in 2016. In conclusion, the current trends of malariometric indices indicate that Nepal is making a significant progress towards achieving the goal of malaria elimination by 2025. The majority of cases are due to *P. vivax,* a complex parasite with latent hypnozoites reservoir causing relapse. In Nepal, imported malaria comprises an increasing proportion of all cases. The Malaria control program in Nepal needs to direct strategies to counter importation of malaria to high risk areas and to conduct collaborative cross border malaria control activities.

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MALARIA IN THE FIRST TRIMESTER, BUT NOT IN THE 2ND AND 3RD TRIMESTER OF PREGNANCY, IS ASSOCIATED WITH MATERNAL ANEMIA: A PRE-CONCEPTIONAL COHORT STUDY IN BENIN

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In Africa, preventive drug strategies against malaria in pregnancy are recommended from the 2nd trimester, and bed nets are rarely distributed before the 1st antenatal care visit at approximately 4 months of pregnancy. Therefore, women remain insufficiently, or not, protected during the 1st trimester, when malaria may be particularly deleterious for the mother and the foetus. For the first time, we assessed the effect of malaria according to the timing of infections during pregnancy on maternal anaemia using a specifically-designed study. From June 2014 to March 2017, 1214 women of reproductive age were recruited and followed up monthly until 411 became pregnant. Pregnant women were then followed up from 5-6 weeks of gestation until delivery. Microscopic malarial infections were detected monthly during pregnancy using thick blood smear. Maternal haemoglobin (Hb) level was assessed in the 1st trimester and in the 3rd trimester of pregnancy. A logistic regression model was used to assess the effect of malaria at different timings during pregnancy on maternal anaemia in late pregnancy (Hb below to 110 g/L). The prevalence of maternal anaemia in late pregnancy was 58.6%. The prevalence of malaria infection was 21.8%, 17.7% and 14.6% in the 1st, 2nd and 3rd trimester of pregnancy, respectively. After adjustment for potential confounders, malaria in the 1st trimester was significantly associated with maternal anaemia (aOR=2.7 [1.4-5.3]; P=0.003), whereas no effect was found for malaria in the 2nd and 3rd trimesters. These results suggest an independent effect of malaria in the 1st trimester of pregnancy on maternal anaemia despite immediate treatment of malarial infections. They argue in favour of additional preventive drug-related measures against malaria starting before or in the 1st trimester of pregnancy.

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HETEROGENEITY OF HUMAN EXPOSURE TO MALARIA VECTOR IN URBAN SETTING BY USING IMMUNO-EPIDEMIOLOGICAL SALIVARY BIOMARKER

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Urban malaria represents an underestimated serious health concern in African countries. This study aims to evaluate the risk of malaria transmission and the effectiveness of insecticide treated nets (ITNs) use for vector control in urban area. New, sensitive and large-scale monitoring indicators which measure the real and quantitative Human-Vector contact was here used. It is based on the human IgG antibody responses to the gSG6-P1 salivary peptide of the *Anopheles* saliva previously validated as pertinent biomarker of Human exposure to *Anopheles* bites, in several epidemiological contexts. Two multidisciplinary cross-sectional studies were undertaken in three neighborhoods (Dar-es-salam, Kennedy and N'gattakro) of Bouaké city (Ivory Coast) during the rainy and the dry

seasons. Blood samples were obtained from children aged 6 months to 14 years-old for immunological tests and ITNs use information's were collected by sociological questionnaire. The specific IgG level of children was significantly higher in rainy season compared to dry season whatever the studied districts (all difference at P<0.001; Mann Whitney test). Interestingly, the specific IgG level was different between to the three neighborhoods in dry season when exposure to *Anopheles* bites decrease (P= 0.032; Kruskal-Wallis test). This result shows the considerable heterogeneity of children exposure to Anopheles vector between neighborhoods in urban context. Surprisingly, no difference were observed between children who declared sleeping always under ITN and those who affirm never sleeping under ITN in rainy season and dry season (p= 0.337; p= 0.094 respectively). This study highlights the high risk of malaria exposure in African urban settings and its heterogeneity within city. The *Anopheles* salivary biomarker could be a suitable tool for accurately and quantitatively assessing the risk of transmission and effectiveness of malaria control methods in urban areas.

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EFFECT OF THE ABO BLOOD GROUP ON SUSCEPTIBILITY TO SEVERE MALARIA AND RELATED OUTCOMES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Understanding how the ABO blood group interacts with *Plasmodium falciparum* malaria will provide insights on the pathogenesis of *falciparum* malaria and provide direction for basic research that may help the development of novel antimalarial treatments and vaccines. Specifically, investigating the genetic basis that reduces susceptibility to severe *P. falciparum* infection may lead the formulation of personalized strategies designed to prevent deaths due to severe *P. falciparum* infection. We systematically summarized information on the effect of ABO blood group on severe *P. falciparum* infection, level of parasitemia and hemoglobin. We searched literature published in Pubmed, Embase, Web of Science, CNAHL and Cochrane Library from inception to February 04, 2017 without restriction. We retrieved 1,923 articles were from five databases: Embase (n=728), PubMed (n=620), Web of Science (n=549), CINAHL (n=14) and Cochrane Library (n=12). After removal of duplicates and two levels of screening, we selected 24 articles for inclusion in the systematic review and 22 for the meta-analysis. A meta-analysis of 15 studies showed an increased odds of severe *P. falciparum* infection (*vs* uncomplicated *P. falciparum* infection) among individuals with blood group A (summary Odds Ratio [OR] 1.34; 95% Confidence Interval [CI] 1.06, 1.62); B (OR 1.48; 95%CI:1.16, 1.79); AB (OR 1.43; 95%CI: 1.00, 1.87); or non-O (A, B or AB) (OR 1.61; 95%CI: 1.31, 1.91) compared with blood group O. A meta-analysis of four studies showed a significantly greater level of *P. falciparum* parasitemia among individuals with blood group A than those of blood group O (mean difference: 1769.2; 95%CI: 848.9, 2689.4). However, a meta-analysis of four studies showed lack of significant difference in hemoglobin level among *P. falciparum* infected individuals of blood group A as compared with those of blood group O. This study suggests that individuals with blood group A, B and AB are more susceptible to severe *P. falciparum* infection and blood group O has a protective effect. However, the ABO blood group may not affect *P. falciparum* infection related reduction in hemoglobin level.

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REACTIVE CASE DETECTION AND EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM* MALARIA IN THE WESTERN KENYA HIGHLANDS

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Identifying asymptomatic infections using index cases can help to understand the epidemiology of malaria and guide control activities. Reactive case detection and the genetic composition of parasites identified through these activities were investigated, between October 2015 and September 2016 in Marani, western Kenya. Fifty index cases were followed up, and 108 index case household members were screened by PCR, as well as 612 neighbours within a 100m radius and 510 controls matched with index cases and located at a distance of ≥ 500 m. In the index case and neighbour households, the prevalence of infection was approximately twice as high as in control households (by PCR: Index cases households: 28.9%, neighbours: 25.3% controls: 12.9%). Assuming all malaria cases were followed up, only a small proportion (<10%) of the asymptomatic reservoir in the population would have been identified. 127 isolates (34 index cases, 11 index household members, 57 neighbours and 25 controls) were genotyped using markers *msp1* and *msp2*. Except for the index cases, (heterozygosity = 0.90), genetic diversity was similar across all the populations (0.80) with higher mean multiplicity of infection in neighbours (1.81) relative to all the study population. Across all population, moderately higher proportions of related genotypes were observed in index case households (27.7%). In conclusion, reactive case detection is effective in identifying additional cases, but likely has limited effect on transmission at the population level. The higher prevalence of infection in index case households and the parasite genetic relatedness indicate small-scale transmission of parasites within high-risk *Foci*.

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EPIDEMIOLOGY OF MALARIA TRANSMISSION IN TWO NEIGHBORING VILLAGES IN THE RURAL COMMUNE OF ANDRIBA, MADAGASCAR

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Malaria remains a major health concern in the island of Madagascar, with *Plasmodium falciparum* and *Plasmodium vivax* as the major parasite species mainly transmitted by *Anopheles gambiae* s.l. and *Anopheles funestus.* However, *Anopheles mascarensis* endemic to the island and *Anopheles coustani* can also act as local or minor vectors. In a region of moderate to high malaria transmission, the prevalence of malaria cases in two nearby villages with apparent similar pattern was significantly different. To try deciphering the basis of this difference, we conducted a multidisciplinary study including Entomology, Parasitology and Immunology. Surveys were conducted from November 2016 to April 2017 at 3 time periods. Mosquitoes were collected by human landing and pyrethrum spray catches. From 1650 caught *Anopheles*, the mean

aggressive density of *An. coustani*, *An. arabiensis*, *An. funestus* and *An. mascarensis* was 10.4, 5.7, 2.5 and 0.9 respectively. The abundance of caught *Anopheles* was similar in both villages. Detection of *Plasmodium* carriage by a TaqMan assay in those mosquitoes is in progress. *Plasmodium* prevalence in the human population was determined by Rapid Diagnostic Test, microscopy and Real-Time PCR. Prevalence and specificity of antibodies against *Plasmodium* antigens as well as an *Anopheles*-specific salivary antigen were determined using a multiplex bead-based serological assay. Malaria prevalence in the population was higher in one village compared to the other, as previously observed. Serological results suggest different kinetics in *P. vivax* transmission between the two villages. No clear serological differences were observed for *P. falciparum*. Our current data confirm the epidemiological variations observed during the malaria transmission season in the two villages surveyed, with an increase in antibodies against *Plasmodium* antigens at the middle of the transmission season. The detail entomological analysis should provide relevant information that might explain the differential pattern of malaria prevalence between the two villages and possibly guide for adapted malaria transmission control.

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PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION IN MOZAMBICAN PREGNANT WOMEN

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Continued monitoring of malaria in pregnancy is lacking in most endemic settings, in spite of its significant disease burden. Increases in malaria-related harmful effects observed among Mozambican pregnant women after drastic malaria declines during the last decade suggest that close monitoring of the transmission is needed to quickly identify rebounds in adverse outcomes, especially in areas embarking on malaria elimination. It has been suggested that malaria prevalence among pregnant women can be a good approximation of the prevalence of malaria in children obtained from household surveys. In Manhiça, malaria hospital admission in the District Hospital followed similar trends to malaria prevalence among pregnant women, pointing towards the value of malaria prevalence among pregnant women delivering at health facilities as a proxy of malaria in the underlying community. We are conducting a three-year *prospective observational* study at three health centers with different levels of malaria transmission in Maputo Province (Manhiça District Hospital [Manhiça District], Ilha Josina Health Center [Manhiça District] and Magude Health Center [Magude District]). The number of women recruited until 9th February 2018 is 640 for Ilha Josina Health Centre; 4493 for Manhica Health Center and 2380 for Magude Health Center. At the first antenatal visits, the prevalence of maternal anemia (Hb < 11 g/dl) was 62.39% in Manhiça; 49.57% in Magude and 56.55% in Ilha josina. At maternity visits, the prevalence of maternal anemia was 64.83% in Manhiça; 56.71% in Magude and 32.14% in Ilha Josina. At the first visit, the prevalence of *Plasmodium falciparum* infection (qPCR) was 9% in Manhiça, 37% in Josina Island and 6% in Magude. Key findings The lower prevalence of *Plasmodium falciparum* infection in Magude (6%) when compared to Manhica (9%) and Ilha Josina (37%) may be a result of the activities carried out in this District in the context of elimination of malaria.

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ECONOMIC BURDEN OF MALARIA: A CASE STUDY OF WORKERS ABSENTEEISM IN A BANANA PLANTATION IN ZIMBABWE

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Absenteeism in this era has attracted attention across a number of areas, as the latter results in lost man hours hence bearing a direct impact on productivity and performance. Man lost hours are as a result of time taken off seeking treatment, this study assessed the impact of malaria on productivity in a banana plantation through absenteeism reducing the labour units on hand and therefore reducing overall farm production as well as economic gains both to the workers and the plantation. The study was carried out at Matanuska banana producing farm in Burma Valley, Zimbabwe. Raw data on absenteeism was obtained in retrospect from the Farm Manager, with malaria infection detected using the Rapid Diagnostic Test (RDT) kit. In the study the following measures of absence were determined; incidence of absence, absence frequency, frequency rate, estimated duration of spells , severity rate, incapacity rate , number of absent days, number of scheduled working days , absenteeism rate. The study followed up a total of 143 employees over a 5 month period in a cohort study. Results reflected malaria positivity to be 21%, 31.5%, 44.8%, 35.7% and 12.6% in January, February, March, April and May 2014 respectively. Initially one spell of absence was observed [194 (86.6%)] followed by 2 spells of absence [30 (13.4%)] and the latter spells were common for all employees. However the duration of spells of absence as a result of malaria ranged from 1.5 to 4.1 working days, with spells dominating among general workers hence the most affected group. An incidence of absence reflected at 93.3% (143/155) with spells of absence over a 5 month period totalling 224. The frequency rate of absenteeism was 1.6 with severity rate of absence being 2.4. The incapacity rate was 3.7. In conclusion, final absenteeism rate was 2.99% (below the 4% excessive rate), but however malaria contributed significantly to worker absenteeism.

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FALCIPARUM PREDOMINANT MALARIA OUTBREAK IN HUNKUND TALUK, BAGALKOTE DISTRICT, KARNATAKA, INDIA, 2015 TO 2016

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India accounts for 58% of malaria cases in South East Asia region. Four villages of Hungund taluk, Bagalkote district in Karnataka state, India reported clustering of malaria cases on 15th of November, 2015. We investigated to describe the outbreak and to give recommendations. Our study population included residents of all four villages, Hirekodagalli, Gugglemaari, Gudur and Hanumnal. We surveyed fever cases in fever clinic, between 20th of November, 2015 and 31 of March, 2016. We defined a confirmed case of Malaria as any patient whose blood smear was positive for plasmodium falciparum or plasmodium vivax. We collected line list of confirmed malaria cases from fever clinics. We calculated attack rates by village, age groups and gender. There were 601 falciparum cases and 213 vivax cases. Sixteen cases had mixed infections. Falciparum cases started on 26th October, 2015, with peak between 27th and 29th October and started coming down after 1st of December, 2015. Last case occurred on 18th of February, 2016. Attack rate of Falciparum cases was 15% (601/4111). Median age was 22 years (range: 1.5 – 81 years). Falciparum incidence was highest (24%) in Gugglemaari village. Vivax cases started on 26th of October peaked between 27th October and 16th of November and last case occurred on 21st of December, 2015. Attack rate of vivax cases was 5% (213/4111). Median age was 22 years (range: 3 to 80 years). Highest attack rate (7%) was in Gugglemaari (69/959) and Gudur (45/668) while attack rate was 4% in Hirekodagalli (83/2024) and 3% in Hanumnal (15/460). In conclusion, there was malaria outbreak between 26th October, 2015 and 18th February, 2016 in four villages of Hungund

taluk. Falciparum accounted for majority of cases and Gugglemaari village was most affected. We recommend identifying entomological and environmental factors for the outbreak.

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CHANGING MALARIA EPIDEMIOLOGY IN KWAZULU-NATAL, A PROVINCE IN SOUTH AFRICA TARGETING ELIMINATION BY 2020

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KwaZulu-Natal, a province in South Africa targeting malaria elimination by 2018 had to revise its timelines due to challenges experienced within the province. Malaria transmission is unstable and seasonal with the peak transmission season being between October and May. The main vector is *Anopheles arabiensis* and the most common parasite, *Plasmodium falciparum*. The majority of the local cases are reported from the Umhlabuyalingana border municipality and imported cases from Ethekwini municipality. Retrospective and current data was sourced from the Malaria Information System that is housed within the Provincial Department of Health. The data was subjected to trends analysis to identify the changing epidemiology in the province. Malaria cases have decreased drastically in KwaZulu-Natal from 42 000 in 2000 to 402 in the first two months of 2017. Due to the decreases in mortality and morbidity, South Africa adopted an elimination agenda in 2012. Although the number of local cases in KwaZulu-Natal decreased to 53 in the 2016/2017 malaria season, the numbers increased to 149 in the first 3 months of 2018. The proportion of imported cases remained consistent at an average of 80% of all reported cases, with the majority of cases originating from Mozambique. The incidence of local malaria increased from 0.07 in 2014 to 0.1 in 2017. Between 2014 and 2017 the number of cases in pregnant women and children under 5 years halved. The main intervention, indoor residual spraying with DDT or a carbamate, was around 69% for 2017. A new potential vector has also been identified in the province which may have contributed to the low levels of transmission. KwaZulu-Natal is on track for achieving elimination but the changing epidemiology continues to be a challenge. The main factors hampering elimination efforts appear to be imported cases that may be causing secondary local cases, as well as the decrease in spray coverage that left more people unprotected. The root causes of the low levels of residual malaria need to be investigated and addressed for KwaZulu-Natal to achieve elimination.

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GAMETOCYTEMIA IN FEBRILE PATIENTS FROM DIVERSE ECO-REGIONS OF KENYA

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While asexual malaria parasitemia determines disease severity, gametocytemia is crucial in the disease transmission. This study used qPCR that targets the 18S rRNA and the Pfs25 mRNA to enumerate asexual parasitemia and gametocytemia respectively in 1,436 blood samples collected from patients presenting with acute febrile illness (AFI) at hospitals located in Kisii highlands, Lake Victoria basin, Nairobi metropolitan, arid and semi arid areas, and the coast region. Overall, the prevalence of asexual parasitemia was 66.3% (952/1436) and 28.8% (414/1436) for gametocytemia. Of the 952 patients with asexual parasitemia, 378 (39.7%) had gametocytemia. There was no correlation between asexual parasitemia density and gametocytemia. Only 9.5% (36/414) of individual with gametocytemia did not have asexual parasitemia. Regionally, the arid and semi arid areas had the highest gametocytemia in absence of asexual parasitemia, 24.8% vs 3.6% in other eco-regions. In addition, gametocyte density in patients without asexual parasitemia was significantly higher ($p<0.0001$) compared to patients with asexual parasitemia. In conclusion, while it's clear that gametocytemia is a function of asexual parasitemia, it remains to be determined why a large proportion of gametocytemia in the arid and semi arid areas is without asexual parasitemia and two, why 60% of asexual parasitemia do not have gametocytemia.

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QUANTIFICATION OF STAGE-SPECIFIC *PLASMODIUM FALCIPARUM* GAMETOCYTE RNA TRANSCRIPTS TO EVALUATE THE IMPACT OF HIV STATUS ON GAMETOCYTEMIA IN WESTERN KENYA

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Plasmodium falciparum and human immunodeficiency virus (HIV) have overlapping endemicities in sub-Saharan Africa, but co-infection remains poorly studied, especially the impact of HIV status on *P. falciparum* gametocyte carriage. We developed a multiplex qRT-PCR panel to quantify the gametocyte RNA transcripts of *Pfs16*, *Pfs48/45*, and *Pfs25*, which are early, intermediate, and late stage gametocyte markers, respectively. Quantitative expression of these markers was used as a sensitive surrogate for gametocytemia in a set of malaria positive samples from adults seeking voluntary HIV testing in Kisumu, Kenya as part of a larger cross-sectional molecular epidemiology study of pre-treatment HIV^+ individuals. The late stage marker *Pfs25* is considered the gold standard for molecular detection of *P. falciparum* gametocytes because *Pfs25* is highly expressed on mature gametocytes, and earlier gametocyte stages are thought to remain sequestered. We found that *Pfs16* was expressed more frequently than *Pfs25* in peripheral samples, and that detection could occur without concurrent *Pfs25* expression, suggesting that there are at least some early stage gametocytes in circulation. Additionally, we compared the expression of our markers of interest between HIV^+ and HIV^- patients in order to determine whether HIV status impacts malaria transmission. Our preliminary studies found that HIV^+ patients have a statistically significant lower overall prevalence of gametocyte detection when compared to HIV^- patients. However, in gametocyte positive samples, expression of *Pfs16* and *Pfs25* is statistically higher in HIV^+ patients as compared to HIV^- patients, and HIV^+ patients are more likely to be concurrently positive for all three gametocyte markers. Thus, HIV^+ individuals are carrying more gametocytes. Additional analyses and *in vitro* work are planned to investigate the mechanism of increased expression of these markers in our HIV^+ cohort. Our data highlights the importance of treating HIV^+ patients with gametocidal drugs to decrease malaria transmission, particularly in areas where co-infection is common.

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ADHERENCE TO ANTIMALARIAL TREATMENT IN THE CONTEXT OF REACTIVE CASE DETECTION IN ZANZIBAR

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Adherence to antimalarial treatment is a central building block of effective malaria control and elimination. While patient adherence to treatment regimens has been investigated in different settings, there is a dearth of evidence on adherence by asymptomatic individuals who are provided

treatment in the context of reactive case detection strategies. This study aimed to assess adherence to the first-line treatment artesunate-amodiaquine (AsAq) by symptomatic and asymptomatic individuals detected and provided treatment by the expanded surveillance system of the Zanzibar Malaria Elimination Programme (ZAMEP). A rolling cross-sectional survey in six districts in Zanzibar followed passively and actively detected infected individuals of all ages on the 3rd day after receiving antimalarial treatment, when they were expected to have completed a full AsAq treatment course. Treatment adherence was assessed by inspection of the blister package and structured interviews with patients or caretakers. A total of 495 study participants were analyzed. The overall patients' adherence to AsAq was 85% (95% CI: 81% - 88%). The adherence by symptomatic study participants was 88% (95% CI: 84% - 93%) and 75% (95% CI: 71% - 79%) by asymptomatic participants. Study participants' health condition was found to be a determinant of adherence to AsAq (OR = 2.8, P = 0.001). Common self-reported reasons for non-adherence were *not feeling sick*, *rapid recovery rate*, *a perception that the drug is too strong*, and *forgetting to take the pills.* In conclusion, we found moderate and high levels of adherence to AsAq among treated asymptomatic and symptomatic study participants respectively. Presence of signs and symptoms prior treatment influenced intake on the treatment. Further efforts focusing on IEC/BCC activities are needed to improve correct and completion of dose uptake by both treated patients.

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PLACENTAL MALARIA AND INCIDENCE OF ANEMIA IN INFANCY

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Several factors contribute to anemia in infancy. In malaria endemic areas, the independent effect of placental malaria (PM) on infant hemoglobin has been sparingly studied. We investigated the effect of PM on the incidence of anemia among a cohort of 1855 mother-infant pairs in the Kintampo area, a high malaria transmission area of Ghana. Placental malaria was determined by placental histology. Haemoglobin was measured using a Micros 60 haematology auto analyzer. Multivariate cox regression analysis was used to determine the association between PM and incidence of first or only episode of anemia. The incidence of first or only episode of anemia was similar among infants of mothers with or without placental malaria, irrespective of gravidity. Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD) was strongly associated with infant anemia (adjusted hazard ratio [aHR] 1.43, 95% Confidence Interval 1.13 - 1.81 p<0.01). Similarly, infants who lived in rural areas had 29% higher incidence of anemia compared with their peers who lived in urban areas. There was no evidence that exposure to PM *in-utero* influenced hemoglobin level during the first year of life. Other factors such as G6PD deficiency, infant exposure to malaria and residence in rural areas are more likely to influence hemoglobin levels in the study area.

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IMPLEMENTATION OF PROACTIVE CASE DETECTION AND COMMUNITY CASE MANAGEMENT IN MODERATE MALARIA TRANSMISSION DISTRICTS IN SENEGAL, 2017

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Since 2014, the Senegal National Malaria Control Program has scaled up a proactive case detection approach to integrated community case management. In addition to being available for consultation (passive case detection), community health workers (CHWs) in low access villages visit every home in their community weekly during malaria transmission season to offer rapid diagnostic tests (RDTs) to people of all ages with fever, artemisinin-based combination therapy (ACT) to those who test positive, and treatment to children < 5 years with diarrhea or pneumonia. From 2014 to 2016, this approach was scaled up in 708 villages in high malaria burden districts, resulting in a three-fold increase in the number of people diagnosed and treated for malaria by CHWs. In 2017, an additional 24 districts with parasite prevalence < 1% implemented this approach. Data reported by the CHWs regarding patients seen during their proactive (in 2017) and passive case detection activities (in 2016 and 2017) were analyzed. In the new districts, 45% (490/1,094) of the CHWs participated in proactive case detection. These CHWs averaged 14 weeks of proactive case detection activities, treating 12,146 children for diarrhea and 12,705 children for pneumonia, and performing 19,136 RDTs (39 per CHW), 2,836 of which were positive (6 per CHW), and 99% of whom received an ACT. In addition, all 1,094 CHWs performed 19,633 RDTs (18 per CHW) and detected 4890 malaria cases (4 per CHW) as part of passive case detection work during the same period. While the total number of CHWs increased by 71% in 2017 compared to 2016, RDTs performed and cases diagnosed during passive case detection increased by 115% and 122%, respectively. Combining proactive and passive case detection, the total numbers of RDTs performed and cases detected by CHWs increased from 2016 to 2017 by 324% and 251%, respectively. While initially targeted to high transmission zones, proactive case detection substantially increased the number of RDTs performed and cases detected and treated in low access villages in low transmission zones, reducing the population infected with malaria, and potentially reducing transmission.

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UNDERSTANDING SUSTAINED FOCAL MALARIA TRANSMISSION IN THE PRESENCE OF REACTIVE CASE DETECTION IN RURAL SOUTHERN ZAMBIA SUSTAINED FOCAL MALARIA TRANSMISSION IN THE PRESENCE OF REACTIVE CASE DETECTION IN RURAL SOUTHERN ZAMBIA

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Reactive case detection is currently conducted in Southern Province, Zambia to enhance surveillance and clear the asymptomatic reservoir. After an index case is confirmed with malaria by RDT, household members and neighbors within 140-meters are tested with an RDT and treated with ACT if positive. A subset of index cases was evaluated by a study team who administered a questionnaire, performed an RDT, and collected a DBS for detection of *Plasmodium falciparum* DNA by qPCR and genotyping. CDC light traps were used for indoor mosquito collections adjacent to sleeping areas and outdoor collections near animal sheds. As part of the study, the screening radius was extended to 250 meters and follow-up visits were performed 30 and 90 days after the initial visit. Enrollment began in March 2016, with data collection concluding in June 2018. The qPCR results will be used to determine prevalence in index and neighboring households and monitor changes over time and space. A molecular barcode will be used to determine parasite genetic relatedness between households and over follow-up. Data on individual characteristics, parasite haplotypes, household characteristics, and indoor and outdoor vector counts will be used to determine factors associated with infection, local transmission, and importation. Individual and household level comparisons will be made between those with and without *P. falciparum* infection.

Households transmission chains will be identified as those with new infections on follow-up visits that are genetically related to previous infections. Household level comparisons will be made between these households, those with no malaria, and those that have infections that are not genetically linked. Results from the first year showed a decrease in prevalence from 11% on the initial visit to 1% on the final visit in index households. Similar decreases were not observed in neighboring households in both screening radii. Within household transmissions were identified between visits in index households only. Additional analyses will provide results essential for understanding factors sustaining transmission in the presence of reactive case detection.

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A COMMUNITY-RANDOMIZED TRIAL ASSESSING THE EFFECTIVENESS OF TARGETED ACTIVE MALARIA CASE DETECTION AMONG HIGH-RISK POPULATIONS IN SOUTHERN LAO PDR: STUDY DESIGN AND BASELINE SURVEY RESULTS

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In the last decade, important progress has been made towards malaria elimination goals in the Greater Mekong Subregion (GMS), and Lao PDR (Laos) is currently committed to national malaria elimination by 2030. However, substantial gaps in early diagnosis and treatment remain, especially in addressing the asymptomatic parasite reservoir in mobile, migrant, ethnic minority and vulnerable (MMEV) populations who may not be captured in formal public sector facilities. Novel diagnostics are needed to address the parasite reservoir not captured by standard rapid diagnostic tests or microscopy have been developed, including the Alere ultra-sensitive RDT (SD Bioline/Roche) for point-of-care *Plasmodium falciparum* detection. To assess the impact of these novel diagnostics on malaria transmission, two novel interventions will be trialed using a split-plot community randomized trial in 56 villages in Champasak province, Lao PDR. This spilt-plot design includes random allocation to intervention or control (standard of care) at two different spatial scales. The primary intervention will be three rounds of mass test and treat (MTAT) with uRDTs among all consenting households in randomized intervention villages. The secondary intervention will be focused test and treat (FTAT) using peer navigators to target MMEV populations in all areas outside villages, randomized by health center catchment areas (HCCAs). The total study population of includes approximately 85,000 persons in 14 HCCAs. A baseline survey to capture household-level data, and to assess the feasibility and acceptability of the intervention was completed December 2017; amongst 4,903 persons tested, there were five persons positive for *P. falciparum*, and one positive for *P. vivax* by rapid diagnostic test; PCR-based results are expected in March 2018, with FTAT scheduled to start in April 2018, and the first round of MTAT to begin in May 2018. Evaluation of each intervention will be based upon a 50% reduction in *P. falciparum* prevalence from an endline survey October-November 2018 and routinely collected incidence data December 2017-November 2018.

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UNDERSTANDING THE EPIDEMIOLOGY OF IMPORTED MALARIA CASES IN VIETNAM AMONG INTERNATIONAL LABORERS

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Vietnam witnessed a 97% decline in malaria cases between 1991 and 2014, prompting a national goal for elimination by 2030. As local malaria transmission declines, a greater focus on the importation and potential reintroduction of malaria parasites is essential to support elimination objectives. Challenges to elimination in Vietnam include an increasing risk of malaria importation from a growing labor force traveling to endemic countries abroad, as well as the continued threat of locally developing or imported multi-drug resistant malaria. Currently, little understanding exists on the epidemiology of imported malaria in Vietnam from returning international laborers. In 2017, a study was conducted at the National Hospital for Tropical Diseases (NHTD) in Ha Noi to describe the clinical and epidemiologic characteristics of suspected malaria patients recently returning from African or other Southeast Asian countries. Eligible participants were comprised of patients admitted to NHTD with a recent history of living or travelling abroad and exhibiting clinical symptoms of malaria. Travel histories, blood samples and clinical data were collected and analyzed. 31 participants were enrolled in the study. 87% (n=27) were males with a mean age of 36.5 years. 77% (n=24) reported to have had malaria at least once, with 16% (n=5) reporting to have had malaria more than 10 times. Angola (48%, n=15) and Cameroon (16%, n=5) were the most common countries visited. Work (68%, n=21) and business (16%, n=5) were the most common reasons for travel. 92% (n=29) tested positive for malaria by blood film examination, with 62% (n=18) diagnosed with *Plasmodium falciparum*. Day 3 parasite clearance rates were 50% (n=9) and 60% (n=3) for *P. falciparum* and *P. vivax*, respectively. Data from these studies indicate that imported malaria from returning international laborers is a potential threat to elimination efforts in Vietnam that requires further attention. Additional scaled research and detailed investigation into appropriate malaria prevention and intervention strategies targeted towards international laborers, both at pre-departure and upon return is required.

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HEALTH CARE PROVIDERS ANTIMALARIAL PRESCRIPTION PRACTICES DURING MALARIA IN PREGNANCY IN LIBERIA

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Malaria is among the top three diseases for outpatient attendance (42%) and the leading cause of inpatient deaths 39% in Liberia. Over the past years, the National Malaria Control Program has had recurrent stock outs of antimalarial commodities (ACTs and mRDTs), quantifications and distribution to health facilities. This challenge affected the full implementation of national malaria treatment guidelines for pregnant women. We retrospectively reviewed medical records of pregnant women who attended selected health facilities from January 2015 to December 2016 to evaluate antimalarial prescription practices amongst pregnant women by health care providers. A total of 931 eligible records were reviewed. The recorded antimalarial prescribed for pregnant women in 2015, ACT accounted for 62%, 95% CI: [59.29-66.23] of all drugs prescribed for the study participants followed by Quinine at 36%, 95%

CI: [32.99-39.18] with Artemether being the least at 0.21% 95% CI: [0.08-0.51] and other drugs accounting for 1.29% 95% CI: [0.56-2.01]. At the same time, 1,317 records were reviewed in 2016 with a declined and slight increment observed in ACT and Quinine prescription at 53% 95% CI: [50.30-55.69] and 44% 95% CI: [41.51-46.88] respectively. Artemether accounted for 0.38% 95% CI: [0.05-0.71] while other was 2.43% 95% CI: [1.60-3.26]. The findings have important Health system and planning implications. Therefore, Service providers should bear in mind that as per the National treatment protocol, quinine and ACT are used - for both second and third trimester, while quinine only is used in the first trimester. However, this result could be a consequence of either non-compliance to protocol or stock out of quinine as quinine is the first line drug of choice for MIP in Liberia.

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DETERMINING THE MEAN TIME INTERVAL BETWEEN TWO SUCCESSIVE EPISODES OF MALARIA AMONGST PREGNANT WOMEN

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In Liberia, malaria is among the top three diseases for out-patient attendance (42%) and the leading cause of inpatient deaths 39%. Over the past years, the National Malaria Control Program has had recurrent stock outs of antimalarial commodities (ACTs and mRDTs), aged specific time interval of malaria episodes (the average number of days from one episode to the subsequent episode), quantifications and distribution to health facilities. This challenge affected the full implementation of national malaria treatment guidelines for pregnant women. The study used health facility-based patients' records to retrospectively determine the mean time interval between two successive malaria episodes amongst pregnant women. A facility-based retrospective, cross sectional survey design was used to collate data across fifteen randomly selected health facilities. Patient's facility-based unique Identification Number (ID) was used to link up the individual health facility attendance and to determine the mean time interval between two successive malaria episodes amongst pregnant women. Ninty-five (95), 95% CI; [85.56-103.96] days was recorded as the mean time interval between 2 successive malaria episodes with minimum interval of 16 and maximum interval of 326 days in 2015. The statistics were slightly different in 2016 as the mean time interval was 99.99 days, 95% CI; [91.79-108.18] with a minimum time interval of 15 days and a maximum time interval of 327 days in 2016. The results have planning implications as well as Health system. Decision makers should consider accounting for the anticipated time interval between two successive malaria episodes in pregnant women thereby carrying out proper quantification during program planning and implementation as the overall implication could lead to over or under planning.

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EVALUATION OF SEROLOGIC MARKERS OF RECENT EXPOSURE TO *PLASMODIUM VIVAX* IN A MODERATE TRANSMISSION REGION OF THE PERUVIAN AMAZONIAN

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Plasmodium vivax is now the dominant parasite throughout the Americas. Key to its elimination is the ability to detect and treat people with asymptomatic blood-stages and those that are at high risk of relapsing. Novel serological markers of recent exposure to *P. vivax* infection have been validated by our team in longitudinal cohort studies in Thailand, Brazil, Papua New Guinea and the Solomon Islands. Using the Luminex® platform, we evaluated responses to a panel of 26 *P. vivax* antigens in 590 Peruvian individuals, who have been followed for between 13 and 37 months in two communities from Loreto, Peru. Healthy donors from non-endemic countries were included as negative controls (n = 274). The prevalence of *P. vivax* by qPCR at the time of serum collection was 21.19%; 93% of these were sub-patent infections. 64% (n = 378) of the cohort participants had at least one qPCR infection in the last 9 months. Using multivariate linear regression models, antibody titers showed a dependent relationship with age and the number of qPCR infections in the last 6 months. In order to identify people at high risk of relapsing *P. vivax* infections, we used classification algorithms and selected a 5-antigen combination that maximizes information on an individual's recent infection (≤ 9 months). After cross-validation, our model accurately classified whether an individual had a recent infection in the last 30 days (AUC = 0.73), 3 (AUC = 0.74), 6 (AUC = 0.79) and 9 months (AUC = 0.83). These analyses suggest that antibodies responses to the 5 novel antigens is higher in individuals with recent exposure to *P. vivax*, even in a setting with high proportions of submicroscopic infections, and thus, potentially allowing the identification of liver-stage carriers.

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RISK FACTORS ASSOCIATED WITH MALARIA IN-HOSPITAL DEATHS IN THREE REFERRAL HOSPITALS FROM A HIGH-BURDEN MALARIA REGION IN NORTHERN MOZAMBIQUE

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Malaria is a leading cause of hospitalization and inpatient mortality in Mozambique. However, few studies have examined the factors associated with malaria related inpatient deaths. The present study describes factors associated with inpatient deaths in a high burden malaria region in Northern Mozambique. This study was based on data obtained from patient records, admitted between June 2015 and May 2016. Data was collected from three health facilities representing the various levels of care: a primary health care clinic, a secondary level facility and a tertiary hospital. These health facilities report the highest number of malaria deaths at their respective attendance level. Logistic regression was used to conduct univariate and multivariate analysis of selected risk factors for malaria mortality. A total of 957 confirmed malaria cases were registered at the three facilities, from which 893 (93.3%) cases had complete data. Among the 893 patients, 390 (43.7%) were children under the age of five years and 488 (54.6%) were women, of which 117 (24.0%) were pregnant. There were 75 cases of death, corresponding to an overall case fatality rate of 8.4%. There were 25 deaths among children under the age of five years. No deaths were recorded among pregnant women. Case fatality was associated with being aged 5 years or older, severity of disease, presence of complications, patient chart inadequately filled and admission to a primary health care facility. Furthermore, although case fatality decreased as length of hospitalization increased, half of the deaths occurred 3 days after admission. Case fatality was not associated with sex, presence of co-morbidities, pre-treatment referral or antimalarial treatment 21 days prior to admission. The malaria case fatality rate remains high in this region. Reducing inpatient malaria deaths will require improvements in early diagnosis and treatment of uncomplicated cases, better identification

and treatment of severe cases, and improvements in the quality of treatment provided during admission, particularly at the primary health care level.

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TRANSFORMING SURVEILLANCE INTO A CORE INTERVENTION; THE PATH TO BUILDING A STRONG MALARIA SURVEILLANCE SYSTEM IN UGANDA

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The 3rd Pillar of the WHO Global Technical Strategy for malaria guides countries to transform malaria surveillance into a core intervention to fast track malaria reduction efforts. It is estimated that only 30% of the malaria cases in Uganda are captured by the surveillance system. Uganda rolled out a systematic multifaceted approach to improve the functioning of the surveillance system. We present current framework, results and experience of the process of strengthening malaria surveillance in Uganda in the period August 2016 to March 2018. First, Uganda conducted an assessment of surveillance monitoring and evaluations (SME) system that subsequently guided their interventions. Strategic policies, guidelines, training circular and tools were developed to support capacity and health systems strengthening activities. The second phase implemented activities as recommended from the SME assessment at all levels. At national level a team of 33 trainers were re-trained in SME, 30 on epidemic preparedness and response and 34 on entomological surveillance. The cascaded training covered 589 health workers trained from 82/116 (71%) districts, entomology surveillance training of 40 expert trainers that cascaded skills to 27 districts. A prioritised national research agenda was developed and national team of 20 trained in mapping. A web-based platform with active dashboard for malaria information developed to ease access and share of evidence on malaria. Follow-up of all malaria deaths, documenting and taking action on circumstances surrounding each malaria mortalities was initiated as offshoot of the weekly reports - with status, action and recommendations shared with stakeholders. Electronic database and capture tools are being update to include IPT3, data validation and data quality analysis performed in an integrated approach. Routine entomological surveillance strategies are also being developed. Implementing partners have been supported to follow-up and take to scale all SME assessment recommendations. Partnerships with members of Parliament (350) who will use the Malaria Score Card tool for decision making have been initiated.

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RTS,S/AS01 MALARIA VACCINE MISMATCH OBSERVED AMONG *PLASMODIUM FALCIPARUM* ISOLATES FROM SOUTHERN AND CENTRAL AFRICA AND GLOBALLY

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The RTS,S/AS01 malaria vaccine encompasses the central repeats and C-terminal of *Plasmodium falciparum* circumsporozoite protein (PfCSP). Although no studies from Phase II clinical trials observed evidence of strain-specific vaccine-induced immunity, recent studies have shown a decrease in vaccine efficacy against non-vaccine strain parasites. In light of goals to reduce malaria morbidity and mortality, anticipating the effectiveness of RTS,S/AS01 is critical to planning widespread vaccine introduction. We deep sequenced C-terminal *Pfcsp* amplicons from 77 individuals living in Luapula Province, Zambia and neighboring Haut-Katanga Province, Democratic Republic of Congo (DRC) and compared the translated amino acid haplotypes to the 3D7 vaccine strain. Further, to contextualize the genetic diversity sampled in in Zambia and the DRC with the global *Pfcsp* diversity, we analyzed 3,809 additional *Pfcsp* sequences from the Pf3k database and constructed a haplotype network representing 15 countries from Africa and Asia. In our study sites in Zambia and the DRC, only ten of the 193 unique *Pfcsp* sequences (5.2%) matched 3D7 at all 84 amino acids. The median number of amino acid differences from 3D7 was seven. The nucleotide diversity observed in our samples was similar to the diversity observed in the global haplotype network. This small sample of *P. falciparum* isolates from the border region between Zambia and the DRC, an area for which little previous *P. falciparum* genetic data has been reported, reveals substantial genetic differences between the RTS,S/AS01 vaccine strain and circulating parasites, consistent with the genetic diversity from 13 other African and Asian countries represented in the Pf3k database. These observations underscore the need for additional research assessing genetic diversity in *P. falciparum* and the impact of PfCSP diversity on the efficacy of RTS,S/AS01.

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ANALYSIS OF WITHIN-HOST EVOLUTION OF *PLASMODIUM FALCIPARUM* DURING TREATMENT

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Antimalarial drugs impose strong selective pressure on *P. falciparum* parasite genomes and leave signatures of selection. The evolutionary basis of drug resistant malaria in endemic and epidemic settings continues to remain an ongoing scientific priority whose solution carries a significant effect on treatment outcomes. To understand these evolutionary changes, we used various approaches to test the neutral models of evolution using *P. falciparum* genomic data which were collected from Kombewa and Maseno in Kisumu, Kenya between 2013 and 2015. The (dS/dN) ratio was used to predict the effect of selection on protein coding loci of the *Pfk13* gene. A logistic regression model was used to test the association between IC_{50s} and the SNPs. mCSM and SDM were used to detect the effects of mutations on the *Pfk13* gene while the PRIMO web server was used to locate the SNPs on the Kelch13 propeller domain. Modeller V9.1 was used to predict the structure of the Kelch 13 propeller domain and the Posview webserver used to predict ACT/kelch 13 interactions. Population differentiation was done using Microsatellite analyzer to calculate F_{ST} and customized R scripts with the relevant population genetics packages were used in the analysis. In 2013, Tajima's D genomic summary statistics was 4.53194, Fu & Li D* 2.13380, and Fu &Li F* 3.62142. However, in 2015 Tajima's D was -2.42910, Fu and Li's D* -5.2712, and Fu and Li's F* -5.0045. The dS/dN in 2013 was 1.0299, while 2015 dS/dN was 2.6884. Kenyan SNPs occur on the intra or inter blade domains on *PfK13* propeller domain. The F_{ST} analysis showed minimal population differentiation of the parasites during treatment. There was no significant association between SNPs and IC_{50} values but SNPs at codon D547E showed association with Artesunate and D559E with AQ and MQ IC_{50} respectively. Even though there is an exponential increase in the number of non-synonymous point mutations in the *Pfk13* gene, the Kenyan *P. falciparum* strains remain sensitive to ACT drugs. Further research needs to be done by deep sequencing this location of chromosome 13 as it will provide more power for finding novel SNPs for further validation.

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GENETIC CHARACTERISTICS OF *PLASMODIUM VIVAX* ISOLATED FROM NORTHERN MALI

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The surprising presence of *Plasmodium vivax* in Mali where the population is mostly Duffy negatives is one more threat to public health. There is a need to investigate the origin and reaction to antimalarial drugs of *P. vivax* isolates in Northern Mali, which is our key objective in the present work. *P. vivax* DNA was extracted from 7 Rapid Diagnostic Tests (RDT) followed by selective Whole Genome Amplification (sWGA). The whole genomes were then sequenced using the Illumina platform, and the Next Generation Sequence data analysed. For population differentiation analysis we used 22 additional *P. vivax* whole genome from 6 other countries, which we downloaded from the European Nucleotide Archive. The population differentiation analysis revealed significant variant differences between *P. vivax* populations in urban and rural areas of Northern Mali. These were located in chromosomes 2, 3, 4, 5, 12, 13 and 14. With regard to variant effects, the ratio of Transition/Transversion was 1.1, while the rate of variants with high effect was 1.62%. We did not identify *P. falciparum* orthologous genes *pvcrt-o* or *pvmdr-1* expressing variants which would lead to antimalarial drug resistance. Pairwise differentiation suggests polymorphisms between *P. vivax* strains isolated in rural and urban areas of Northern Mali. The Neighbour-Joining analysis shows clearly that taxa from Mali cluster together and are genetically distinct to those from Mauritania which share a border with Mali. The strains isolated in Northern Mali are genetically closer to those from Madagascar, India and Latine America. *In conclusion,* the results suggest that Northern Mali strains remain sensitive to Chloroquine and Primaquine. This study confirms that *P. vivax* strains, genetically distinct from those of Mauritania, are circulating in Mali.

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PARTIAL GENOME DRAFT OF *PLASMODIUM SIMIUM*, A VIVAX-LIKE PARASITE OF NEW WORLD MONKEYS

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Plasmodium simium was originally described in non-human primates from São Paulo, southeast Brazil, and subsequently was found in two genera of the Atelidae family, *Alouatta caraya and A. clamitans*, of the Atlantic Forest of South and Southeast Brazil. Studies suggest that *P. simium* is morphologically and genetically indistinguishable from *P. vivax*, which is consistent with one or more host switches between humans and monkeys in recent evolutionary times. The direction of the host switch (whether from monkeys to humans or vice-versa) remains under debate, although available phylogenetic data renders a lateral transfer from New World monkeys to humans very unlikely. Here we describe the first genome draft of the *P. simium*, isolate RS, derived from a field-collected blood sample from a howler monkey in Southeast Brazil. Because of extensive contamination with host DNA, we carried out selective whole-genome amplification using two previously described two different primer sets (pvset1 and pvset1920) and protocols (Cowell, et al, 2017, doi: 10.1128/mBio.02257-16). A DNA library was prepared using the TruSeq Nano DNA Kit (Illumina) and sequenced using Illumina HiSeq 2500. We mapped high-quality reads (quality scores >30) onto two *P. vivax* reference genomes, Sal-I version 10.0 and PvP01, using BWA. We used GATK version 2.0 for SNP calling. GATK Best Practices was used to annotate SNPs after SNPs files from different set of primers were merged in one single file. We obtained an average coverage of 129× (pvset1) and 76× (pvset1920), with a G+C content of 44-50% Overall, between 92-96% of the reads were identical to *P. vivax* reference genomes[MU1] , but only 35-51% of the Sal-I and PvP01 genomes, respectively, were covered by at least 6 reads from the *P. simium* isolate RS. A total of 3083-2658 SNPs (pvset1-pvset1920) were found compared to Sal-I and 4152-3898 SNPs (pvset1-pvset1920) compared to PvP01. These preliminary genome data may help to illuminate the origins and phylogenetic relationships of this little studied zoonotic malaria parasite.

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HUMAN MIGRATION AND THE SPREAD OF MALARIA PARASITES TO THE NEW WORLD

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The Americas were the last continent to be settled by modern humans, but how and when human malaria parasites arrived in the New World is uncertain. Here, we apply phylogenetic analysis and coalescent-based gene flow modeling to a global collection of *Plasmodium falciparum* and *P. vivax* mitogenomes to infer the demographic history and geographic origins of malaria parasites circulating in the Americas. Importantly, we examine *P. vivax* mitogenomes from previously unsampled forest-covered sites along the Atlantic Coast of Brazil, including the vivax-like species *P. simium* that locally infects platyrrhini monkeys. The best-supported gene flow models are consistent with migration of both malaria parasites from Africa and South Asia to the New World, with no genetic signature of a population bottleneck upon parasite's arrival in the Americas. We found evidence of additional gene flow from Melanesia in *P. vivax* (but not *P. falciparum*) mitogenomes from the Americas and speculate that some *P. vivax* lineages might have arrived with the Australasian peoples who contributed genes to Native Americans in pre-Columbian times. Mitochondrial haplotypes characterized in *P. simium* from monkeys from the Atlantic Forest are shared by local humans. These vivax-like lineages have not spread to the Amazon Basin, are much less diverse than *P. vivax* circulating elsewhere in Brazil, and show no close genetic relatedness with *P. vivax* populations from other continents. Enslaved peoples brought from a wide variety of African locations were major carriers of *P. falciparum* mitochondrial lineages into the Americas, but additional human migration waves are likely to have contributed to the extensive genetic diversity of present-day New World populations of *P. vivax.* The reduced genetic diversity of vivax-

like monkey parasites, compared with human *P. vivax* from across this country, argues for a recent human-to-monkey transfer of these lineages in the Atlantic Forest of Brazil.

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GENETIC ANALYSIS OF RELAPSING *PLASMODIUM VIVAX* FROM INDONESIAN SOLDIERS RETURNING TO A MALARIA-FREE AREA USING TARGETED AMPLICON DEEP SEQUENCING

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In the era of malaria elimination, identification of *Plasmodium vivax* relapse is a key to reduce malaria transmission. Hypnozoite dormancy and reactivation, largely remains a mystery. Relapse episodes in clinical settings are often confounded by re-infection in malaria endemic areas. We performed genetic studies in cohorts of soldiers returning from high-endemic area in Papua, Indonesia to non-malaria endemic areas using amplicon deep sequencing. 127 relapsing isolates collected from 94 individuals which were amplified in duplicate at a 117bp hypervariable pvmsp1 region were sequenced using Ion-Torrent technology. A median of 4314 pvmsp1 high quality sequencing reads in each isolate was used to identify unique variants and determine multiplicity of infection (MOI) via Seekdeep. 28 pvmsp1 haplotypes were detected among 94 individuals, with two-thirds (19/28) appearing in more than one isolate. Multiplicity of infection (MOI) in the returning soldier relapse episodes was relatively low (mean MOI = 1.6, range 1-4). 55% of first relapses were monoclonal, 71% of second relapses were monoclonal ($p = 0.05$), with the majority of paired relapses exhibiting a similar or decreased genetic complexity over time. Of the 33 twice relapsers, 8 displayed the identical clone at both relapses, while 11 were completely heterologous/different. Overall, our findings suggest the soldiers were exposed to multiple parasite genotypes during the yearlong deployment, but were more likely to experience clonal relapses, with decreasing genetic complexity over time, suggesting that early relapse genotypes were more prevalent, or simply decreasing hypnozoite burden over time.

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GENETIC VARIABILITY IN THE *PLASMODIUM VIVAX* VACCINE CANDIDATE ANTIGENS FROM CENTRAL INDIA

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The genetic diversity and evolutionary plasticity constitute major obstacles for malaria control and its elimination. Outside sub-Saharan Africa, *Plasmodium vivax* is the most widespread. Unlike *P. falciparum*, *P. vivax* forms dormant liver stages (hypnozoites) which causes recurring (Benign tertian) malaria. The unique biology of *P. vivax* and its epidemiology makes elimination more challenging with existing tools. Considering the high antigenic diversity in *P.vivax*, a next generation multi-allelic or multivalent vaccine is urgently needed to achieve the elimination. In this study, we have investigated genetic diversity in *P. vivax* vaccine candidates expressed during different stages of parasite lifecycle in field isolates, which includes merozoite surface protein -3 (*Pvmsp-3*), circumsporozoite protein *(Pvcsp*) and transmission blocking ookinete surface protein (*Pvs25*) . Samples were collected at Janakpur CHC hospital, district Korea Chhattisgarh, India. Amplification and DNA sequencing of *Pvcsp*, *Pvs25* and *Pvmsp-3* genes was performed using designed primers through polymerase chain reaction (PCR). Out of 58 samples, 40 samples were successfully sequenced and analyzed using reference strain *of P.vivax* (Salvador-I). *Pvcsp* gene analysis shows that majority of samples harbored VK210 type (72.5 %) in comparison with VK247 (27.5%) whereas in case of *Pvs25* gene, all sequenced isolates contains two non-synonymous nucleotide substitutions (G289C, T389C) which gives corresponding amino acid changes (E97Q, I130T). Subsequently, highly polymorphic *Pvmsp*-3 marker gene showed presence of two allelic size variants in field isolates. The frequency of Type-A variant was highest in 92.5% isolates followed by 7.5% of Type-B. Overall, a total of 25 polymorphisms were detected among all *Pvmsp*-3 allelic types. This study gives a best picture of antigenic repertoire in field isolates from a highly endemic Chhattisgarh state (Central Indian isolates) of India. Better understanding the geographic distribution of genetic diversity of this vaccine candidate gens will be key to designing and implementing efficacious vaccines.

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GENETIC DIVERSITY OF *MSP1*, *MSP2* < *GLURP* GENES ACCORDING TO HUMAN GENETICS RESISTANCE FACTORS (HEMOGLOBIN AND G6PD) IN *PLASMODIUM FALCIPARUM (P.F)* ISOLATES FROM SUBJECTS IN BURKINA FASO

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The genetic diversity &lt antigenic variation of the parasite are main factors responsible &gt the slow acquisition of protection against malaria. There are innate genetic factors mechanisms that allow human to resist malaria. A number of genetic association studies have shown a role &gt human genetic variation in resistance to infections. However, little attention has been devoted to the possible influence of human genetic variation on genetic diversity of *P.f*. *Msp1*, *msp2* &lt *glurp* have been widely used as markers to study genetic diversity, multiplicity of infections, level of malaria immunity transmission. The aim of this study is to analyse the genetic diversity of *msp1*, *msp2,* &lt *glurp* genes of *P.f* according to human genetic resistance factors as hemoglobin &lt G6PD deficiency from subjects with uncomplicated malaria, living in malaria endemic area. This was a cross-sectional study, carried out at Banfora &lt Sapone (Burkina Faso) where 464 subjects were enrolled. Samples were extracted &lt then analyzed by a nested PCR of *msp1, msp2* &lt *glurp* genes while the human genetic background was assessed by RFLP-PCR. The distribution of the *msp1* &lt *msp2* allelic families was not different according to the type of hemoglobin ($p=0.669$ &lt 0.925 respectively) but there were different according the type of G6PD ($p=0.004$ &lt 0.033 respectively). The K1 allele family (91.59 %) is the most widespread *msp1* allelic family while in the *msp2* alleles , the 3D7 family is the most common (89.22% versus 69.18% FC27) &lt this difference is statistically significant ($p<0.0001$). The *glurp* gene (92.03%) is the most widespread gene &lt there was no difference in its distribution according to the human factors of resistance. Our results on the polymorphism of *msp1* &lt *msp2* genes according to the type of G6PD suggest a correlation between the distribution of G6PD types &lt the severity of malaria.

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WHAT DRIVES THE SPATIAL AND TEMPORAL PATTERNS OF GENETIC DIFFERENCES BETWEEN *PLASMODIUM FALCIPARUM* MALARIA INFECTIONS IN KILIFI COUNTY, KENYA?

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Knowledge of how malaria infections spread locally is important for the design of interventions to reduce and interrupt transmission. A previous analysis of 1602 genotyped infections in Kilifi found an interaction between time and geographic distance on genetic distance. The mean number of single nucleotide polymorphisms (SNP) different was lower for pairs of infections with both a short time interval and short geographic distance. We sought to determine the processes and parameter values that are consistent with this observed pattern. We estimate the distribution of geographic distances between parent and offspring infections, and investigate whether specific immunity functions are necessary to account for the observed pattern. Methods for a setting with moderate transmission and a low proportion of the total infections sampled are limited. We developed a stochastic simulation model of households, people and infections parameterizing the model for the total number of infections, population and house density for Kilifi. The acquisition of new infections, mutation, recombination, geographic location and clearance were simulated. We used 53 SNPs with minor allele frequencies greater than 5%, and fit the model to the observed numbers of SNP differences. The method was able to recover the mean geographic distance from simulated data with known values. The observed interaction could be reproduced for at least some parameter values for the baseline model and the model variants with immunity functions. Although we cannot rule out genotype-specific immunity or a limit on the number of infections, they are not necessary to account for the observed pattern. The measure of goodness-of-fit did not support mean distances of less than 400m alone. However, the results were consistent with a mixture of distributions of longer and shorter mean distances, perhaps through human and vector movement. This is the first study that we know of which has attempted to estimate the mean distance between parent and offspring infections from data with a low coverage of infections in a setting with moderate transmission, and has implications for the design of studies.

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WHOLE GENOME SEQUENCING TO MEASURE COMPLEXITY OF INFECTION AND GENETIC DIVERSITY IN *PLASMODIUM VIVAX* CLINICAL ISOLATES FROM THE CHINA-MYANMAR BORDER

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Plasmodium vivax malaria has been refractory to malaria elimination efforts along the China-Myanmar border. Better understanding of the genomic epidemiology of *P. vivax* could inform these efforts by improving our understanding of parasite population structure, potentially leading to tools for distinguishing between local and imported infections. Most population genetic studies of *P. vivax* have relied on small numbers of microsatellite markers or single nucleotide polymorphisms or targeted a few polymorphic protein-coding genes, limiting their power to measure population structure or to detect the presence of multiple, genetically-different parasites in a single infection. The objective of this study was to assess complexity of infection and genetic diversity of *P. vivax* in clinical isolates collected from individuals symptomatic for malaria and living near the China-Myanmar border, a region that has experienced a recent increase in *P. vivax* malaria cases. We analyzed whole genome sequence data of 69 leukocyte-depleted, RDT-positive, field samples with low *P. vivax* genomic DNA obtained from a refugee camp located adjacent to the border between Laiza Township in Myanmar's Kachin State and the town of Nabang in China's Yunnan Province. The average sequencing coverage ranged from 34 - 411X for each sample, with more than 76% of *P. vivax* Salvador I reference genome covered with atleast 20 reads. Preliminary results show that the vast majority of the infections were monoclonal (90%) with a single strain significantly different from the reference strain, 5 infections with two strains (50:50, 30:70, 20:80 frequency) and 2 infections with three or more strains in varying proportions. Our analyses show that the whole genome sequence data can be used to conduct high resolution complexity of infection inference in field-collected samples in this region of low transmission. The sequence data obtained is being analyzed to assess genetic diversity and will facilitate the study of *P. vivax* geographical variations that may help to estimate parasite movement and distinguish transmission sources and sinks in this elimination setting.

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COMPARING AND VALIDATING GENE CO-EXPRESSION NETWORKS IN *PLASMODIUM FALCIPARUM* PARASITE

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Biological systems are complex, requiring comprehensive interrogation. Traditional differential expression approaches to the analysis of whole transcriptome data generally consider each gene in isolation outside of its broader regulatory context, failing to leverage the information available in the interactions among genes. The application of network science approaches to large-scale biological data can provide a global view of the intricate webs of interactions that make up these biological systems while also facilitating more integrative analyses. Given the wealth of available data, ample opportunity exists to generate new insights into gene-gene interactions, gene neighborhoods, gene function, and pathways by utilizing network views of these systems. Network science is a new and rapidly evolving field that relies on a wide range of network construction methods and thresholding criteria, each with distinctive strengths and weaknesses. With these methods oftentimes resulting in very different networks, development of network validation can provide a measure of how well each of the networks captures relevant biological information. By relying on the expectation that genes with similar function are more likely to be co-expressed, we have established a validation pipeline for *P. falciparum* gene co-expression networks that determines how well the structure of the network captures relationships between genes that are known to be functionally related. Using this pipeline and a published gene expression dataset, we have investigated and ranked the performance of several network construction methods to establish a high-quality baseline network for further investigations in *P. falciparum*.

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HUMAN MOBILITY RELATED TRANSMISSION OF NEW PARASITE GENETIC CLUSTERS IN MAZAN BASIN IN THE PERUVIAN AMAZON

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Plasmodium vivax is the main cause of malaria in the Peruvian Amazon and in recent years malaria incidence is rising in this region. Population genetic tools could be key components for the design and targeting of control strategies. These tools allow to measure the individual contribution of the factors associated with transmission and can discriminate between the endogenous transmission and imported cases. In this work, infections caused by *P. vivax* were collected in 2 basins, Mazan (MZ) and Napo (NP), located in the Peruvian Amazon. Thus, 173 infections were genotyped with 16 microsatellites. The genetic diversity and level of inbreeding in each basin was evaluated, and a Bayesian method was used to identified the number of genetic clusters, and autochthonous, introduced and imported infections. Finally, epidemiological factors associated with the genetic clusters and its effect on the clinical manifestations were determined. Results showed that MZ presented a greater genetic diversity (*He* = 0.71), high proportion of polyclonal infections (35.2%), and lower linkage disequilibrium (I_A^S 0.07) with respect to NP (*He* = 0.39, 18.8%, I_A^S 0.21). Five genetic clusters and 20 (22.7%) imported/introduced infections were identified in MZ, while in NP only 2 clusters were observed and just 3 (3.5%) infections were imported. In addition, in MZ cluster 2 was associated with people who traveled outside their community in the last month (OR = 4.33, p = 0.04); while the parasitemia of the infection (OR = 1.10, p = 0.04), being infected by a parasite belonging to cluster 1 (OR = 4.83, p = 0.03) , and living with someone who has left the community in the last month (OR = 4.48, p = 0.01) increased the probability of presenting symptoms. Our results suggest that human mobilization encourages the entry of new genetic clusters in MZ, so malaria control strategies that consider this factor are required.

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PLASMODIUM FALCIPARUM INFECTION INDUCES MEASURABLE IGA RESPONSES ON PROTEIN MICROARRAYS

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Previous studies of antibody responses to *Plasmodium falciparum (Pf)* malaria have focused on IgG, less frequently, IgM, and rarely, IgA, which is typically associated with response to antigens presented to mucosal sites. However, when we comprehensively assessed antibody responses in a controlled human malaria infection (CHMI) study, we observed IgA responses to *Pf* on protein microarrays in U.S. malaria-naïve volunteers who received 1-5 bites from aseptic *Anopheles stephensi* mosquitoes infected with the *Pf* NF54 strain. We then examined IgA responses to naturally acquired malaria in 1- to 6-year-old Malian children (median = 4 years) exposed to intense seasonal malaria transmission. Serum from the malaria-naïve group collected at baseline and 4 weeks following CHMI (n=35 pairs) and from Malian children collected 6-41 days before and 14-42 days following a clinical malaria episode (n=48 pairs) was probed with a protein microarray containing 823 *Pf* antigens from the 3D7 clone of NF54. For each antigen, a paired *t* test with α=0.05 was used to compare baseline and post-infection $\log_2$-transformed antibody levels. In the naïve group, IgA increased for 64% (n=527) of the *Pf* proteins. In Malian children, the IgA response rate was lower, with increased antibodies compared to baseline for 10% (n=85) of *Pf* proteins; 64% of the 85 proteins (n=54) were also the target of antibodies in the naïve group. For most antigens, IgA responses were higher in the naïve group than in Malian children. We conclude that the immunoproteome following first infection in a malaria-naïve adult by CHMI differs from the immunoproteome of a child living in a high transmission setting. Lower levels of seroreactivity in Malian children may reflect their nascent immune systems and/or exposure to diverse *Pf* strains, as opposed to the CHMI strain that is homologous to the protein microarray antigens. Future directions include exploration of possible sites of IgA induction, including mucosal-associated invariant T cells in the liver and gut-associated gamma-delta T cells, leading to new insights into *Pf* immune responses.

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A SINGLE NUCLEOTIDE POLYMORPHISM IN A *PLASMODIUM BERGHEI* APIAP2 TRANSCRIPTION FACTOR ALTERS THE DEVELOPMENT OF HOST IMMUNE RESPONSE

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Malaria is a deadly infectious disease caused by parasites of the *Plasmodium* genus that results in the deaths of over 400,000 individuals each year, mostly among young children in Africa. Antibodies play a central role in malaria immunity but the acquisition of protective antibodies in malaria endemic regions is a remarkably slow process that takes years of repeated infections, suggesting that the parasite itself may interfere with the generation of B cell immunity. However, at present we have little knowledge of *Plasmodium* virulence factors that influence host immunity. Here we provide evidence that a single nucleotide polymorphism resulting in a serine (S) to phenylalanine (F) change in the DNA-binding domain of an ApiAP2 transcription factor of the rodent parasite *Plasmodium berghei* (Pb) NK65 strain alters the outcome of infection in a mouse model. Infection with PbNK65F as compared to PbNK65S resulted in the differential expression of only 46 genes, most of which are predicted to play roles in antigenic variation and immune invasion. Although both infections were lethal, compared to PbNK65S infection, infection with PbNK65F resulted in a larger expansion of germinal center B cells, plasma cell lineage B cells and T follicular helper cells by a γ-interferon dependent process and higher levels of infected red blood cell-specific TH1-type IgG2a and 2c antibodies. Indeed, under the cover of the antimalarial drug chloroquine, or following sublethal infection, PbNK65F but not PbNK65S

infections resulted in the generation of protective immunity to subsequent challenges. Thus, our results demonstrate that a Pb ApiAP2 transcription factor functions as a parasite virulence factor in *Plasmodium* infections.

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EFFECT OF ALLELIC POLYMORPHISM ON MALARIA PARASITE SPECIFIC *EX VIVO* IFN-Γ RESPONSES TO APICAL MEMBRANE ANTIGEN 1 IN A MALARIA EXPOSED POPULATION

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Despite extensive genetic diversity in the *P. falciparum* Apical Membrane Antigen 1 (AMA1), the leading vaccine candidate antigens, vaccines continue to be formulated using recombinant antigens representing a few strains. There is limited data on the potential effect of antigenic polymorphisms on T cell responses, hence knowledge on the efficacy of vaccines based on such peptides is limited. This study was designed to investigate the effect of allelic polymorphism on malaria parasite *ex vivo* T cell-specific IFN-γ response to the malaria parasite vaccine candidate antigen AMA1, in a malaria exposed population. Seven study subjects with known HLA A and/or B super types were recruited for the study. The known HLA class 1 allele types were used to predict their recognition of 9-10mer peptides from the *P. falciparum* AMA1 antigen (3D7 strain) using the NetMHC algorithm. Full length sequences of the 3D7 strain *Plasmodium falciparum* AMA1 peptides were aligned with corresponding AMA1 sequences from 7G8, FVO, tm284, FC27 and AAN35928 parasite strains for identification and chemical synthesis of sequences with regions showing variability. 133 synthesized peptides grouped into 65 allelic sets, were used to stimulate study subjects' peripheral blood mononuclear cell (PBMCs) in IFN-γ ELISpot assays. A total of 4/7 (57.14%) of the study subjects responded positively to stimulations from one or all two/three corresponding peptides, in 22 allelic sets. Stimulation responses (sfc/million) within corresponding peptides in 10/22 (45.45%) of the allelic sets were found to be significantly different ($p < 0.05$). The site of amino acid substitutions at positions 1 and 6 of the allelic peptides accounted for 53.33% of all amino acid substitutions. In conclusion, although only 45.45% of the tested allelic sets were found to produce significantly different responses, the position of substituted amino acid could be a determining factor in differentiating between immunologically relevant and irrelevant allelic polymorphism in the study strains.

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IGG AND IGE RESPONSES TO *PLASMODIUM FALCIPARUM* AND INTESTINAL PARASITE ANTIGENS IN PEOPLE FROM SOUTHERN MOZAMBIQUE

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Exposure to multiple parasites in African people may lead to harboring two or more simultaneous infections. These co-infections could generate immune responses with different profiles that may impair the ability of the immune system to fight one of the coexisting pathogens. Intestinal parasites mainly induce a T_H2 (and IgE) response, whereas immunity to *Plasmodium falciparum* is acquired through a T_H1 (and IgG) profile. We have previously found that the induction of certain T_H2 cytokines is associated with a lack of malaria protection in RTS,S vaccinated children. We also found that CSP, the main component of RTS,S, and certain *Plasmodium falciparum* blood stage antigens, can induce IgE responses, and high levels of IgE against merozoite surface proteins were associated with a higher risk of developing clinical malaria. We hypothesize that the induction of T_H2 cytokines and IgE by *P. falciparum* antigens are due in part to an immune deviation caused by previous or current infections with intestinal parasites. In order to investigate the possible role of parasite co-infections on immune deviation, we have applied the quantitative suspension array technology to measure total IgG, IgG_{1-4} and IgE responses against a multiplex panel with antigens of *P. falciparum* (AMA-1, EXP-1, EBA-140, LSA-1, MSP-1, MSP-2, MSP-5*), Giardia lamblia* (VSP3), *Cryptosporidium parvum* (Cp17), *Ascaris lumbricoides* (As16, As37), *Trichuris trichiura* (Tm-WAP, Tm16), *Necator americanus* (Na-GST-1, Na-SAA-2), *Ancylostoma duodenale* (Ay-CP-2) and *Strongyloides stercoralis* (NIE), using plasma samples from children and adults from Southern Mozambique, for which their infection status for malaria, intestinal helminths and protozoa is known. We will present results on the possible mutual influence of intestinal parasites and *P. falciparum* infections on the respective total IgG, IgG_{1-4} and IgE responses. Future studies will focus on the study of IgM responses, the profiling of cytokines, chemokines and growth factors and cellular populations.

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SIGNALING INDUCED BY TLR2 AND TLR4 COLLECTIVELY MEDIATE LUNG AND LIVER PATHOLOGY IN MOUSE MALARIA MODELS

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Malaria still remains a major health problem in many countries around the world and is caused by the *Plasmodium* family of parasites. Like many other infectious diseases, malaria is a host immune response-driven disease that presents various multiple systemic clinical conditions as well as single or multiple organ-related pathologies, including cerebral malaria, acute respiratory disease syndrome, and liver pathology. Despite several decades of extensive research efforts worldwide, neither an immunotherapeutic to treat the disease nor an effective vaccine is available. A major constraint in realizing these goals is thought to be due to incomplete understanding of molecular and cellular processes involved in the development of immunity to malaria. Although malaria immunity has been extensively studied in mouse models and in humans for the past several decades, efforts have been predominantly focused on adaptive immunity, aiming to identify vaccine candidates. Thus, there have been relatively meager efforts in dissecting host-parasite interaction and cellular signaling mechanisms that initiate innate immunity. In-depth knowledge of these processes is important since innate immunity is the key for the development of suitable adaptive immunity. Previously, we and others showed that TLR-mediated signaling plays important roles in malaria parasite-induced innate immune responses, which contribute to protective immunity or pathogenesis. In the present study, using mouse malaria models, we found that TLR2 and TLR4 specifically contribute to malaria-induced acute respiratory syndrome and liver pathology. Parasite sequestration in the organs and consequent TLR-mediated signaling and inflammatory responses initiated by a parasite factor results in the recruitment of leukocytes, leading to exacerbated inflammatory responses in the organs causing tissue damage and pathology. The molecular and cellular mechanisms that contribute to the observed organ pathologies will be discussed.

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SELF-REACTIVE IMMUNOGLOBULIN G CONTRIBUTES TO ASYMPTOMATIC *PLASMODIUM FALCIPARUM* MALARIA IN AN ENDEMIC AREA OF IVORY COAST

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Despite considerable research, the mechanisms of natural acquired immunity to malaria in asymptomatic carrier are only partially understood. Among them the role play by self-reactive antibodies has not been clarified yet. This study aims to analyze the contribution of self-reactive antibody repertoire in asymptomatic malaria in Ivory Coast and also determine the impact of transmission level on these auto-antibody responses. Blood samples, n = 94 of patients consulting for symptomatic malaria (MM) attacks and living in three different malaria endemic settings (rural and peri-urban), n=47 asymptomatic carriers (AS) of parasites and n= 38 endemic control (EC) were enrolled. Anti-IgG antibodies against a whole parasite (3D7), brain extract and Anopheles salivary gland peptide gSG6-P1 were quantified by the enzyme-linked immunosorbent assay (ELISA). Human self-reactive antibody repertoires were analyzed by quantitative immunoblotting (Panama blot). The association of self- and parasite-specific- antibody repertoires was evaluated using Kruskal-Wallis test and Spearman's rank correlation. The level of total IgG response against 3D7 in AM was lower than those of MM. Moreover, children with AM exhibited a high level of auto-antibodies recognizing brain antigen as γ- , β-actin, Glutaryl-CoA dehydrogenase, Succinate-CoA ligase, and Acetyl CoA dehydrogenase. In addition, a significant correlation between the exposure marker (IgG against gSG6-P1) and auto-antibody responses was found ($p<0.01$). Then the reactivity of serum IgG against antigenic bands S6, S10, S11, S12, and S13 of brain extract (molecular weight between 50-75 kDa) discriminate different transmission sites. These results showed that exposure or parasite pressure seem to cause a quantitative and qualitative change in self antibody immune reactive profile. More the intense production of autoantibody in asymptomatic malaria carriers with decreased IgG to 3D7 represents an active immune response and highlights their potential role in mediating disease resistance or tolerance. However, their functional protective role needs further investigation.

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IMPACT OF RECURRENT MALARIA ON VΔ2 ΓΔ T CELL *IN VITRO* ANTI-PARASITIC ACTIVITY

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Clinical immunity to the most deadly human malaria parasite, *Plasmodium falciparum (Pf),* develops slowly over an individual's lifetime; while immunity limits symptomatic infections in older children and adults, protection against parasite replication is partial. Immune mediators, such as pro-inflammatory cytokines produced from innate-like γδ T cells, can reduce parasitemia but can also lead to excessive inflammation that exacerbates pathology. Repeated malaria exposure among Ugandan children is associated with reduced percentages of the Vδ2 γδ T cell subset, decreased cytokine production and proliferation in response to malaria antigens, and increased expression of immunoregulatory genes. This loss and dysfunction of the Vδ2 γδ T cell subset is further associated with a reduced likelihood of symptoms upon subsequent *Pf* infection. Aiming to investigate the role of attenuation of the γδ T cell pro-inflammatory response in the development of clinical anti-malarial immunity, we are currently examining the mechanistic processes driving altered function of γδ T cells after repeated malaria infection. We optimized an *in vitro* assay to test inhibition of *Pf*-infected red blood cell (iRBC) growth by Vδ2 γδ T cells and observed a significant reduction in parasite reinvasion after γδ T cells from malaria-naïve individuals were incubated with *Pf*-iRBC. We are evaluating how recurrent *in vivo* malaria impacts the ability of γδ T cells to inhibit parasite reinvasion using samples obtained longitudinally from Ugandan children with varying malaria exposure. Further, we will test the hypothesis that altered γδ T cell functional responses after repeated infection are due to transcriptional and/or epigenetic regulation. By deepening our understanding of the molecular mechanisms driving inefficient acquisition of antimalarial immunity in children, this work could enable novel therapeutic approaches that reverse this phenomenon.

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THE ROLE OF REGULATORY T CELLS IN THE CONTROL OF PARASITE GROWTH AND CLINICAL OUTCOMES FOLLOWING CONTROLLED HUMAN MALARIA INFECTION IN SEMI-IMMUNE KENYAN ADULTS

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The understanding of naturally-acquired immunity to malaria, caused by *Plasmodium falciparum,* has been hindered by a lack of appropriate animal models and conflicting results from immunoepidemiological studies. Controlled human malaria infection (CHMI) provides a more appropriate and reliable model to investigate protective and non-protective immune responses to malaria in more detail. 144 semi-immune Kenyan adults were each infected by direct venous inoculation (DVI) with 3,200 aseptic, purified, cryopreserved *P. falciparum* sporozoites (Sanaria® PfSPZ Challenge) and followed up for 21 days with twice-daily (days 7 – 14) or daily (days 15 – 21) qPCR monitoring of parasitaemia. 45.8% (*n*=66) of participants developed parasitaemia and met the diagnostic threshold for treatment (500 parasites/µl of blood) before day 21. 41% (*n*=59) exhibited low parasite growth or managed to clear parasites before day 21 and did not meet the criteria for diagnosis. 13.2% (*n*=19) had no parasites detectable by the qPCR assay at all timepoints. In contrast, in prior studies in malaria-naïve populations in the US or EU, all subjects administered 3,200 PfSPZ by DVI met the diagnostic threshold for treatment. We hypothesise that varying levels of naturally-acquired immunity underlie the differing parasite growth rates observed with CHMI. Regulatory T cell (Treg) activity has been associated with increased *P. falciparum* growth rates in malaria naïve adults undergoing CHMI, but their role in naturally acquired immunity is unclear. We will examine whether Treg frequency and transcriptional phenotype, before and during CHMI in semi-immune adults correlates with parasite growth and clinical outcomes. We will then determine if functional relationships exist between Treg activity, antigen-specific effector $CD4^+$ and antibody responses using *in vitro* assays. We will also determine whether the balance of pro- and anti-inflammatory cytokines can predict outcomes following CHMI. These data will improve our understanding of which components of the immune response are important in malaria, to inform vaccine design. A complete dataset will be presented at the meeting.

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PROPORTIONS AND FUNCTION OF *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE-SPECIFIC B CELLS IN VACCINATED AND NATURALLY EXPOSED INDIVIDUALS

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Repeated exposure to *Plasmodium falciparum* (*Pf*) infections results in the expansion of atypical memory B cells (aMBCs) that have been shown to be dysfunctional in-vitro suggesting that Pf impairs the B cell/antibody response to malaria. It is unclear whether PfCSP-specific memory B cells induced by either vaccination or natural exposure are functional. The aim of this project is to characterize the B cell response to circumsporozoite protein (RTS, S), the antigen in RTS, S/AS01 vaccine, in vaccinated and naturally exposed individuals. We hypothesize that only a subset of antigen-specific B cells are able to produce antibodies that neutralize *Pf*. Specifically, we used PfCSP-tetramer multiparameter flow cytometry and ELISA to determine the proportions of CSP-specific B cells and antibody levels in children with varying amounts of exposure to malaria following vaccination with RTS, S/AS01. PfCSP specific B cells and antibody levels are maintained at higher levels in RTS, S vaccinees compared to controls (p= 0.01 and <0.0001 respectively) at 6.5 months of immunization. The proportions of PfCSP- specific B cells in the vaccinees decrease significantly by month 74, however vaccinees maintain higher anti-CSP serum antibody levels compared to controls (p=<0.0001). To determine whether the function of CSP-specific B cells is compromised, we will compare gene transcription profiles of these cells with those of the more effective tetanus vaccine isolated from the same individual using single cell RNAseq. Additionally, recombinant CSP-specific antibodies will be made and their ability to neutralize sporozoites *in vitro* and *in vivo* tested. The data will help determine whether the deficit in protection following immunization with RTS, S is at least partly the result of the quality of the antibodies produced, a problem of the level of the B-cell response itself, or a result of both.

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NATURALLY ACQUIRED IMMUNITY TO *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-1, DUFFY BINDING PROTEIN AND CIRSCUMSPOROZOITE PROTEIN AND RISK OF RECURRENCE FOLLOWING RADICAL TREATMENT

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Plasmodium vivax (Pv) is the most geographically widespread parasite causing malaria worldwide and represents a challenge to malaria elimination. Currently some vaccine candidates such as Merozoite surface protein-1 (MSP-1), Duffy binding protein (DBP) and Cirscumsporozoite protein (CSP) are in pre-clinical or early phase-I clinical trials. However, little is known about the relationship between naturally acquired immunity response against these vaccine candidates and recurrence events. In order to understand this relationship we measured antibodies against MSP-1, DBP and CSP using plasma samples from a clinical trial aimed at assessing the efficacy of 3 Primaquine regimes to prevent possible Pv relapses in the Peruvian Amazon. Patients were followed for 210 days after treatment and from 485 patients we identified 90 recurrent cases. Six microsatellite markers were used to distinguish homologues (probable relapse) or heterologous recurrence (probable re-infection). Relationship between antibodies titters and the risk of recurrence was analyzed. Our results showed that the median of antibodies titters IgG against MSP-1 in non-recurrence group was 4.1 and 3.6 times higher vs. homologous (n=48) and heterologous (n=42) recurrence, respectively (p<0.001). Furthermore, titters of antibodies IgM against MSP-1 in non-recurrence group was 0.4 and 0.6 times lower vs. homologous and heterologous recurrence, respectively (p<0.001). Antibodies IgG against DBP in non-recurrence group was 1.6 and 1.1 times higher compared with homologous and heterologous recurrence, respectively (p=0.054). We did not observe difference in IgG titters against CSP between groups. Multivariate regression model showed strong protection against recurrence associated with upper quintiles 4-5 (OR=0.08, p<0.001) of antibodies IgG against MSP-1 and higher protection against homologous recurrence. Our results suggest that the intensity of antibodies IgG against MSP-1 during acute infection is associated with protection against recurrence and potentially relapses compared with DBP or CSP and support the potential of MSP-1 as a Pv vaccine candidate.

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MALARIA IN PREGNANCY: IMPACT OF MICROSCOPIC AND SUB-MICROSCOPIC INFECTIONS ON NEWBORN CHILDREN'S CORD BLOOD IMMUNE RESPONSES

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In endemic regions, newborns are often exposed to malaria *in utero*. Such exposure affects susceptibility to malaria through mechanisms that are poorly understood. We examined the relationships between burden and timing of malaria in pregnancy on newborn's cellular immune responses. 363 Kenyan women were followed from early pregnancy until delivery. Malaria infection was detected at prenatal visits and delivery by standard microscopy and PCR. Their newborns' cord blood lymphocyte responses to malarial blood stage-antigens were analyzed for proliferation and production of TNF-α, IL-6, IFN-γ, IL-2, IL-12p70, IL-5, IL-13, IL-10 cytokines. Cytokines were quantified via the Bio-Plex assay. The Prevalence of malaria parasites among mothers was 47% (172/363), more than half (60%) of these were sub-microscopic. Newborns from mothers with submicroscopic infection expressed higher levels of inflammatory ($P < 0.01$), Th1 ($P < 0.001$), Th2 ($P < 0.001$), and regulatory ($P < 0.01$) cytokines as compared to the unexposed. In contrast, there was no significant difference in newborns from mothers having microscopically detected infections to the unexposed. Parasitemia occurring at prenatal visits, but not at delivery, yielded a mixed pro- and anti-inflammatory response pattern at birth, whereas parasitemia occurring only at delivery was associated with elevated IL-13 response. Density and timing of MiP appear to influence fetal effector responses. The observed patterning may be important for subsequent anti-*Plasmodium* response during early life.

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PLASMODIUM FALCIPARUM STRAINS SPONTANEOUSLY SWITCH INVASION PHENOTYPE IN SUSPENSION CULTURE

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The extensive redundancy in the use of invasion ligands by *Plasmodium falciparum*, and its unique ability to switch between invasion pathways have hampered vaccine development. *P. falciparum* strains Dd2 and W2mef have been shown to change from sialic acid (SA)-dependent to SA-independent phenotypes when selected on neuraminidase-treated erythrocytes. Following an observation of increasing ability of Dd2 to invade neuraminidase-treated cells when cultured for several weeks while shaking, we systematically investigated this phenomenon by comparing

invasion phenotypes of Dd2, W2mef and 3D7 strains of *P. falciparum* that were cultured with gentle shaking (*Suspended*) or under static (*Static*) conditions. While *Static* Dd2 and W2mef remained SA-dependent for the entire duration of the investigation, *Suspended* parasites spontaneously and progressively switched to SA-independent phenotype from week 2 onwards. Furthermore, returning *Suspended* cultures to *Static* conditions led to a gradual reversal to SA-dependent phenotype. The switch to SA-independent phenotype was accompanied by upregulation of the key invasion ligand, reticulocyte-binding homologue 4 (RH4), and the increased invasion was inhibited by antibodies to the RH4 receptor, CR1. Our data demonstrate a novel mechanism for inducing the switching of invasion pathways in *P. falciparum* parasites and may provide clues for understanding the mechanisms involved. The data further demonstrate the likely role of non-immune factors to phenotypic variation in *P. falciparum*, and highlights the possible contribution of in-vivo physiological conditions towards parasite diversity.

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EFFECT OF MALARIA PATHOLOGY ON CD4 CELLS

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Understanding immunity in *Plasmodium* infection can help in the treatment and vaccine production. The effect of malaria pathology on CD4 cell count was carried out at a Teaching Hospital in Enugu Nigeria between March–July 2017. Patients on doctor's provisional diagnosis of malaria were examined for *Plasmodium* infections and the degree of parasitaemia (0, +, ++, and +++). Positive samples (+, ++ and +++) and negative ones (0) were thereafter examined for their CD4 cell counts. 30 patients were studied. All the *Plasmodium*-negative specimens showed normal range of CD4 cell count (509 – 1488); with mean value of 1109. The + parasitaemia showed lower ranges of CD4 count (262 – 954); mean = 613; with immune falls in three (262, 425 and 427). The ++ parasitaemia showed crash in the CD4 cells count (35, 152, 22 and 494) except one with 542 count; mean = 291. The +++ parasitaemia showed not much low ranges of CD4 cell count (318 – 994) because most parasitaemia were not due to P. *falciparium*; with a crash in only one (318); mean = 566. The CD4 cells falls and crashes were detected only in *Plasmodium falciparium* parasitaemia infections. In conclusion, *Plasmodium falciparium* infection causes immunosuppression in patients. Corollary, it means is that malaria infection in the immunodeficients and AIDS patients will accelerates the complications as well as death, unlike prevailing reports.

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PRODUCING MALARIA INDICATORS THROUGH DISTRICT HEALTH INFORMATION SOFTWARE: PRACTICES, PROCESSES AND CHALLENGES IN KENYA

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Globally, there is increasing interest in malaria indicators produced through routine information systems due to their potential to provide near real-time data for driving malaria control decisions. Deficiencies in routine health information systems are well recognized and interventions such as the computerization of District Information Systems have been implemented to improve data quality, demand and use. However, little is known about the micro-practices and processes that shape routine malaria data generation at the frontline. This study critically examined how data for constructing routine malaria indicators are constructed and reported through the District Health Information Software (DHIS2) in Kenya. The study was conducted over 18-months in four frontline health facilities and two sub-county health records offices located in two malaria endemic counties. The study employed an ethnographic approach to data collection which involved observations, document review, records review, and interviews (n=27). Data were analysed using a thematic content analysis approach. Routine malaria data generation at the frontline was undermined by a range of factors such as understaffing, human resource management challenges, stock-out of essential commodities, poorly designed tools, and unclear instructions for data collection and collation. In response to these challenges, health workers adopted various coping mechanisms such as informal task shifting and use of improvised tools which sustained the data collection process but undermine data quality. Data quality problems were concealed in aggregated reports entered in the DHIS2. Problems were compounded by inadequate data collection support systems such as supervision. Challenges to routine malaria data generation and reporting are embedded within the broader challenges faced by the health system. The DHIS2 is one component of the health system and interventions to improve reporting practices will shape and be shaped by the functioning of other system components. Any intervention seeking to improve routine malaria data generation must, therefore, look beyond malaria or the health information system-specific initiatives to also include those that address the broader contextual factors that shape malaria data generation.

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IDENTIFICATION OF THE MALARIA OXIDOREDUCTASE ENZYME INVOLVED IN HYDROGEN PEROXIDE PRODUCTION FROM PRIMAQUINE METABOLITES

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Malaria is a major vector-born disease that affects populations in tropical and subtropical areas. The achievement in malaria elimination and control requires effective tools to block transmission and to prevent re-establishment of malaria. Primaquine is the only currently available anti-malarial that has gametocytocidal activity against *Plasmodium falciparum* and ability to kill liver stages, including hypnozoites in relapsing strains (*P. vivax* and *P. ovale*). In terms of the malaria elimination and control agenda, primaquine becomes one of the most important tools we have for deployment alongside other anti-malarial drugs targeting asexual stages. However, primaquine cannot be given safely to all malaria patients because of haemolytic toxicity in patients who suffer from glucose-6-phosphate dehydrogenase deficiency. Despite more than seventy years of investigation, the mechanism of action of primaquine remains poorly understood. Primaquine efficacy requires biotransformation from the unmodified primaquine core to as yet undefined active metabolite/s. Recent literature shows clear evidence for a role of CYP2D6 in the production of active metabolites. It is hypothesised that the anti-malarial activity occurs via hydrogen peroxide, oxidative stress, and oxidative damage produced from the redox cycling of CYP 2D6 mediated primaquine metabolites. However, this still doesn't explain the unique susceptibility of malaria parasites to primaquine or it's metabolites. Here, for the first time we report on a *P. falciparum* enzyme having reductase activity that is able to complete the redox cycling of primaquine metabolites within the parasite. The gene was cloned and expressed in *E. coli*. The purified protein shows an ability to use primaquine metabolites in the generation of hydrogen peroxide. Our observations do support a clear mechanism of action primaquine that will prove invaluable in the design of newer safer analogues.

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MEASURING COVERAGE OF TREATMENT WITH ACT AMONG UNDER-FIVES IN POPULATION SURVEYS: THE IMPORTANCE OF VISUAL AIDS, PACKAGING AND PRESCRIPTION REVIEW FOR SURVEYS IN SETTINGS WITH A DIVERSE DRUG MARKET AND IMPERFECT HEALTH WORKER ADHERENCE TO NATIONAL TREATMENT GUIDELINES

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Nationally representative household surveys are the standard approach to monitor access to and treatment with artemisinin-based combination therapy (ACT) among children under five years (U5). A prospective case-control study was performed in Mali to validate caregivers' recall of treatment received by U5s when seeking care for fever from rural and urban public health facilities, community health workers (CHWs), and urban private facilities. Clinician-recorded consultation details, without independent direct observation, were the gold standard. Consenting caregivers were followed- up for interview at home within two weeks using standard questions from Demographic and Health Surveys and Malaria Indicator Surveys. Among 1,602 caregivers in the study, recall of ACT received by their child had sensitivity of 43% and specificity of 90%. Higher sensitivity of ACT recall was associated with seeking care from a CHW, being literate, and higher socioeconomic status. Use of visual aids showing common drugs, review of prescriptions, and retained packaging improved ACT recall sensitivity to 92%, with specificity of 72%. Participants enrolled during the seasonal malaria chemoprevention (SMC) campaign had higher false-positive reports of ACT received by their child, compared to those enrolled pre-SMC. Caregivers rarely reported receiving the SMC drug combination from consultations at facilities or CHW sites. Health worker adherence to case management guidelines was inconsistent. Only 50% of febrile U5s were tested for malaria. Most (95%) of the 573 children with confirmed uncomplicated malaria received any antimalarial drug, but only 63% received ACT; 25% injectable artesunate; and a few received artemether, amodiaquine, or quinine. Furthermore, 36% of children with a negative test were prescribed ACT. Use of injectable artesunate was highest at public rural facilities. These findings emphasize the need to include visual aids, prescription, or packaging review alongside standard survey questions to assess ACT coverage, particularly in a context where clinicians do not consistently prescribe ACT for uncomplicated confirmed malaria.

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DETECTION OF *PLASMODIUM SP.* IN NON-HUMAN PRIMATES AND MOSQUITOES IN FOREST FRAGMENTS IN COLOMBIA

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Plasmodium parasites infect non-human primates (NHP) in tropical regions around the world. In Latin America, NHP are potential reservoirs of *Plasmodium brasilianum*/*Plasmodium malariae*, and some NHP species have been found infected with *Plasmodium falciparum* and *Plasmodium simium*, as reported previously. Considering the risk of infection to humans associated with the presence of NHP and *Anopheles* infected with *Plasmodium spp.,* this study aimed to determine the circulating species of *Plasmodium* in vectors and NHP species living in fragmented forest areas in Colombia. Sampling was conducted in five different forest fragments. Primates were followed and faecal samples were collected immediately after defecation, and placed in tubes with RNA*later* solution. Samples from 75 *Ateles hybridus,* 25 *Cebus versicolor*, 58 *Alouatta seniculus* and eight *Aotus griseimembra* were collected. Blood samples from four *A.hybridus,* nine *C. versicolor*, five *A. seniculus* and seven *A. griseimembra* were obtained. Adult mosquitoes were sorted after capture and most female *Anopheles* were preserved in RNA*later* buffer, the remaining were kept dry for taxonomic identification. *Plasmodium sp*. detection was performed through PCR, and second reactions were conducted for *Plasmodium falciparum, Plasmodium malariae* and *Plasmodium vivax* detection, using ribosomal and mitocondrial primers. PCR products were visualized on agarose gel and positive samples were sequenced. Mosquito identification was confirmed through DNA barcoding. From faecal samples it was found *P.falciparum* infecting *A.seniculus*, while *P. malariae* infected *A. seniculus, A. hybridus* and *A. griseimembra*. *P. vivax* infected *A. hybridus, A. seniculus* and *C. versicolor*. From blood samples it was found *P. malariae* infecting all the four NHP species tested and *P.vivax* infected *A. hybridus*. Infection with *P.vivax* was confirmed in three *Anopheles* species and one was positive for *P. malariae.* This scenario suggests important epidemiological implications in the human - NHP interface and the associated risk of Malaria transmission.

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QUALITATIVE INSIGHTS INTO HUMAN BEHAVIOR AND RESIDUAL MALARIA TRANSMISSION IN UNGUJA ISLAND, ZANZIBAR: FINDINGS FROM IN-DEPTH INTERVIEWS AND DIRECT OBSERVATION OF COMMUNITY EVENTS

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Zanzibar has experienced dramatic declines in malaria transmission, owing to its comprehensive malaria control program. This includes wide-scale deployment of insecticide treated nets (ITNs) and targeted indoor residual spraying (IRS). Despite high coverage of these indoor interventions, low-level residual transmission persists. This study used complementary qualitative approaches to better understand human behaviors that can drive residual transmission. A total of 62 in-depth interviews were conducted with community members and local leaders across six sites in Unguja, Zanzibar. 19 semi-structured community observations were conducted to capture nighttime activities and special events. Data was coded and analyzed using a thematic approach. Nighttime activities, sleeping patterns, prevention measures, and migration patterns emerged as important themes. Nighttime activities were largely categorized into routine social activities, household chores, livelihood activities and special events. Many activities took place outdoors, in the peri-domestic setting, or away from home, with some lasting throughout the night. Gender variations were observed, with men routinely spending more time outdoors and away from home than women and children. Outdoor sleeping was reported and observed, especially during 'special' socio-cultural events, including weddings, funerals, and religious ceremonies. Participants reported having few methods of prevention while outdoors, when traveling, or away from home, and generally perceived higher risk of infection while outdoors. Travel and migration, particularly seasonal workers coming from mainland Tanzania, was also perceived as a key

factor related to malaria transmission. Study findings highlight gaps in prevention when people are outdoors, away from home, traveling, or at large social gatherings. Supplemental prevention measures are needed where ITNs and other indoor-based interventions are insufficient. Programs aimed at providing education and prevention tools at the community level to visitors should also be explored.

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SIMULTANEOUS ZOONOTIC MALARIA AND SYLVATIC YELLOW FEVER HUMAN INFECTION IN RIO DE JANEIRO ATLANTIC FOREST, BRAZIL

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The number of autochthonous malaria cases has been increasing in the Atlantic Forest (AF) of Brazil (Rio de Janeiro state) since 2015-16. The cases have been diagnosed as *Plasmodium vivax*, based on the morphology of the parasites. As the *P. vivax-like* non-human primate malaria parasite species *Plasmodium simium* is locally enzootic, we performed a molecular epidemiological investigation to determine whether zoonotic malaria transmission was occurring. Blood samples of humans from 2015-16 presenting malaria as well as from local howler monkeys were examined. Additionally, sequencing of the parasite mitochondrial genome was applied. This study shows that these parasites are *P. simium*, a closely related parasite species whose natural hosts are non-human primates (NHP) native to the AF. Some, if not most, of the autochthonous cases previously diagnosed as *P. vivax* in the region are likely to have been *P. simium* acquired via mosquitoes infected from monkeys, thereby making this part of Brazil the site of a second global focus of zoonotic malaria. Since late 2016, Brazil has been suffering a rapid expansion of a severe sylvatic yellow fever virus (YFV) outbreak, which has reached one of the most populated zones of the country, heretofore a yellow fever-free zone for more than 70 years. In 2017-18, an outbreak of YFV started in the same area of the AF were *P. simium* has been reported. The natural host of the virus and *Plasmodium* are the same. Interestingly, from 24 YF confirmed human cases (by qRT-PCR), we also identified co infection with *Plasmodium* in two individuals by retrospective PCR. The mechanism by which a patient may become infected by both *Plasmodium* and YFV is consecutive bites from two different infected mosquitoes or species (e.g., anopheline vectors for malaria and *Haemagogus* or *Sabethes* spp. for YFV). Thorough screening of the local NHP is required to evaluate the extent of this newly recognized zoonotic threat to public health in Brazilian Atlantic Forest. This situation has immediate implications for public health in this region, and further and more profound consequences for the control and eventual elimination of malaria and YF in Brazil.

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THE CHALLENGE OF SCALING UP AND SUSTAINING DIGITAL SOLUTIONS: "SMS FOR LIFE" - A CASE STUDY FROM A HEALTH SYSTEMS INTEGRATION PERSPECTIVE

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Malaria remains a major public health concern in Tanzania with 5.2 million reported cases in 2016. The program "SMS for Life" used Short Messaging Service (SMS) to monitor antimalarial stock levels at public health facilities and address the stock-out problem in Tanzania. After a successful pilot in 2010, the proportion of health facilities that had no stock of one or more antimalarials declined from 78% during the first week of the program to 26% at week 21. The nation-wide scale up was implemented across all 5600 public health facilities. However, a study in 2013 showed that Tanzania continued to experience antimalarial stock-outs. The program was discontinued without replacement in 2016. This case study documents the evolution of the program from a health systems integration perspective to draw lessons for mHealth approaches to supply chain management at scale. We reviewed internal and external documentation on "SMS for Life". Using purposive and snowball sampling methods, semi-structured interviews were conducted among identified relevant stakeholders at international, national, district and health facility levels. Data from semi-structured interviews and document review were triangulated and analyzed. Additionally, a social network analysis was done to describe the relationship among the actors involved in the program. We analyzed the implementation of the program in terms of system thinking against health system building blocks framework and the WHO MAP framework. The results provide details on the roles of stakeholders, as well as, the variables and evidence considered in the decision-making processes. We also identified factors that led to the termination of the program in Tanzania. "SMS for Life" was viewed to have performed well on a technical level, but insufficient ownership and system integration were identified as major barriers to sustainability at scale. A new version of "SMS for Life" is currently being planned and the identification of the bottlenecks in Tanzania will inform planning to ensure integration and long-term results. Findings of this study will also help overcome similar barriers in other mHealth programs.

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MASSIVE OVER-TREATMENT OF UNCOMPLICATED MALARIA CASES WAS ASSOCIATED WITH IN-SERVICE TRAINING AND SMS INTERVENTION IN A QUASI-EXPERIMENTAL STUDY IN GHANA

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Failure to test for malaria before treatment and failure to follow negative test results are still prevalent despite the current in-service training strategy deployed by the National Malaria Control Program (NMCP) of Ghana to improve prescriber adherence to malaria case management guidelines. Addition of a one-way SMS intervention post training may improve adherence to guidelines through knowledge retention. The aim herein was to assess the effectiveness of this addition in the Greater Accra Region (GAR). A quasi-experimental trial with a non-equivalent control group was conducted in La-Nkwantanang Madina (Arm 1), Ga South (Arm 2) and La Dade-Kotopon (Arm 3). Prescribers in Arms 1 and 2 received a three-day standard training on malaria case management. Within 8 weeks, prescribers in Arm 1 were repeatedly sent 10 discrete SMS messages every other week. Prescribers in Arm 3 received no intervention. Data were managed and analysed using Stata version 13.0. The primary outcome was the proportion of febrile patient records with negative malaria tests results prescribed ACTs. Analysis was by intention to treat. The effects of the interventions were estimated using differences in difference analysis after adjusting for confounding. As much as 2519 and 2356 patient records at baseline and end line respectively were compared. At baseline, 40.0% of the febrile patients were tested whilst 42.7% of the remaining febrile patients were treated presumptively. 93.0% of 158 febrile patients

with confirmed malaria were treated with ACTs and 21.4% of 679 febrile patients with negative malaria test results were prescribed ACTs at baseline. The training alone intervention resulted in only a 21.2% percentage point increase in the proportion of febrile patients tested for malaria. The training plus SMS intervention was associated with a 34.9 percentage point increase in overtreatment. Increasing test rates can potentially lead to overtreatment if the diagnostic capacities of facilities for other febrile illnesses and alternative treatment options for prescribers are not enhanced.

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INVESTIGATING THE FUNCTION OF PUTATIVE MITOCHONDRIAL PROTEINS OF *PLASMODIUM FALCIPARUM* IN *SACCHROMYCES CEREVISIAE*

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Plasmodium falciparum contains a minimalistic, but essential mitochondrion in all stages of its life cycle. Several mitochondrial processes such as division, genome replication, membrane biogenesis that are very well understood in higher eukaryotes are very unclear in *Plasmodium*. Furthermore, very little is known about the regulation of mitochondrial structure and function. Understanding the molecular mechanisms of some of these essential processes would require functional studies of the key proteins. Here, we use the yeast heterologous system, *Sacchromyces cerevisiae* to understand the function of three putative mitochondrial proteins that may be involved in key processes. We show that PfSURF1, the putative orthologue of human and yeast SURF1/SHY1, involved in cytochrome c oxidase assembly, partially complements a haploid *S. cerevisiae* strain harboring the null allele *shy1*. Prohibitins (PHB1 and PHB2) are mitochondrial proteins, and have been shown to regulate the structure and function of mitochondria in other eurkaryotes. Using the yeast two-hybrid system, we show that putative *Plasmodium* prohibitins (PfPHB1 and PfPHB2) interact with each other, which suggests that they could form supercomplexes of heterodimers in *Plasmodium*, the functional form required for optimum mitochondrial function.

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COMPENSATORY HEME SCAVENGING MECHANISMS IN MALARIA-INFECTED PREGNANT WOMEN CORRELATE WITH BIRTH OUTCOMES

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Malaria caused 445,000 deaths globally in 2016, with pregnant women and children at the highest risk. Malaria in pregnancy involve severe symptoms and outcomes including anemia, higher rates of miscarriage, premature delivery, low birth weight neonates, intrauterine, neonatal and maternal death. Current effective treatment for malaria targets parasite burden. Therefore, there is an urgent need to understand the causes of poor birth outcomes and to identify novel interventions as well as predictive biomarkers to determine at-risk individuals. However recent studies have shown that malaria pathogenesis is mediated by parasite derived factors as well as host factors such as heme, a by-product of parasite infected erythrocyte destruction. Our lab has previously determined that free serum heme levels and cytotoxicity in pregnant women were dependent on the robustness of their heme scavenging systems. We hypothesize that individuals with effective heme scavenging mediators will result in improved birth outcomes and that alternatively, those individuals with poor heme regulation will have poor birth outcomes. We assessed archived serum samples obtained from pregnant women in Ghana for heme- oxygenase 1 (HO-1), haptoglobin, and hemopexin levels to correlate results with birth outcomes. The results indicate that high serum levels of HO-1, haptoglobin and hemopexin in pregnant women asymptomatic for malaria had better birth outcomes than malaria-infected pregnant women that did not have high serum levels of these mediators. We conclude that the heme scavenging mechanisms heme-oxygenase 1, haptoglobin and hemopexin have the potential to provide novel prognostic biomarkers for pregnant women at risk for poor birth outcomes.

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SETTING PRIORITIES IN MALARIA RESEARCH FOR MALAWI: THE PROCESS

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Research plays a pivotal role in understanding social and economic trends and disease epidemiology. In addition, it is vital in informing development of health interventions and health systems innovations and taking them to scale. In resource-limited settings like Malawi, it is vital that public funds are used wisely to maximal effect. It is of great importance therefore to ensure that priorities for research are within context, evidence informed and set apriori for health research investments. We describe the process undertaken to develop priorities in malaria research for Malawi. The National Malaria Control Programme engaged stakeholders comprising of malaria researchers, experts, academicians, program implementers and development partners to define malaria themes, identify and analyse gaps in research. At the start, the stakeholders received an orientation to evidence based guideline development as well as evidence synthesis. This was followed by a series of stakeholder meetings to analyze current malaria control interventions; review current and previous research conducted in Malawi; develop broad priority research areas; map research evidence on the identified priorities to determine the research landscape and gaps and finally rank the identified priorities using the Essential National Health Research (ENHR) strategy. A comprehensive evidence informed five year national malaria research agenda for Malawi was formulated. The agenda highlights broad research priorities under the four main malaria themes which include case management, vector control, malaria in pregnancy and cross cutting (monitoring and evaluation and behavior change and communication). These research priorities were appropriately ranked using the ENHR strategy to guide appropriate allocation of the limited resources. In conclusion, in settings with limited resources, the process of formulating a national research agenda should involve thorough consultations and review of the appropriate evidence to support the need for further research.

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CLINICAL FEATURES AND OUTCOME OF MALARIA IN CHILDREN SUFFERING FROM SICKLE CELL DISEASE: A RETROSPECTIVE DESCRIPTION OF 35 CASES

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The Democratic Republic of Congo (DRC) has two remarkable features, the extremely high prevalence of malaria and sickle cell disease (SCD). Both conditions bring together a high rate of morbidity and mortality in children mostly. Therefore, there has been only one report on the clinical features in the sickle cell pediatric population suffering from malaria in our country. The aim of this study was to contribute to the improvement of the management of malaria in children with sickle cell disease. We carried out a retrospective study in Evangelical Hospital of Vanga in rural zone

of Kwilu Province, Democratic Republic of Congo, for the period from January 2015 to December 2016, to children aged from 0 to 15 years. We analyzed the data of 35 children with SCD who developed malaria and which files were available. Of the 35 homozygous sickle cell children with malaria, the most represented were between 5 to 10 years (60%) with a F: M ratio of 1.19. The pain crisis (100%), fever (60%), severe anemia (51.42%) and jaundice (40%) were the most frequent clinical manifestations The hemoglobin level of less than 6 g / dL was found in 51.43% of children in the series. Malaria affected 45.72% of the studied population and caused 8.57% of deaths; 50% of hospitalized children were treated as severe malaria.

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THE ETHIOPIAN EXPERIENCE IN ESTABLISHING MALARIA SENTINEL SITE SURVEILLANCE

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The Ethiopian Ministry of Health embarked on eliminating malaria from low transmission settings in 2020 and throughout endemic areas in 2030. However, the country experiences complex malaria epidemiology mainly occurrences of both *P.falciparum* and *Plasmodium vivax* and associated challenges in implementation. This calls for strong surveillance system that track epidemiological and entomological as well as monitoring seasonal climate forecast at local level for targeted action. Accordingly, in 2016, the National Malaria Control Program identified and approved 25 health centers to enhance health-facility-based malaria surveillance. Next, a characterization survey was conducted using interview and observation of potential entomological investigation posts from April to May 2017. GPS reading of the 25 sentinel sites and adjacent entomological posts was recorded for mapping. Above two-thirds of the sites were recruited from lowland (below 1,500m) and the rest from highlands (above 1,500-2000m). There was high variability among the sites in terms of institutional capacity. Annual malaria cases treated *per* annum vary. Findings were summarized for each site for further recommendation in implementing phased-approach of implementation.

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EVALUATING THE CONTRIBUTION OF THE PRESIDENT'S MALARIA INITIATIVE TO REDUCTION OF MALARIA BURDEN IN PRIORITY UNTRIES IN SUB-SAHARAN AFRICA USING GENERALIZED STIMATING EQUATIONS AND MATCHING PROCEDURES

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The President's Malaria Initiative (PMI) launched in 2005 as a key player in malaria prevention and treatment in sub-Saharan Africa (SSA). Several country-specific evaluations have demonstrated great progress in reducing under-five mortality associated with scaling up malaria interventions in PMI priority countries. However, documentation of PMI's specific contributions was limited, until the publication of Jakubowski. et al. 2017. It used difference-in-difference analysis to show higher reduction of under-five mortality in PMI-supported countries than in others. To generate more evidence, this study used rigorous statistical analyses to assess reduction in mortality attributable to PMI support. The study employed Generalized Estimating Equations (GEEs) and a series of matching procedures to evaluate the impact of PMI on under-five mortality and on population coverage of insecticide-treated nets (ITNs), indoor residual spraying (IRS), and artemisinin combination therapy (ACT) in SSA. The analyses used country-level secondary data and controlled for several country-level characteristics assumed to influence outcome measures of interest, PMI program participation, or both. The Mahalanobis distance metric, with 1:1 nearest neighbor matching adjusting for bias in population size and the particular country, showed a reduction in under-five mortality by approximately 12 per 1000 live births (95% CI: 20.6-3.1; p=0.012). There were statistically significant increases in the population coverage of ITN, IRS, and ACTs in PMI countries over the implementation period. ITN use in the population was 0.23% higher (95% CI average treatment effect on the treated: 0.17-0.30; p<0.001) in PMI- recipient countries than non-PMI countries. PMI contributed significantly to increasing coverage of malaria control interventions and reducing under-five mortality in SSA.

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CONTRIBUTION OF QUARTERLY MALARIA DATA REVIEW AND VALIDATION TO DATA QUALITY AND MALARIA SERVICES IMPROVEMENT

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The PMI-funded Improving Malaria Care Project (IMC), aims to improve the quality of prevention, diagnosis and treatment of malaria in Burkina Faso. IMC also supports the NMCP to improve the quality of malaria data collected and used for decision-making through quarterly review of provider-collected malaria data. In 2016, IMC supported the NMCP to develop the malaria data review and validation manual, including a data review sheet. IMC supports 25 Health Districts to organize a quarterly malaria data review workshop with all health facility managers (HFM) who collect data. Before the workshop, the district data manager (DDM) captures data from monthly reports sent by health facilities (HF) in the online national DHIS2. The regional data manager (RDM) extracts malaria data from DHIS2, pastes in the data review file and creates a data sheet. Automatically, the data review sheet highlights in red the cells of each HF where the system detected an error. During the workshop, the RDM shows the errors for each indicator and asks the HFM to verify their reports. Generally 3 kinds of errors are noted; errors in understanding (e.g. confusing number of confirmed malaria cases treated with number of suspected malaria cases treated), typing errors, and miscalculations. By correcting these errors, the RDM and the DDM explain indicator definitions and prevention and treatment guidelines to providers. After 2 rounds of malaria data review in Boromo, Dano and Koupela districts, we noted that providers improved their understanding of indicator definitions and knowledge of prevention and treatment guidelines. The on-site data review in 41 HF showed that 88% (192/218) of providers comply with data reporting and case management guidelines. Furthermore, the percentage of HF with mistakes in their data dropped from 87% to 41% in Boromo, from 94% to 38% in Dano, and from 84% to 32% in Koupela. The indicators with errors in understanding decreased from 15 to 3 in Boromo, from 16 to 3 in Dano, and from 13 to 4 in Koupela. Thus, by improving data quality through review at the district level with providers, we are building provider's capacity and improving the quality of malaria services.

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IMPACT OF ANTIMALARIAL INTERVENTIONS ON MALARIA MORBIDLY AND MORTALITY IN HOSPITALS FROM 2001 TO 2017, SENEGAL

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The Senegal National Malaria Control Program and partners accelerated malaria control in the last decade, with nationwide roll-out of artemisinin-

based combination therapy (ACT) starting in 2006, and rapid diagnostic tests (RDTs) starting in October 2007. Since 2003, insecticide-treated nets (ITNs) have been distributed through health facilities to pregnant women and children under five years, and since 2008, mass campaigns of ITN distribution have targeted children under five years, with universal coverage in 2010, 2014, and 2016. Since 2007, indoor residual spraying has been implemented in seven districts. We assessed the trends of malaria cases, hospitalizations and deaths at 37 hospitals and in all 76 districts during the period of the scale-up of malaria control interventions. Data collected from all hospitals and districts from 2001-2017 were used to assess the impact of accelerated malaria control. Numbers of outpatient and inpatient cases and deaths were compared between the 2001-2007 period and the accelerated - intervention period of 2008-2017. From 2001 to 2007, the proportion of suspect cases confirmed increased slightly from 2.2% to 4.0%. With the introduction of RDTs, this increased to 99.55% in 2017, with a mean test positivity rate of 19.5%. The proportion of all consultations due to malaria decreased from 39.7% in 2001 to 26.9% in 2007 (pre-RDT). After the introduction of RDTs and change in definition from clinical to biological onfirmed, the proportion of all consultations due to malaria decreased from 9.1% in 2008 to 3.26% in 2017. The proportion of patients hospitalized for malaria accounted for 11.5% of all hospitalizations in 2001 and decreased to 4% in 2017. From 2001 to 2007, the proportion of hospitalized deaths attributable to malaria decreased from 24.5% in 2001 to 8.1% in 2007. From 2008 to 2017, the proportion of hospitalized deaths attributable to malaria decreased from 7.1% to 1.73%. We conclude that malaria morbidity and mortality has decreased substantially during the scale up of malaria control interventions

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FROM PRESUMED TO CONFIRMATION DIAGNOSIS: IMPROVING TESTING AND TREATMENT OF MALARIA IN CHILDREN UNDER FIVE IN RURAL COMMUNITIES IN MADAGASCAR

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Since 2008, the National Malaria Control Program (NMCP) of Madagascar has scaled-up the implementation of Community Integrated Management of Childhood Illnesses with the introduction of malaria rapid diagnostic tests (RDT) at the community level. With support from the President's Malaria Initiative, the USAID Mikolo Project supports community health volunteers (CHV) to manage uncomplicated malaria cases. We assess the effectiveness of applied fever cascade management at the community level in improving malaria treatment among children under five (CU5). The USAID Mikolo Project has trained and equipped 5,364 CHVs throughout eight regions of Madagascar to correctly manage fever cases based on the 2013-2017 NMCP objectives: test over 80% of fever cases for malaria using RDTs, and treat over 80% of confirmed (RDT-positive) cases with artemisinin-based combination therapy (ACT). In order to ensure quality case management, the project introduced a quality improvement approach consisting of continuous supervision, training, and performance evaluations. Additionally, the project established a community logistics system that tracks and forecasts stock needs to ensure continuous supplies of RDTs and ACTs. Between 2013 and 2017, around 440,000 fever cases in CU5 were correctly managed by CHVs. With the implementation of the quality improvement approach for better case management, and with the establishment of the community supply chain system ensuring availability of stocks, the percent of fevers tested with RDTs increased from 70% in 2013 to 89% in 2017, and the rate of confirmed malaria cases treated with ACT increased from 35% in 2013 to 86% during the same period. Finally, presumptive treatment of fevers (treating with ACT without a confirmed RDT) has decreased considerably—from 39% in 2013 to almost 0% in 2017 (the 14% not treated by ACT by CHVs were directly referred to a health center instead of presumptively treated). Overall, the increased quality of fever case management services provided by CHVs has improved the cascade of malaria treatment in CU5 at the community level in Madagascar.

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USE OF INSECTICIDES-TREATED NETS (ITNS) AND ASYMPTOMATIC MALARIA CARRIAGE IN AREA WITH HIGH TRANSMISSION, KALIFABOUGOU, MALI

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Much progress has been made in the fight against malaria. However, it remains the major public health problem in worldwide (216 million cases) and particularly in sub-Saharan Africa (194 million cases) according to WHO malaria report 2017. Malaria is still one of the top five causes of under-five mortality with 80% of morbidity in Sub-Saharan Africa (WHO 2018). In Mali, malaria morbidity and mortality are still high, 1.700.000 and 2.309 respectively (PNLP 2015). The incidence of the disease is still high although the coverage of ITN use is increasing (54%) in Sub-Saharan Africa (WHO 2017) and 93% in Mali (PNLP 2015). In the context of malaria eradication and elimination, we would like to assess the role of ITNs on asymptomatic malaria. This study was undertaken to 1) evaluate the association between the use of ITNs and malaria incidence in a cohort from May to November 2013; 2) to determine the incidence of malaria in the study population; 3) determine the frequency of the use of ITNs in the study population and 4) to determine the association between the use of ITNs and the incidence of malaria, asymptomatic malaria carriers and the episodes number. This study is part of a cohort study of 582 volunteers initiated in May 2011 in Kalifabougou, Mali. However this assessment was conducted on data collected from May 2013 to December 2013. Data were collected on asymptomatic malaria measured during monthly visits using blood smear. ITNs used was also assessed in May and December 2013. ITNs was used by 98% of the participants of which 81% were regular users. Permanent users of ITNs represented 75% while non-permanent users represented 74% (p>0.05). There was an association between the use of ITN and the occurrence of asymptomatic malaria (p=0,032). 14% were carriers of malaria parasites, among ITNs regular users, versus 22.2% among the non-regular users. In conclusion, our results showed that the permanent use of ITNs are associated with the reduction of asymptomatic malaria carriage.

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CHANGES IN CARDIAC REPOLARIZATION FOLLOWING REPEATED DOSING OF DIHYDROARTEMISININ-PIPERAQUINE IN PREGNANCY

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Malaria in pregnancy (MiP) remains a key cause of adverse maternal and infant outcomes. Alternatives to sulphadoxine-pyrimethamine (SP) are needed for intermittent preventive treatment (IPTp). Dihydroartemisinin-

piperaquine (DP) is a long acting antimalarial with the best potential to replace SP for IPTp, but it prolongs the cardiac QT interval. The effect with repeated dosing is not known. As part of a larger trial comparing the efficacy of IPTp-DP to IPTp-SP for prevention of MiP, we assessed QT prolongation with repeated dosing of DP. A subset of 34 pregnant women randomized to receive IPTp-DP monthly from enrolment (16-28 weeks gestational age) to delivery were included. Pre- and post-dose (obtained 4-6 hours after the last daily dose) 12-lead electrocardiograms (EKG) were obtained with each course. Women were excluded from the study for a machine reported QT corrected by Fridericia's formula (QTcF) ≥450ms at baseline or ≥480ms at any subsequent visit. A cardiologist reviewed all EKGs and calculated the QTcF and QT corrected with Bazett's formula (QTcB). Mean difference in the QTcF (ΔQTcF) and QTcB (ΔQTcB), between the post and the baseline measurement, as well as the course specific pre-dose measurement, was calculated for each dose. Post-dose EKG measurements were obtained from 30 women who received between 1-6 courses of IPTp-DP. At enrolment, the average QTcB was 427msec (range 384-465) and QTcF was 403msec (range 372-436). The pre-dose measurement was shorter at subsequent time points when compared to enrolment: ΔQTcB range -6.9 to -0.4 msec and ΔQTcF -11.8 to -1.7 msec. The mean difference from baseline to successive post-dose measurements was 16.1 and 15.6 msec overall for ΔQTcB and ΔQTcF, respectively, and 21.1, 14.9, 12.1, 12.4, 19.2, and 16.0msec for ΔQTcB and 24.4, 15.4, 11.3, 9.0, 12.4, 9.0 for ΔQTcF, for the 1st, 2nd, 3rd, 4th, 5th, and 6th doses, respectively. The mean differences comparing the post-dose to the pre-dose measurement for each specific course were similar. While IPTp-DP was associated with an increase in the QT interval, this was on average small, and did not increase in magnitude with successive doses.

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FACTORS ASSOCIATED WITH INSECTICIDE-TREATED NET USAGE AMONG WOMEN OF CHILDBEARING AGE IN MALAWI: A MULTILEVEL ANALYSIS

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Use of insecticide treated net (ITN) has been the cornerstone for malaria prevention in most malaria-endemic countries. Despite reportedly high ITN coverage in Malawi, discrepancies in ITN use have been observed. Prior studies have identified individual factors that affect the use of ITNs among pregnant women. However, studies have generally not assessed how community factors, and women empowerment status influence ITN usage among women of childbearing age (WOCBA). This study aimed to identify individual- and community-level factors influencing ITN usage among groups of WOCBA in Malawi. Individual- and community-level factors influencing ITN usage in Malawi were assessed through interviews with 16,130 WOCBA (15-49 years) across 850 communities who participated in the 2015-16 Malawi Demographic Health Survey. Multilevel logistic regression analysis was used. ITN use was similar between pregnant women and nonpregnant women with children under 5 years (45.9% and 46.9%, respectively) but slightly lower among nonpregnant women without children under 5 years (39.1%). Both individual and community characteristics were associated with ITN use among WOCBA and varied significantly across subgroups. Specifically, nonpregnant women with children under 5 years living in communities where women had high autonomy in health care decisions had an 18% greater odds of using an ITN compared with those from communities where women had low health care autonomy (adjusted odds ratio [aOR] = 1.18; 95% confidence interval [CI]: 1.00-1.38). Distance to health care facility influenced ITN usage among pregnant women; those who did not regard distance as a problem had a 44% greater odds of using an ITN than those for whom distance was seen as a problem (aOR = 1.44; 95% CI: 1.09-1.89). Number of household members, region, urbanization, and community ITN coverage influenced ITN usage across all WOCBA groups. The findings confirmed the importance of assessing various factors affecting ITN usage among groups of WOCBA. Both individual- and community-level factors should be considered when designing and implementing ITN programs in Malawi.

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THE EFFECTIVENESS OF INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY WITH ALTERNATIVE ANTIMALARIALS COMPARED TO SULFADOXINE-PYRIMETHAMINE FOR THE PREVENTION OF LOW BIRTH WEIGHT: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Despite widespread drug resistance rendering malaria treatment with sulfadoxine-pyrimethamine (SP) ineffective, intermittent preventive treatment during pregnancy with SP (IPTp-SP) remains a key strategy to prevent low birth weight (LBW). Although alternatives to SP for IPTp have demonstrated increased effectiveness for malaria outcomes, results for the prevention of LBW have been mixed. We conducted a meta-analysis comparing the effect of IPTp with alternative antimalarials to IPTp-SP for the prevention of LBW and malaria parasitemia. We searched multiple databases in accordance with PRISMA guidelines for systematic reviews. Studies comparing IPTp-SP to IPTp with non-SP or SP combination antimalarials (alternative antimalarials) and reporting LBW prevalence (primary outcome) were included. Meta-analyses with study-specific random effects were used to estimate pooled risk ratios (RR) and 95% confidence intervals (CIs) for the prevention of LBW and peripheral and placental malaria infection at delivery by microscopy (secondary outcomes). Our search identified 1,179 records; 6 full-text articles were included in meta-analyses following screening of titles, abstracts, and full-text articles for eligibility. IPTp with alternative antimalarials provided no additional protection against LBW (RR: 0.94, 95% CI: 0.84, 1.05) compared to IPTp-SP, but significantly reduced the prevalences of both peripheral (RR: 0.62, 95% CI: 0.42-0.91, p=0.015) and placental (RR: 0.65, 95% CI: 0.44-0.97, p=0.035) malaria infection at delivery. The lack of pooled effect of IPTp with alternative antimalarials on LBW but significant reductions on malaria outcomes compared to IPTp-SP suggests that either IPTp-SP may be preventing LBW through a non-antimalarial mechanism (i.e. as an antibiotic) or that the maximum protection against LBW has been achieved by IPTp-SP. Future studies should examine the effect of IPTp with alternative antimalarials in combination with an antibiotic. Malaria control programs should continue to promote IPTp-SP among pregnant women living in areas of high malaria transmission in accordance with current guidelines.

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ACCELERATING IPTP3 UPTAKE IN MALAWI

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In 2013, Malawi adopted the World Health Organization's updated policy recommendation on use of Intermittent Preventive Treatment in pregnancy (IPTp). Support from the U.S. President's Malaria Initiative, working through USAID funded health projects, enabled collaboration between the National Malaria Control Program (NMCP) and the Reproductive Health Directorate (RHD) of the Ministry of Health to build capacity to implement the updated IPTp policy. These partners updated the National Malaria Treatment Guidelines to be in line with the new policy and developed appropriate training manuals. Over 90% of health workers from 603 facilities in Malawi were trained on the new IPTp policy and guidelines.

Post-training assessment scores of health staff increased by an average of 40 percentage points. Frequent supportive supervisions, coaching, and mentoring followed trainings to ensure compliance to new IPTp guidelines. Community volunteers and local community based organizations, such as community health action groups (CHAGs), were also engaged in order to identify and solve local problems, as well as encourage pregnant women to attend antenatal care (ANC) and receive malaria prevention services. Significant gains in IPTp3 coverage have been observed in Malawi since 2014 following policy change. In 2014, IPTp3 coverage in the Malaria Indicator Survey (MIS) was 13%, it increased to 30% in 2015 (Malawi Demographic Health Survey), and 43% in 2017 (MIS). Malawi's experience shows that collaboration between NMCP and RHD, as well as between clinics and communities has not only improved knowledge of the new policy, but resulted in increased uptake of services and protection of pregnant women from malaria.

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DECREASE OF MALARIA BURDEN AMONG CHILDREN UNDER FIVE YEARS AND OTHER AGE GROUPS IN SMC REGIONS IN SENEGAL

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Senegal, a West African country, has made great strides in malaria control during the last decade, but its four southeastern regions continue to suffer a high malaria burden. In 2014, Senegal introduced seasonal malaria chemoprevention (SMC), a treatment of sulfadoxine-pyrimethamine and amodiaquine monthly during the rainy season, for children aged 3 -120 months in the four high transmission regions. The Ministry of Health also increased access to care, particularly for children under five years, through a variety of initiatives, and increased access to malaria diagnostic testing. We analyzed routine malaria information system data, provided by all publicly supported health facilities, for the four regions that received SMC and for which completeness of reporting was more than 99%, for the period from 2013 (the year before introduction) to 2017. Data were analyzed for children under 5 years and for non-pregnant patients 5 years and older. From 2013 to 2017, the number of severe malaria cases among children under five decreased from 1384 to 819 corresponding to 40,8% ; in the same period and for the same age group , the proportional mortality (proportion of deaths due to malaria out of all malaria deaths) also moved from 36,6% to 9,4%. We also observed the same results in all population from 2013 to 207 with a decreasing of severe malaria cases from to 6092 to 3592 corresponding to 40,4% and a reduction of proportional mortality from 20,4% to 7,6%. In the other age groups, we noticed in the same period a decrease of 36,6% in severe malaria cases among non-pregnant patients 5 years and older 54,5% among pregnant women. The proportional mortality was also reduced from 17% to 7% and 3,8% to 2,4% respectively among non-pregnant patients 5 years and older and pregnant women. This results in each age group show the contribution of Seasonal Malaria Chemoprevention in the reduction of malaria burden in the Southeastern part of Senegal.

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PERCEPTION OF G6PD DEFICIENCY AND PRIMAQUINE RISK AND BENEFIT IN A MALARIA RISK POPULATION IN NORTHWEST CAMBODIA

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The treatment of Plasmodium vivax (Pv) malaria with primaquine (PQ) can result in life-threatening hemolysis if given to a glucose-6-phosphate dehydrogenase deficient (G6PDd) patient. Therefore, patients should be educated on the limitations of testing and the risks and benefits of taking PQ. We conducted operational research to gain a better understanding of the knowledge of G6PDd screening and PQ treatment among populations from a malaria endemic area in northern Cambodia. Educational materials were developed on G6PD testing, PQ benefits and the risks associated with both single low dose (SLD) and two-week radical cure treatment. Health care workers and village malaria workers used these materials to counsel healthy male test volunteers. Between December 2017 and March 2018, 1,543 G6PD test volunteers were recruited of which 251 (16%) were classified as being G6PDd. Eight hundred fifty-five volunteers (55%) completed pre- and post-counseling questionnaires. Pre-counseling, 653 (79.73%) participants said they would take SLD PQ if recommended, increasing to 816 (99.63%) post-counseling. Similarly, while 656 (79.32%) indicated a willingness to take radical cure PQ treatment before counseling, this increased to 819 (99.03%) after counseling. One hundred thirty six (15.98%) volunteers perceived SLD PQ as low risk for G6PDd patients, before counseling, this increased to 730 (85.78%) post counseling. 164 (19.34%) considered SLD PQ low risk for G6PD normal patients pre counseling vs. 814 (95.99%) post-counseling. Before counseling, 128 (15.13%) volunteers perceived radical cure PQ treatment a high risk for G6PDd patients, this increased to 804 (95.04%) after the counseling. 157 (18.62%) volunteers thought radical cure PQ treatment was a low risk for G6PD normal patients, compared to 808 (95.85%) post counseling. Although malaria elimination efforts have highlighted the need to deploy radically curative doses of PQ, this is the first study to our knowledge seeking to characterize community level understanding of G6PD and the risk and benefits of PQ for vivax radical cure. Additional results will be presented.

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STRATEGY FOR IMPROVING THE TREATMENT OF SEASONAL MALARIA CHEMOPREVENTION AMONG CHILDREN FROM 3 TO 120 MONTHS OF AGE IN GOUDOMP HEALTH DISTRICT, SENEGAL: A 3-DAY DIRECTLY OBSERVED TREATMENT INITIATIVE

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Despite significant progress, malaria remains a public health problem in sub-Saharan African countries. In high-transmission areas, more than two-thirds of deaths occur in children under five. WHO recommended in 2012, Seasonal Malaria Chemoprevention (SMC) whose goal is to maintain therapeutic antimalarial drug concentrations throughout the period of highest malaria risk.Senegal has introduced this new intervention in its malaria prevention system and management policy since 2012. Goudomp health district in South Senegal started this intervention in 2014 and recorded 95% coverage in 2016 mass campaign. However, a survey leaded by parasitology unit in 2016 showed only 46% of full treatment coverage, meaning children have taken all doses. The research hypothesis was the SMC through the Direct Observed Treatment (DOT) and communication approach by community health workers improve the coverage up to 90%. The choice of units included was reasoned, taking into account the location of health structures and certain accessibility criteria. Volunteers were trained to administrate drug and deliver messages to parents and caregivers for 2 days, following the first administration made by these latter. All visit were done by volunteers. A social mobilization committee had been set up to strengthen communication and management of refusal cases. Mothers and care givers were informed and sensitize to report any occurred adverse effects. During the mass campaign, 3375 households were daily visited by volunteers. We obtained 90.5% coverage of full treatment under DOT in six units; 41 cases of minor side events have been reported and managed with good clinical response. During household visits, key messages were delivered to parents and caregivers on the importance of SMC for malaria prevention. DOT intiative to strengthen SMC coverage for full treatment with integrated communication has been successful in Goudomp health districts, and scale up with follow up of side events and efficiency studies can be considered

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EXPLORATION OF FACTORS ASSOCIATED WITH BED NET MAINTENANCE IN KENYA USING A NOVEL BED NET CARE ADHERENCE SCORE

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Bed net care practices are important to their effectiveness as malaria prevention tools. Using data from a cross-sectional household survey conducted in high (>1500m) and low (<1200m) altitude sites in western Kenya, we developed a bed net care adherence score combining washing, drying, retreating, and repairing behaviors. Households were surveyed about bed net perceptions, ownership, use, and barriers to use from July-August 2015. We hypothesized knowledge of net care practices and *Plasmodium* transmission, perceived malaria risk, and believed ability to prevent malaria to be associated with net care adherence. There were 1,217 surveyed households, of which 82.4% owned ≥ 1 net. Of highland households, 203 (31.6%) own no nets compared to 11 (1.9%) of lowland households. Reported care practices of 1,766 nets were scored and categorized into low, moderate, and high adherence to recommendations. Low adherence was rare (4.5% of nets); moderate adherence was common (54.1%). Among households with high net care knowledge, 145 of their nets (78.4%) received highly adherent care. Among households that believe they can prevent malaria, 224 of their nets (59.7%) received highly adherent care compared to 331 (39.4%) of households that are not confident they can prevent malaria (p<0.001). Net care knowledge and confidence to prevent malaria may be associated with high net care adherence. Of households with accurate *Plasmodium* transmission knowledge, 307 of their nets (48.5%) received highly adherent care compared with 12 (33.3%) nets among households with low transmission knowledge (p<0.001). Interestingly, 89 (17.7%) of nets owned by households that perceive high malaria risk received highly adherent care, where 147 (22.5%) nets of households that perceive low malaria risk receive highly adherent care (p<0.001). High malaria risk perception may not be associated with greater net care. Further evaluations are planned to measure the relationships of these factors with high adherence. Results will guide malaria education interventions to tailor messaging, and ultimately aid in the effectiveness and longevity of bed nets as malaria prevention tools.

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PROGRESS TOWARD UNIVERSAL BED NET COVERAGE IN WESTERN KENYA HAS NOT ENSURED UNIVERSAL USAGE, PARTICULARLY AMONG SCHOOL-AGE CHILDREN

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Insecticide-treated nets (ITN) are an effective tool for malaria control, and the WHO recommends universal coverage of one net for every two people in endemic areas. However, universal coverage does not guarantee universal usage, defined as all household members sleeping under an ITN. We assessed whether household ITN ownership in western Kenya meets the WHO target, and investigated the alignment between universal coverage and universal usage. Households from high (>1500m) and low (<1200m) altitude sites in western Kenya were randomly selected for a cross-sectional survey from July-August 2015. The lowlands experience perennial, highly endemic malaria; the highlands have lower prevalence with seasonal outbreaks. Household members completed detailed surveys about ITN ownership, use, and barriers to use. Household net ownership was categorized as adequate (≤2 people per net), inadequate (>2 people per net), or none. The survey included 574 lowland and 643 highland households, with 1,679 and 2,742 members, respectively. ITN ownership was common in the lowlands, with only 11 households (1.9%) having none, but inadequate coverage remains, with 149 households (26.0%) below the WHO target. In the highlands, only 241 households (37.5%) owned adequate nets. Inadequate coverage still resulted in high usage in the lowlands but not the highlands, with all members using a net in 81% of inadequately covered lowland households but 35% of highland households. In households with adequate nets, there was no significant difference in use by age (~3% of all ages being non-users), but school-age children (5-15 years) were significantly less likely than other age groups to use ITNs in households with inadequate nets (37% non-users versus 10% of children under 5 and 17% of adults ≥16 years, p<0.0001). ITN ownership is high in our study sites in Western Kenya, particularly in the endemic lowlands, but additional efforts are needed to reach WHO recommended coverage levels. In cases of inadequate coverage, perceived risk of malaria may drive prioritization of use among household members, with implications for continued sources of transmission in these regions.

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UNDERSTANDING THE GAP BETWEEN ACCESS AND USE OF INSECTICIDE-TREATED NETS IN GHANA: A QUALITATIVE STUDY ACROSS THREE ECOLOGICAL ZONES

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Mass and continuous distribution channels have significantly increased access to insecticide-treated nets in Ghana. Despite these gains, a gap remains between access and use. A qualitative research study was carried out to explore individual and contextual factors influencing net use, among those with access. The study was carried out across three purposively selected sites from the three epi-ecological zones in Ghana. A total of 18 focus group discussions (FGDs) and free listing and ranking activities were carried out with community members, health workers, and community leaders. Seven case studies were created based on follow-up home visits and interviews with select FGD participants. FGDs and case study interviews were audio recorded, transcribed verbatim, and analyzed thematically. An iterative process was used by which emerging themes were shared and built upon during data collection. Key barriers included environments where ITN use is difficult, such as outdoors, the perception that ITNs provide limited value due to exposure to mosquito bites during early evening hours and nighttime activities, experiences with skin irritation even after airing the ITN, lack of airflow and congestion in the sleeping space, and in some cases, a lack of information on ITNs and malaria prevention. Having a personal experience getting malaria, or having a loved one fall ill, was one of the most powerful motivators of consistent ITN use. Growing up using an ITN, or developing a habit of use, were also listed as facilitating factors. The economic benefit associated with prevention over treatment was also discussed. Providing visual representations on how to use an ITN in challenging environments, positioning ITN use within the broader context of malaria prevention, increasing saliency of malaria risk, highlighting the cost and time benefits of prevention over treatment, development of a net use culture beginning in primary schools, and increasing knowledge of malaria transmission were identified as opportunities. This information will be used to inform social and behavior change messaging and innovative approaches to addressing the net use gap in Ghana.

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INTERMITTENT PREVENTIVE TREATMENT WITH SULPHADOXINE PYRIMETHAMINE (SP) AMONG PREGNANT WOMEN IN KINTAMPO AREA OF GHANA - FINDINGS FROM 2011 TO 2015

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Malaria in pregnancy is a major public health problem in Ghana. It continues to contribute to the risk of spontaneous abortion, preterm delivery, low birth weight, and increases the risk of perinatal mortality. The World Health Organization (WHO) and the Ghana National Malaria Control Program recommend Intermittent Preventive Treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP) to prevent malaria-related adverse outcomes. However, the coverage of the recommended three or more doses of IPTp-SP has been found to be low in some parts of Ghana. The objective of this study was to determine the coverage of IPTp-SP among pregnant women in the middle belt of Ghana, and to explore the impact of socio-demographic characteristics on the uptake of IPTp-SP. Data from the Kintampo Health and Demographic Surveillance System (KHDSS) on the use of IPTp-SP among pregnant women from 2011 to 2015 was used. A logistic regression model was used to assess the determinant of IPTp-SP. In accounting for the possible correlation induced by the repeated observations, Generalized Estimating Equation (GEE) was applied. A total of 17,484 pregnant women from 2011 - 2015 were included in the study. The coverage of the recommended three or more doses of IPTp-SP among all pregnant women in the period was 40.6% (N= 4,065), 44.0% (N= 4,570), 45.9% (N= 4,547), 20.9% (N= 4,295), and 32.4% (N= 3,870) for 2011, 2012, 2013, 2014 and 2015 respectively. In the adjusted model, participants with secondary education (OR 1.5, 95% CI 1.25 - 1.75), aged 20 – 29 years (OR 1.5, 95% CI 1.30 - 1.65), primigravidae (OR 1.2, 95% CI 1.05 - 1.27), married (OR 1.2, 95% CI 1.07 - 1.25), and the least poor (OR 1.5, 95% CI 1.36 - 1.71) were significantly more likely to take the recommended three or more doses of IPTp-SP. However, participants in medium households (OR 0.8, 95% CI 0.71 - 0.87), Muslims (OR 0.8, 95% CI 0.71 - 0.84), and those from Northern tribes (OR 0.9, 95% CI 0.77 - 0.93) were significantly less likely to take three or more doses of IPTp-SP. These factors should be considered in the development and implementation of strategies aimed at improving the intake of IPTp-SP.

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PREDICTORS OF INSECTICIDE TREATED NET USE AMONG UNDER-FIVE YEARS OLD CHILDREN IN MAINLAND TANZANIA: FURTHER ANALYSIS OF MALARIA INDICATOR SURVEY 2015-16

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Tanzania is currently under epidemiological transition from meso-endemic to hypoendemic levels. The country is characterized by marked heterogeneity. The use of Insecticide Treated Net (ITN) is one of the key intervention to protect vulnerable groups including under-five children. According to the 2015-16 Malaria Indicator Survey (MIS), the average net use among children under five was 54.4% with some variation across regions; highest in Geita (86%), Katavi (85%) and lowest in Manyara (13%), Rukwa (15%) regions. Further analysis of the MIS datasets for Mainland Tanzania aimed at exploring predictors of ITN usage among under-five children focusing socio-economic and geographical factors to explain variability, differentials and relationship between variables. Data were analyzed using STATA software version 15.1 whereby "svy" commands were used for performing bivariate and logistic regression on ITN use among under-five children. A total of 9,843 records from this age group were reviewed. Permission to use these datasets was granted by ICF Macro. Result shows that the estimated odds of ITN use for older children is 8.2% less than young children. The odds for children living in high altitude areas (>1750 meters above sea level), rural setting, and from households with more than 5 people shows they are less likely to use ITN by 57.7%, 29.7% and 49.8% respectively. ITN use is not associated with the distance of the household to the nearest health facility. The estimated odds of mosquito net use are 60.6% more for each additional mosquito net in the household while wealthier families were 50.9% more likely to use mosquito net than those living in the poorest families. In addition, mosquito net use is high among households with sufficient number of mosquito nets. Finding reveals children living in high altitude are less likely to use mosquito net than those living in low altitude; probably due to low temperature leading to low mosquito vector density. Hence, stratification of malaria interventions should be considered to ensure effective use of diminishing resources.

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PERCEPTION OF THE MOTHERS AND THE CHILD MINDERS OF THE REGION OF SÉDHIOU ON THE SEASONAL MALARIA CHEMOPREVENTION IN 2017: ARE THE ABSENCES AND THE DISEASES OF THE CHILDREN - NO CASES OF DISGUISED REFUSALS?

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In the South of Senegal, the region of Sédhiou applies the Seasonal Malaria Chemoprevention (SMC) recommended by the WHO at the children from 3 to 120 months with 3 passages a year. Used Amodiaquine can give unwanted effects which could be sources of refusal. However according to the passages, the number of refusals decreases compared with the cases of sick or absent children by which the number increases that is why we wondered if it did not constitute cases of disguised refusals. The documentary revie) and the survey investigates qualitative were used with 8 focuses group and 19 individual interviews. And was noted 544 cases of vomitings, on 2079 of sick children and 4621 of the absent children while we noted that 116 cases of refusal. All the women recognized that the CPS served to prevent the malaria and some people ask for it even for them. The posology of the SMC is known as well by the women and they give medicine rather in the evening to avoid the side effects. **FD 26 years mother of 4 children:** " my children run to put themselves under the bed as soon as they see the agents giving medicine because they are bitter and they vomit when they take them but I make them go out so that they take them and for the second and the third dose I give them to them in the evening like that, they have nothing, it is because I know the value of this medicine ". For the absent and sick cases of child, the conversations are in favour of real cases of disease and the absent children took medicine left with their mom their return. It was noted as well as children declared absent were found hidden in the rooms of their parents. The importance of the SMC is well known by the women however the unwanted effects especially digestive create reluctances for the parents who think that the medicine is too powerful but effective for the prevention of the malaria so that they developed a particular strategy to limit these effects to know the taking in the evening in the bedtime. However the increase of the absent and sick children seems to be a new shape of refusal.

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THE ROLE OF ACTIVE CASE DETECTION IN MALARIA ELIMINATION: *WHAT ARE THE CHALLENGES?*

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Zanzibar has made significant progress in reducing malaria burden, with malaria prevalence reported to be 0.7% by microscopy in children under five in the 2015-16 Tanzania Demographic and Health Survey. Considering this and other key impact indicators, in 2014 the Ministry of Health changed the name and strategies of the program from Zanzibar Malaria Control to Zanzibar Malaria Elimination Program. In this context intensive malaria surveillance, which includes case investigation and classification, was implemented in 2013. In our program, tracking and follow up investigations and prompt reporting of each malaria case is imperative. Active case detection involves both reactive and proactive case detection. Reactive is triggered whenever a case is identified by passive case detection at health facility. It involves visits to the households of index case, screening family members and neighbors. Proactive is the screening of a focal population in hotspots with at least five index cases reported in a village within 7 days. This study presents findings of reactive case detection. Reactive case detection is conducted by the district response teams, in collaboration with village leadership. In 2017, reactive case detection was conducted in 32 villages following reporting of 3609 confirmed index cases. A total of 16,163 household's members were screened for malaria parasites from the targeted 19,617 community members. Eighty three (0.5%) members were found positive. Reactive case detection requires adequate resources to ensure intended target is reached. Regardless of intensive community involvement refusal among the community members is always reported . In some instances, non-targeted community members request for malaria testing of which interferes with the planned budget and logistics. Reactive case detection is one of the core interventions towards malaria elimination. The system requires high involvement of community members, and sufficient resources. Active case detection is an expensive and complex intervention which needs proper planning.

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THE RISK OF PLASMODIUM VIVAX PARASITAEMIA AFTER PLASMODIUM FALCIPARUM INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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In Thailand, there is a high risk of *Plasmodium vivax* parasitaemia following treatment of *P. falciparum* infection. To inform the benefits of universal radical cure for patients with *P. falciparum*, the risk of *P. vivax* after *P. falciparum* was quantified across a range of co-endemic settings. A systematic review identified prospective clinical studies of antimalarial efficacy for uncomplicated *P. falciparum* malaria, published before January 2018 undertaken in areas co-endemic for *P. vivax*. The primary outcome was risk of *P. vivax* parasitaemia following *P. falciparum* infection at day 42. Secondary outcomes were risk of *P. vivax* at day 28 and 63 and risk of any parasitaemia. Estimates were pooled using meta-analysis and heterogeneity was investigated using meta-regression. We included 153 studies enrolling 31,262 patients. The risk of any recurrent parasitaemia by day 42 was 18.4% (95%CI 15.2-21.8; I^2=94.8%; 117 estimates) with 37.1% (28.2-46.2, I^2=92.2%) of these due to *P. vivax*. The risk of *P. vivax* parasitaemia was 5.6% (4.0-7.5; 92.7%; 117 estimates) by day 42 and 24.0% (18.0-30.6; 95.2%; 30 estimates) by day 63. *P. vivax* appeared later than *P. falciparum* recurrences and the risk was greater following rapidly-eliminated drugs and in studies undertaken in areas of short-relapse periodicity. The risk of *P. vivax* parasitaemia within 42 days of treatment with artemether-lumefantrine was 15.3% (4.1-31.4; 97.8%; 10 estimates). Partner drugs such as mefloquine or piperaquine delayed recurrence compared to lumefantrine, however, the risk of *P. vivax* parasitaemia was >15% by day 63 following all ACTs. In summary, following *P. falciparum* treatment, the risk of *P. vivax* parasitaemia is far greater than expected from reinfection alone. In co-endemic settings, universal radical cure with

an ACT and hypnozoitocidal agent, for patients with *P. falciparum* or *P. vivax*, has the potential to reduce all-cause recurrent parasitaemia and facilitate malaria elimination.

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GENETIC STRUCTURE OF *PLASMODIUM FALCIPARUM* IN AN ENDEMIC AREA OF THE PACIFIC COAST OF SOUTH AMERICA

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Ecuador plans to eliminate malaria by 2023; indeed, the country has seen a decrease in the number or cases from more than 100 000 in 2001 to only 558 in 2015. Nevertheless, the number of cases has increased in recent years and approximately 1280 infections were reported in 2017, 30 % of which were caused by *P. falciparum.* Most malaria population genetics studies performed in Latin America indicate high clonality and clear structure of *P. falciparum* populations. An outbreak of *P. falciparum* in Northwest Ecuador was the result of a clonal expansion of parasites circulating at low levels in the country or re-invading Ecuador from neighboring territories. However, general characteristics of *P. falciparum* circulating in Ecuador have not been determined. The main goal of this study was to genetically characterize and geographically map the population structure of *P. falciparum* in Northwest Ecuador and determine how the *P. falciparum* population structure changed across time. For this purpose, seven neutral microsatellites markers in two groups of samples from two locations were used (79 samples collected from 2002 to 2006 and 109 samples collected from 2013 to 2016). We found that the genetic population structure of *P. falciparum* in Ecuador has changed from 2002 to 2016 while diversity decreased. Our analyses showed that parasites from border locations have higher diversity than parasites from inland locations and that Ecuadorian *P. falciparum* share genotypes with both Colombian and Peruvian parasites. In addition, *P. falciparum* genotypes not previously reported, were found in Ecuadorian locations. The *P. falciparum* diversity found in Ecuador could be a product of migration or the result of haplotypes circulating in the country in low proportions. Studies of the genetic characterization of *P. falciparum* in eliminating areas help determine the possible origin of parasites in order to create strategies to prevent the entrance of new lineages and achieve local elimination of malaria.

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A MIXED METHOD STUDY OF HEALTH SEEKING BEHAVIOR FOR FEBRILE ILLNESSES AND ITS IMPLICATIONS FOR MALARIA CONTROL AND ELIMINATION IN SAVANNAKHET PROVINCE, LAO PDR

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Health seeking behavior is shaped by interactions between individual and societal factors as well as health services. Analyses of the determinants of health seeking behavior are important for malaria control and elimination. The main objective of this study was to explore factors affecting the health seeking behavior for febrile illnesses in Lao PDR. Household heads or their representatives (n=281) were interviewed using a structured questionnaire. 8 to10 people from each study village (n=100) were included for focus group discussions (FGDs). Most respondents were *Lao Theung* (269/281; 95.7% that comprised ethnic groups: *Mang Kong*: 200/281; 71.7% and *Tree*: 64/281; 22.7%), males (201/281; 71.5%) and almost half were from the age group 31-50 years (138/281; 49.1%). Geographic proximity to a health centre (AOR=6.5; CI=1.74-24.25; for those < 3.5km versus those > 3.6km) and previous experience of attending a health centre (AOR=4.7; CI=1.2-19.1) were both strong predictors of visiting a health centre when febrile symptoms were experienced as opposed to traditional healers. Attending local health centers/hospitals was often constrained by the transportation and finances. The first choice for treatment for most participants was local health centres, even though there was a mix of seeking health care from traditional healers as well. Participants indicated that they navigate more than one type of health care system (health centre/hospitals and traditional healers). Decisions about where and when to attend formal health care facilities depended on finances, travel capabilities (distance to the health centre, road conditions, availability of transport), severity of symptoms and recognition of the illness (more likely to attend health centres/hospitals if considered severe). Reducing health care costs and increasing the ease of access to health care facilities may lead to improved health care attendance. Current and future malaria control programs can only benefit by addressing these factors in addition to collaboration with the existing network of health workers, village health volunteers and traditional healers.

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IMPACT OF MOSQUITO LARVAL SOURCE REDUCTION ACTIVITIES OF TRAINED SCHOOL AGED CHILDREN IN THE CONTROL OF MALARIA IN NIGERIA

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Malaria prevention in Nigeria is focused almost entirely on the use of Long Lasting Insecticide Treated Bed-Nets (LLIN) with little or no attention on Larva Source Reduction (LSR). Involvement of school-aged children in health interventions have been found to be positive, therefore, this study was carried out to measure the impact of mosquito LSR activities of school-aged children (6-12 years) in the control of malaria. A total of 24 primary school-aged children were trained and engaged for LSR activities in the intervention community while no training was carried out in the control community. There were pre and post intervention assessment of social, entomological and parasitological indices in the study communities. The activities of the children were observed to significantly ($P<0.05$) reduce potential mosquito breeding sites and containers as well as larval abundance in the intervention community with a reduction in House Index (HI), Container Index (CI) and Breteau index from 66.67% to 16.67%, 46.91% to 8.02% and 211.1 to 33.3 respectively. Population of Indoor resting mosquitoes and an overall malaria parasite prevalence also reduced significantly in the intervention community from 40.8% to 23.0% ($P<0.05$). There were no significant reductions in any of the parameters measured in the control community. Knowledge of participants about mosquito breeding sites, consciousness to cover any water-holding containers and environmental hygiene were all observed to improve significantly ($P<0.05$) in the intervention community with 84.6% of respondents attributing their behavioral changes to the influence of the activities of the children. The LSR activities of the trained children in this study were seen to positively impact the intervention community; therefore, children participation in mosquito LSR may be a good strategy for malaria elimination

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CASEIN DIETS PREVENT EXPERIMENTAL CEREBRAL MALARIA BY ENHANCING HOST PRO-INFLAMMATORY IMMUNE RESPONSES

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Malaria and malnutrition are the major causes of morbidity and mortality in under-five children in developing countries. Based on the fact that casein hydrolyzate can regulate host immune responses, we investigate the related immune mechanisms in casein diet preventing experimental cerebral malaria (ECM). Female 6–8-weeks-old C57BL/6 mice were fed with different doses (5%, 20%, 35%) of casein diet or control diet for one month before infection with Pb ANKA to establish ECM. Thymus was weighed and αβ T cells and γδ T cells were assayed by FACS on Day 0 post infection. Splenic DCs, Th1 cells, Tregs and macrophages were determined by FACS on Day 3 post infection. The levels of cytokines from splenic supernatants were measured by ELISA or Greiess assay. 11 amino acids in the serum were assayed by API 4000 LC-MS/MS with Non-derivatized MSMS kit. Casein diets can reduce the parasitemia and prolong survival of ECM mice. Thymus atrophy was found in 5% casein diet group while γδ T cells were significantly increased in all casein diet groups. On d3 p.i., casein diets increased splenic Th1 cells and macrophages ($P<0.05$), and the similar trend was observed in IFN-γ, TNF-α and NO levels ($P<0.05$). Casein diet didn't affect the number of Tregs and IL-10 levels. L-Pro/L-Leu/L-Val/L-Tyr/L-Arg levels in the serum were significantly elevated in 20% Casein diet group. These data present the mechanism of casein diets in preventing ECM by improving host pro-inflammatory immune responses to control malaria infection.

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IMPACT OF MASS TREATMENT WITH DIHYDROARTEMISININ PIPERAQUINE ON MALARIA TRANSMISSION DYNAMICS IN THE GAMBIA: A PROSPECTIVE STUDY

Julia Mwesigwa[1], Jane Achan[1], Miriam Wathou[1], Nuredin Ibrahim Mohammed[1], Musa Jawara[1], Aurelia Prom[1], Fatoumatta Kanuteh[1], Jean-Pierre Geertruyden[2], Umberto D'Alessandro[1]

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Mass drug administration (MDA) aims to reduce malaria transmission in addition to on-going control interventions. This study aimed to determine the impact of MDA on the dynamics of malaria, clinical disease, efficacy of dihydroartemisinin piperaquine (DP) and risk of re-infections immediately after and during the transmission season. All residents older than 6 months were enrolled in a prospective cohort from six village pairs. Annual single round MDAs with DP were carried out in 2014 and 2015 to all residents at the start of each transmission seasons (June). Individual finger prick samples were collected monthly (July to December) for slide microscopy and PCR. A total of 4312 subjects were enrolled, in both intervention years, both coverage (3 doses) (2014: 68.22%: 2015: 65.60%) and compliance (2014: 83.11% and 2015: 85.93%) were high. Monthly malaria prevalence was significantly lower in from July to September following MDA than in April 2014 and was significantly lower during the 2014 transmission season, after MDA, than in the 2013 transmission season. DP efficacy to malaria at days 28 and 42 for three doses was (AOR=0.62, 95% CI:0.38-0.99), and (AOR=0.51, 95% CI: 0.29-0.90) respectively and for clinical malaria in 2014 (AOR=0.44, 95% CI: 0.30-0.64) and (AOR=0.59, 95% CI:0.46-0.74) in 2015. Gametocyte carriage declined in July (4/55, 7.27%), August (2/70, 2.85%) and November after the first MDA round. Individuals infected before MDA had 2 times at higher odds of re-infection in July (AOR=2.53, 95% CI:1.49-4.29) and during the transmission season (AOR=2.49, 95% CI:1.72-3.62). A single annual MDA round was able to reduce both malaria prevalence and clinical disease incidence, at least in the first months of the transmission season. However, several MDA rounds, some of them targeting the human reservoir of infection during the dry season, may be needed to obtain a more marked reduction of transmission.

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MOTIVATION AND MANAGEMENT OF PARTICIPANT FATIGUE IN LONG -TERM RESEARCH: LESSONS FROM TARGET MALARIA RESEARCH

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Despite registered progress in malaria control in sub-Saharan Africa, malaria still ranks high on the research agenda for development of new malaria control interventions. Scientists and donors derive motivation for continued research from anticipated progress/ acknowledgment on their contributions to solutions and increased knowledge base on malaria vectors and control methods. On the other hand, malaria endemic communities face the sting of the disease by deaths witnessed, high treatment expenses and declining efficacy of some existing methods. Paradoxically, it is in such communities that researchers seek cooperation of participants to contribute to research. Gaps in stakeholder engagement point to participant fatigue and declining levels of motivation hence Target Malaria's strategy to have a dedicated team of stakeholder engagement experts at the onset to ensure acceptance and consents for research activities. Early laboratory results indicate promise of the technology using genetically modified vector strains as a tool to reduce

malaria transmitting vector populations. Though still in its early stages, Target Malaria recognizes the value of co-development and has used qualitative research methods of observation, key informant interviews, focus group discussions with stakeholders to collect stakeholder concerns, complaints and suggestions to inform progress of the research. One of the challenges so far encountered is the quest by research participants seeking tangible (such as material and monetary) benefits as motivation to contribute to research progress at field study sites. In conformity to the ethical requirements against payment of research participants - undue influence/ or coercion, lessons have been learnt on innovative approaches to motivate research participants without giving tangible benefits while addressing participant fatigue. Analysis of emerging concerns point to the value of co-development in long-term research as a factor that increases motivation, reduces fatigue among participants and has potential to increase uptake of innovative malaria control technologies.

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IMPLEMENTING REACTIVE CASE TREATMENT TO REDUCE MALARIA PARASITE CARRIAGE: HOW ADAPTABLE ARE LOCAL COMMUNITY STRUCTURES?

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Interventions to clear the human reservoir of infection typically involve mass treatment of at-risk populations. These campaigns usually operate as vertical programs, in parallel to local health systems, requiring substantial investments to set up and operate, and face the challenge of adequate coverage and compliance. The Gambia has recorded significant reductions in malaria burden, especially in its western and central regions, and is considering options for elimination. As part of a trial on the impact of reactive treatment of contacts of clinical malaria cases on parasite carriage, we considered the feasibility of delivering treatment to affected compound members using the existing local health and social structures. In a stepwise iterative approach, we designed and evolved a process to deliver a full, weight-derived course of antimalarial treatment to all members of a compound within 24 hours of a case of malaria being diagnosed. Drug distribution and reporting of adverse events were progressively devolved from study personnel to the local village health workers (VHW). The study team monitored treatment adherence and comprehension of dosing via phone calls and house visits when required. There were 17 cases from 12 compounds (population 367) in five villages reported during the 2017 transmission season. Most (16/17) were diagnosed and treated by the VHW who also delivered treatment to 273 of the 350 (78%) compound members present at the time of the event. There was no refusal to participate. Adherence, measured by number of individuals who completed three doses, was 94% (253/273). There were 27 mild adverse effects reported which resolved without intervention. Community systems, VHWs and compound heads represent an effective and acceptable platform for the delivery of reactive treatment to at-risk populations. The feasibility of this approach for other interventions and settings needs further evaluation.

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SOCIAL STRUCTURAL IMPLICATIONS OF MALARIA VACCINE TRIALS IN EQUATORIAL GUINEA

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The Equato-Guinean Malaria Vaccine Initiative (EGMVI) was established in 2014. The goal of the EGMVI is to reach elimination on the island of Bioko, in conjunction with the Bioko Island Malaria Control Program. New strategies to achieve this goal are being planned through. the inclusion of the PfSPZ Vaccine with other standard malaria control interventions. The EGMVI recently completed the second phase of clinical trials to determine product safety and efficacy, in route toward applying the vaccine on Bioko's population. Before entering Phase III trials, it will be important to understand the community perception of the candidate malaria vaccine. The primary goal is to understand the potential impact of malaria vaccine clinical trials on socio-economic and health (SEH) aspects of trial participants in their respective communities. Understanding attitudes towards the vaccine, perceived benefits and concerns, are important for designing communication strategies, and will inform how SEH dynamics may impact subsequent vaccine uptake. Mixed-methods will used to analyze data from participants from several neighboring communities where clinical trials are held. Data will come from qualitative focus group discussions and in-depth interviews. Quantitative surveys will be administered to capture data on individuals from trial and non-trial communities. Health professionals and community stakeholders will also be surveyed. Findings suggest that participant motivation to participate and to recommend their family members and friends to participant in trials will influence subsequent vaccine uptake. Identified desired channels of vaccine administration and its implications for the cost effectiveness of a mass vaccination on Bioko Island will be discussed.

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UNDERSTANDING MOBILE POPULATIONS BEYOND MOBILITY: INSIGHTS FROM SOCIAL INQUIRY FOR MALARIA ELIMINATION

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Recent studies acknowledge the impact of human population movements on maintaining (a)symptomatic malaria reservoirs, spreading (resistant) parasites and increasing exposure to vectors. Drawing on social inquiry into human mobility as part of interdisciplinary research into malaria transmission dynamics and the effectiveness of malaria control strategies carried out by the authors in South-East Asia (SEA), Sub-Saharan Africa (SSA) and Latin America, we aim to highlight overlooked factors in defining mobile populations despite their key importance for heterogeneous elimination settings. Effective control strategies require scientific knowledge to distinguish and characterize different types of mobile populations as each group represents specific risk factors and hence different and appropriate interventions. Absent populations are even more difficult to deal with: nation states and more specifically public health institutions lack the capacity and the tools to efficiently target these populations. This is made more difficult by the lack of data on mobility as this is a common exclusion criterion in most biomedical and clinical studies, limiting available data on infection, transmission dynamics

and vulnerability. An additional, rarely recognized challenge is that for specific ethnic groups, mobility is a defining characteristic of their social identity, often constructed in opposition to sedentary populations whose attitudes historically vary between trust and distrust. Indeed, nomadic cattle herders in SSA often distrust 'sedentary' health interventions while ethnic minorities in SEA (*e.g.* Vietnam), representing the main hotspots of residual malaria transmission, hesitate between assimilation and resistance to official state-based policies, including malaria control. Similar considerations apply to indigenous people in the Americas (*e.g.* Ecuador and Peru). Malaria elimination will be possible only if mobile populations are adequately targeted and involved by interventions.

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COMMUNITY LAB OF IDEAS FOR HEALTH (CLIH): HORIZONTAL DIALOGUE AND NEGOTIATION OF RELEVANCE FOR MALARIA ELIMINATION TRIAL IN THE GAMBIA

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Despite decades of the scientific attention and financial efforts, malaria remains as a global threat. It is therefore timely to consider innovative medical but also social approaches, such as community participatory strategies. "Community Lab of Ideas for Health (CLIH)" approach was developed in the context of a clinical trial on reactive case detection and treatment for malaria in The Gambia (*Reactive Household-based Self-administered Treatment: RHOST)*. The trial treated both passively detected malaria patients and all individuals (possibly asymptomatic) residing in the patient's compound in order to interrupt transmission. As part of the trial, a mixed-methods anthropological study was conducted to understand communities and tailor the intervention to the local context. A platform (*lab*) was created for the dialogue between communities and the project including researchers from different collaborating disciplines. This CLIH served to exchange ideas and co-create the required relevance of and trust in the intervention for both the project and communities. This relevance was largely defined around the economic burden of malaria instead of its definition in terms of health outcomes. Income opportunities lost and the spiral of poverty functioned as a common language around which this relevance could be negotiated. An additional key function was the reinforcement of mutual trust through negotiating the accountability of the project in relation to the micro-politics of the community and the reinforcement of local resources, in this case the Village Health Worker, to sustain and enhance the existing health system. Adherence to the treatment (Dihydroartemisinin-Piperaquine) for reactively identified contacts of malaria patients, at least in large part attributable to the CLIH, was as high as 94%, suggesting the relevance and additional potential for CLIH and other participatory applications.

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IMPROVING THE EFFICIENCY OF REACTIVE SCREEN-AND-TREAT FOR MALARIA ELIMINATION IN SOUTHERN ZAMBIA

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Malaria screen-and-treat (Step-D in Zambia) is a reactive case detection strategy in which malaria cases confirmed at health centers trigger community health workers (CHWs) to screen for secondary cases within 140 m of the index case household with P*f*HRP2 RDTs. Few studies have evaluated whether an evidence-based strategy using environmental features that characterize household surroundings can improve the efficiency of secondary case identification. This study conducted in the catchment area of Macha Hospital, Choma District, Zambia, extended the screening radius to 250 m and assessed whether local environmental factors can guide CHWs to identify secondary cases more efficiently. Demographic information, bed-net use, and household construction were obtained from surveys. Households were stratified into malaria positive and negative secondary households using RDT and qPCR results. High resolution satellite imagery was imported into ArcGIS and used to generate local environmental features (i.e. number of animal pens within 100 m, distance to nearest animal pen, distance and elevation difference between index and secondary houses) and large-scale features (i.e. distance to main road and nearest stream category). Generalized estimating equations were used to estimate the difference in odds between positive vs. negative secondary households for each predictor. From January 12, 2015 to July 26, 2017, 4,202 individuals residing in 692 households were enrolled. 165 participants tested positive for malaria by RDT or qPCR, 66% of whom resided in index households. Parasite prevalence was 8.6% and 1.9% in index and secondary households, respectively. Stratification resulted in 488 negative secondary and 45 positive secondary households. Results from the regression model revealed 50%, 90%, and 50% significantly higher odds of a secondary household being malaria positive if the nearest stream category was 3, 4, or 5, respectively. Screening for secondary households within a low-transmission setting in rural southern Zambia could be optimized by using streams as environmental features to guide more efficient reactive case detection.

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QUALITY CONTROL AND QUALITY ASSURANCE: RECOMBINANT HUMAN G6PD PLUS HEMOGLOBIN AS A RESOURCE FOR ROBUST G6PD TESTING IN *PLASMODIUM VIVAX* RADICAL CURE

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Quality Control and Quality Assurance: Recombinant human G6PD plus hemoglobin as a resource for robust G6PD testing in *Plasmodium vivax* radical cure Maria Kahn, ChangCheng Zhu, Sampa Pal and Gonzalo Domingo PATH, Seattle, WA

Robust glucose-6-phosphate dehydrogenase (G6PD) tests are currently under development to meet the needs for radical cure of *Plasmodium vivax* with 8-aminoquionlines. A large gap for the support of point-of-care testing is the availability of reagents to support quality control of G6PD diagnostic test along the supply chain from the manufacturer to the end user. While reagents and systems exist to support QC of laboratory screening tests for G6PD deficiency, they are not configured appropriately to support point-of-care testing. Here we have demonstrated the feasibility of using lyophilized recombinant human G6PD as a QC reagent for novel point-of-care tests for G6PD tests. For the calibration of quantitative G6PD tests, a combination of recombinant G6PD plus hemoglobin was used to create panels of normal, intermediate and deficient controls. Both recombinant human G6PD and hemoglobin were combined and lyophilized with appropriate excipients to produce the integrated G6PD-Hb control reagent in different concentrations. Lyophilized material demonstrated stable G6PD enzyme activity and hemoglobin concentration on reconstitution. Real time and accelerated stability data performed over at least 6 months and over five different temperatures will be presented. These control reagents will be essential to support the framework for a sustainable QC/QA system of robust point-of-care G6PD testing for *Plasmodium vivax* radical cure.

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MALARIA ELIMINATION IN ZANZIBAR - WHY HAS IT NOT YET SUCCEEDED ?

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Malaria control in Zanzibar has successfully and uniquely reduced the hyperendemic situation before 2004 to presently low hypoendemicity, a pre-elimination stage with an astounding public health impact. However, epidemiological analyses reveal several obstacles and challenges that necessitate new tools and strategies in order to achieve full elimination on the isles. Some challenges relate to genetic shifts of the parasite and mosquito populations to escape diagnosis and treatment as well as vector control interventions respectively . Piloted new interventions and strategies have only provided partial impact, not significant enough to fully respond to the new challenges. The Zanzibar malaria control/elimination coverage, impact and transmission dynamics will be presented and a research and operational road map will be proposed in order to bend the present curve and potentially provide proof of concept of malaria elimination from a high malaria endemic area typical of sub-Saharan Africa.

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PREVENTION OF MALARIA REINTRODUCTION THROUGH ELIMINATION ACTIVITIES AND MALARIA CASE INVESTIGATION AND CLASSIFICATION IN SAMPOV LOUN OPERATIONAL DISTRICT, CAMBODIA

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Since July 2015, the Cambodia National Malaria Program (CNM) has been implementing malaria elimination activities in Sampov Loun Operational District (SPL OD) with support from USAID | PMI. A 1-3-7 active surveillance strategy is applied to ensure every case detected is notified within 24 hours, investigated within 3 days, and responded to within 7 days. Through the 1-3-7 strategy, after a case is notified, a team led by the OD malaria supervisor with health facility staff and a village malaria worker, conducts a case investigation. The investigation collects the patient profile, current diagnosis result, travel history during the last three weeks and malaria history during the last three months. Case classification determines if the case is locally transmitted (indigenous) or imported from outside SPL OD. Simultaneously, response activities through reactive case detection (Re-ACD) surrounding the index case include screening of index household (HH) members, co-travelers, and surrounding HH members who present suspected malaria symptoms, assessing net availability and top up, and providing health education. From July 2015 to February 2018, results show a high percentage of timely notification, investigation, and response increasing from 20-52% within the first 3 months to 85-100% in all 1-3-7 categories. To-date, 596 cases were investigated and classified. From July 2015 - March 2016, 28 indigenous cases were identified within 9 of 127 villages. Since March 2016, no indigenous *Pf* malaria cases were detected. Other response activities included health education to 3,091 people, 250 insecticide-treated nets (ITNs) topped up and 1,752 individuals surrounding index cases screened through Re-ACD. Re-ACD identified no cases among index and surrounding HHs, however 3% of co-travelers were diagnosed with malaria. These findings suggest a positive impact from the package of response activities in preventing reintroduction of locally transmitted *Pf* cases in SPL OD since April 2016 (as of February 2018). Screening household and neighbor contacts identified no new infections and is likely not a cost-effective use of resources in this setting.

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VILLAGE-BASED STRATIFICATION IN MYANMAR FOR MONITORING AND TO INFORM SELECTION OFMALARIA INTERVENTIONS

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Current malaria endemicity in Myanmar shows focal characteristics. Even within one township, the level of malaria transmission can significantly vary depending on environmental and socio-economic-demographic factors. In 2013, University Research Co.(URC), implementer of the PMI-supported Control and Prevention of Malaria Project, developed a Village-Based Stratification (VBS) framework using Annual Blood Examination Rate (ABER) and village-based Test Positivity Rate (TPR) to monitor malaria endemicity and choose appropriate interventions. The project applied the VBS by specifying village population, collecting patients' addresses, monitoring adequacy of community diagnosis with an ABER ≥10%, and stratifying villages according to the number of cases reported in the previous year. Villages were stratified into 3 categories using the TPR criteria: high transmission (TPR ≥5%, red color), moderate-low transmission (TPR >0-<5%, yellow), and no transmission (no cases detected the past year, green). The VBS framework was applied to 272 villages of South Rakhine State and Tanintharyi Region with a sufficient ABER≥10% for 4 consecutive years and the following results were obtained: 100 villages with TPR ≥5%, 103 villages with a TPR >0-<5%, and 69 villages with no malaria, as stratified in September 2014, changed into TPR ≥5% for 34 villages, TPR >0-<5% for 68 villages, and no malaria for 170 villages, in September 2017. In 2016, URC modified the VBS according to the new stratification criteria adopted by the National Malaria Control Program using Annual Parasite Incidence (API) instead of TPR for categorization into high-moderate-low transmission, potential transmission (receptive with no current transmission), and malaria free. Villages with malaria in the previous year are stratified by tercile, tailoring API data for best applicability. Stratification can be done using existing routine surveillance data and is very useful for monitoring progress and providing strategic information to target appropriate interventions for different strata in developing countries with limited resources.

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ASSESSMENT OF MALARIA SERVICES OFFERED BY PRIVATE PROVIDERS IN RURAL, UNDER-SERVED AREAS OF TANINTHARYI REGION, MYANMAR

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In Myanmar, a move towards malaria elimination requires expanding coverage of malaria services to all populations at-risk, in particular those living in rural, under-served areas where neither health facilities nor village malaria workers are available. To assess the extent and type of malaria services provided by private providers (PPs) in under-served areas, the PMI-supported Defeat Malaria project conducted in August 2017 a mapping of PPs in 179 villages of Tanintharyi Region. The survey identified 118 private practitioners and 1,145 drug stores, of whom only 12 (1%) sold anti-malarial drugs. Of the 130 PPs offering malaria services (118 private practitioners and 12 drug sellers), 41 (32%) had not received any malaria training, 42 (32%) did not have any malaria rapid diagnostic tests (RDT), and 45 (35%) did not have artemisinin-based combination therapy (ACT) or other anti-malaria drugs. Eighty-five of the 130 PPs were identified as prescribers of anti-malarial drugs, and 14 (16%) of them had artesunate and/or artemether monotherapies, while the remaining used ACTs. PPs were present in 86 (48%) of the 179 villages, and people from the other 93 villages received malaria health services from nearby public facilities. From qualitative key informant interviews by convenience sampling with 20 (15%) PPs it emerged that anti-malarial drugs were not available in most drug stores due to low demand, restriction of selling unregistered drugs and anti-malaria monotherapy, and free provision of drugs by NGOs and public facilities. Most PPs weren't aware of the national malaria drug policy. Ninety-one (70%) PPs had previously referred patients to public facilities or NGOs. Not all PPs could provide malaria services due to lack of malaria training, RDTs or ACTs, or an appropriate referral mechanism. PPs from rural, under-served areas should be appropriately trained, supported for malaria service provision, encouraged to engage in a timely referral mechanism and case reporting, and receive information on Myanmar's national malaria treatment policy.

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MALARIA ELIMINATION IN NORTHERN AND CENTRAL LAO PDR: ASSESSING TECHNICAL, OPERATIONAL, AND FINANCIAL FEASIBILITY

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Northern and Central Lao PDR is rapidly accelerating towards malaria elimination. In 2017, there were 454 reported malaria cases across 13 provinces, accounting for only 5% of the national malaria burden. Nearly two-thirds of cases occurred in just four districts. Based on these trends, the Center for Malariology, Parasitology, and Entomology (CMPE) established goals to eliminate *Plasmodium falciparum* malaria by 2020 and *P. vivax* malaria by 2025 from these areas of Lao PDR. In support of these goals, the first malaria elimination feasibility assessment for Northern and Central Lao PDR was conducted in early 2018 to inform strategic decisions and operational planning. In accordance with the 2014 WHO elimination scenario planning tool, this assessment examines technical, operational, and financial feasibility. The technical assessment integrates national surveillance data analysis, findings from a population-based parasitemia and risk factor survey and qualitative research, and mathematical modelling to predict the impact of various intervention scenarios on malaria transmission. A comprehensive literature review and semi-structured interviews with key stakeholders were also conducted. Achieving elimination in Northern and Central Lao PDR in line with national strategic timelines is feasible using currently available tools. Key technical strategies required include strengthening case management (training, supportive supervision, expanded community-based care); improving coverage and targeting of vector control; and introducing case-based surveillance and rapid response. Operational capacity must be strengthened to support these technical strategies, including improvements in availability of trained staff, access to district funds, stock management, communications, and program management. Interventions, training, and staffing were costed using an activity-based approach to estimate resource needs. Sustained investment in high quality interventions and operations will be required to achieve malaria elimination and prevent resurgence in Northern and Central Lao PDR, paving the way for national elimination.

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COMBINING SEROLOGICAL AND CLINICAL INCIDENCE METRICS FROM EASY ACCESS GROUP SURVEYS AND ROUTINE SURVEILLANCE TO GUIDE ELIMINATION ACTIVITIES IN HAITI

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The island of Hispaniola is the last remaining endemic area of malaria in the Caribbean. In Haiti, nation-wide PCR/RDT prevalence was below 1% in 2011, 2012, 2015 and 2017. However, heterogeneity is pronounced and there is limited insight to date as to how cases detected through routine surveillance relate to malaria transmission in the community. We aim to compare transmission intensity estimates by health facility catchment area using antimalarial antibody responses in multiple populations sampled to support elimination activities by Haiti's Ministry of Public Health and Population. Data sources included a sample of easy access group venues (EAG; 9 health facilities, 23 schools; n=4233) and routine health surveillance (RHS; 9 health facilities) in the Artibonite Valley in Haiti. Antibody responses (IgG) against 23 malarial antigens were assessed in the EAG survey using a multiplex bead assay (including 6 markers of sero-incidence). All the cases recorded at health facilities were confirmed with malaria rapid diagnostic tests. Spearman's rank sum agreement was used to assess the crude agreement according to venue type and metrics by age groups. The optimal combination of antibody metrics from the Artibonite data was then applied to data from an EAG survey (n=4922; 16 health facilities, 25 schools) and RHS (16 health facilities) in the Grand'Anse Department in southern Haiti. Serological metrics from EAG surveys may help in identifying communities with ongoing malaria transmission to guide elimination activities in Haiti.

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PERCEPTION OF MALARIA RISK AND ACCEPTABILITY OF REACTIVE, FOCAL ADMINISTRATION OF PRESUMPTIVE TREATMENT AND INDOOR RESIDUAL SPRAYING, A QUALITATIVE STUDY FROM THE MALARIA ELIMINATION SETTING OF NAMIBIA

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To reduce and interrupt malaria transmission in elimination settings, reactive, focal presumptive treatment and indoor residual spraying (IRS)

target the parasite reservoir in humans and mosquitoes. However, low acceptance of treatment without evidence of malaria infection, or of reactive, focal IRS could limit widespread implementation and success. As part of a cluster randomized trial in Zambezi Region, Namibia, comparing reactive focal screening and treatment to presumptive treatment, and reactive focal IRS to no IRS, qualitative data collection was conducted prior to (August 2015) and during two rounds of trial implementation (June - August, 2016 & 2017). Data collection included: 15 focus group discussions (FGDs) and six interviews prior to interventions, and 34 FGDs and four key informant interviews during and after the interventions. Pre-trial findings show that most participants believed malaria interventions are effective, but IRS and long-lasting insecticide treated nets (LLINs) are not always available. Some respondents expressed discomfort accepting medication without symptoms or testing, but with education would be more likely to accept. Potential barriers included use of traditional healers, distrust of public sector health staff and interventions for their potentially stigmatizing effects. Practices that could influence acceptance included offering community-based care and ongoing community sensitization and education. During the trial, perceptions of malaria risk were closely related to presence of illness in the community. Reasons for participating included effective recruitment, the opportunity to learn, feeling unwell, and level of disease in the community. Most refusals were attributed to being busy or away from home. When considering improvements, members suggested they be informed of visits in advance to prepare, more interventions in more areas, and to fill gaps in LLIN distribution. The community preferences identified during the trial led to an increase in efforts to inform communities in advance and better coordination of the clinical teams to increase acceptance of trial interventions.

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THE CONTRIBUTION OF MALARIA VULNERABLE PEOPLE LIVING WITH HIV/AIDS TO MALARIA CONTROL

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Ghana has made many strides in the fight against malaria. However, the shared geographical location of HIV and malaria may influence interactions of these diseases. People living with HIV/AIDS are vulnerable to malaria in malaria-endemic settings. Our research looked at diagnostics of malaria in people living with HIV/AIDS (PLHA) using a rapid diagnostic test that is locally common, expert microscopy and PCR. We also studied their knowledge and practices with regard to malaria prevention in rural and urban Ghana. Locally available HRP2-based malaria rapid diagnostic tests were comparable to the PCR gold standard. However, few false negatives detected did not test positive for the *pfhrp2/3* gene. Residents in the rural area were less informed of malaria and its preventive measures than those in the urban areas. Their knowledge was associated with increased odds for malaria. The common practice in both areas was the low use of insecticide treated mosquito nets even though many people owned them. Further focus of national malaria control program may help in the exponential decline of malaria in Ghana. Perhaps, an integrated control program for both HIV and malaria may prove to be beneficial.

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RETROSPECTIVE ANALYSIS OF HEALTH FACILITY-REPORTED MALARIA CASES IN HIGH FOREST-RELATED MALARIA AREAS IN ACEH, INDONESIA

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Passively collected routine health facility data are an underutilized source of information for estimating the burden of malaria, identifying remaining hotspots, and developing risk maps for targeting scarce resources. We extracted routinely collected monthly health facility data from January 2013 until December 2016 to describe risk factors, identify hotspots, and generate spatial risk maps of malaria in Aceh Besar and Aceh Jaya districts, in Aceh Province, Indonesia. Demographic, employment, location of residencies, and parasitological data, including date of febrile, date of diagnosis and date of treatment were assembled from Primary Health Center registry books. Case locations were geo-located based on village names using Google Maps and other map resources. The resulting geo-database was then integrated into a Geographic Information System using the open-source QGIS software. We included 2,412 malaria positive individuals from both districts in the analysis. Number of malaria positive individuals decreased from 1,532 patients in 2013 to 133 patients in 2016. *Plasmodium vivax* was the most dominant infection (88.4%), altogether with other species of *Plasmodium* malaria infections, such as *P. falciparum* (9.2%), *P. malariae* (0.1%), *P. knowlesi* (0.2%) and mixed infections (3%). Most individuals with positive malaria were forest-related workers (96.4%), such as miners (77.1%), farmers (11.9%) and loggers (7.3%). Multivariable logistic regression will be used to establish socio-demographic and environmental risk factors including forest cover, and geostatistical analyses will be conducted to produce species-specific risk maps.

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QUANTIFYING MOVEMENT PATTERNS OF MOBILE MIGRANT POPULATIONS DURING A MALARIA FOCAL TEST AND TREAT INTERVENTION IN SOUTHERN LAO PDR

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In the Greater Mekong Subregion, mobile and migrant populations (MMPs) are considered at higher risk for malaria infection due to forest-going and travel-related behaviors. In the Lao People's Democratic Republic (PDR), MMPs engage in a diverse set of forest-based activities for economic gain and general livelihood, increasing their risk for malaria. Much remains unknown about MMPs in Lao PDR, and more evidence is needed to properly characterize and respond to these at-risk populations. In particular, movement patterns are an important component of MMP behavior and necessitate further research to better clarify links between mobility and malaria occurring outside of village settings. As part of a larger active case detection trial in Champasak province, Southern Lao PDR, a focal test and treat intervention employing 30 peer navigators

(PNs) to seek out non-village based MMPs is underway across 15 health center catchment areas (HCCAs). An estimated subset of 50 PN-identified MMPs will be recruited to carry "i-Got-U" GPS loggers to characterize and quantify mobility patterns. Evaluated outcomes will include time spent outdoors, outdoor movement during evening and dawn hours, time spent in the forest, distances traveled, and frequency of travel by MMPs. MMP travel proximity to borders, bodies of water, forest fringe, and deeply forested areas will also be assessed. Data collection is ongoing through September 2018 and will be followed by a cluster analysis to compare mobility patterns of PNs and identified MMPs. PN movement will be analyzed in the context of the study to reveal coverage across HCCAs and to support analyses of MMP movement patterns. Collected GPS data will be used to examine the locations of identified MMPs, MMPs with malaria, and areas with the highest densities of MMPs, as well as possible transmission hotspots. These parameters of MMP movement patterns will be used to inform development of mathematical models of malaria transmission in high-risk mobile forest-goer populations. Findings from this study will help to further define MMP behavior and its possible impacts on malaria transmission in Southern Lao PDR.

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MODELLED PREDICTED PUBLIC HEALTH IMPACT AND COST-EFFECTIVENESS OF CHILDHOOD RTS,S/AS01E MALARIA VACCINE IN MALAWI, USING A MARKOV STATIC MODEL

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The RTS,S/AS01E is the most advanced malaria vaccine to date being assessed in Malawi, Ghana and Kenya as part of a large-scale pilot implementation programme involving over 700,000 children. As its incorporation into existing immunisation programmes will have substantial cost implications, cost-effectiveness studies provide an essential tool for countries to consider vaccine introduction. We explored the predicted health impact and cost-effectiveness of the RTS,S/AS01E malaria vaccine on the childhood population of Malawi. We calculated the Incremental Cost Effectiveness Ratio (ICER) per disability adjusted life years (DALYs) averted by vaccination and compared it to Malawi's mean per capita Gross Domestic Product. We used a previously validated and published Markov model, which simulated the malaria progression in a 2017 birth cohort of Malawian children less than 15 years old. In the simulation, we used a 46% vaccine efficacy as demonstrated in the phase 3 trials, 75% RTS,S/A01 coverage, estimated $5 cost of the vaccine, treatment costs for clinical malaria from previous publications and Malawi specific indicators for malaria interventions such bet net use and antimalarial use. Both health service and societal perspectives were explored. The ICER/DALY was $115, lower than the 6 year mean GDP per capita of $398.6 for Malawi. Sensitivity analyses exploring the impact of variation in vaccine costs, vaccine coverage rate and coverage of 3 doses and 4 doses showed vaccine implementation would be cost-effective across a wide range of scenarios. RTS,S/AS01 was predicted to avert a median of 93 940 (range 20 490-126 540) clinical cases and 394 (127-708) deaths for the three-dose schedule, or 116 480 (31 450-160 410) clinical cases and 484 (189-859) deaths for the four-dose schedule, per 100 000 fully vaccinated children. We predict the introduction of the RTS,S/AS01 vaccine in the Malawian EPI to be to be highly cost effective.

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MODELLED PREDICTED PUBLIC HEALTH IMPACT AND COST-EFFECTIVENESS OF CHILDHOOD RTS,S/AS01E MALARIA VACCINE IN MALAWI, USING A MARKOV STATIC MODEL

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FUNCTIONAL ACTIVITY AND HOST IMMUNE RESPONSE OF *PLASMODIUM VIVAX* RETICULOCYTE BINDING PROTEIN 2P1

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Plasmodium vivax only infect immature red blood cells called the reticulocytes. This host cell tropism is believed to be mediated by the reticulocyte binding protein family of the parasite. Sequencing of cDNA from clinical *P. vivax* isolates confirmed the presence of six genes of this family that are transcriptionally active and likely encode proteins (PvRBP1a, PvRBP1b, PvRBP2a, PvRBP2b, PvRBP2c, and PvRBP2p1). Among these proteins, PvRBP2p1 is most similar to PfRH5, a strong candidate for vaccine development against *P. falciparum*. Both PfRH5 and PvRBP2p1 are composed of only the N-terminal red blood cell binding domain and lack the transmembrane domain possessed by other members of the family. This research aims to evaluate the roles of PvRBP2p1. Using *E. coli*, we expressed full-length recombinant PvRBP2p1. Using this protein in erythrocyte binding assays, we found that, unlike some well characterized PvRBPs, PvRBP2p1 binds both mature erythrocytes and reticulocytes. We also found that treatment of erythrocytes with trypsin or chymotrypsin significantly reduces binding activity. To localize the

protein, antibody was raised in rabbits against recombinant PvRBP2p1 and used in immune-fluorescence assays. The protein was found only in very mature schizonts and localized at the apical end of merozoites. We then used ELISA to measure antibody levels to PvRBP2p1 in *P. vivax* infected individuals. Interestingly, a statistically significant negative correlation was found between PvRBP2p1 antibody level and parasite density in blood. Interestingly, the levels of anti-PvRBP2p1 antibody in patients were generally lower than that of an asymptomatic carrier. These results suggest that the anti-PvRBP2p1 antibodies may play a protective role, possibly by inhibiting erythrocyte invasion. In summary, we provide evidence that PvRBP2p1 may be a good candidate for vaccine development against blood stage *P. vivax* infection.

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VOLUNTEERS PERSPECTIVES OF CLINICAL TRIAL PROCEDURES: LESSONS FROM THE PILOT STUDY FOR MALARIA CLINICAL TRIAL PROCEDURES OF RECRUITMENT AND SCREENING OF ADULT VOLUNTEERS WITH OR WITHOUT HIV IN TANZANIA

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Malaria Control strategies have resulted in a successful reduction of malaria transmission in Africa. This success raises the optimism and possibility of elimination and eradication of malaria through the use of the vaccine.Clinical trial volunteer recruitment and retention are essential to the success of any clinical study. Yet little is known about perspectives of volunteers on clinical trial procedures and what makes them opt for participating in the trial. This study was ubdertken to explore volunteers perspectives on recruitment and screening procedures in Malaria clinical trial in Tanzania. This was a was cross-sectional study conducted in September and December 2017. An exit structured questionnaire was administered to the purposefully selected 148 volunteers who participated in a pilot study of Malaria clinical trial procedures of recruitment and screening of adult volunteers aged 18 to 45 years with or without HIV in Tanzania.The descriptive analysis of the collected data was done by using STATA statistical package. A total of 148 participants were interviewed with Overall mean ± SD age was 29.8 ± 6.7 years. Almost two-thirds (62%) of the volunteers to participate because they wanted to know their health status.More than 90% of the volunteers strongly approved study procedures.Majority of the volunteers (91.5%) had a positive perception regarding female volunteers in reproductive age to take measures for prevention of pregnancy while participating in future trials. Lastly, the majority of volunteers (92.4%) had a positive perception regarding the storage of urine, stool and blood samples up to 15 years for further analyses with the appropriate ethical approval as required. In conclusion, the results of the study indicated that volunteers hold a positive perception of the study procedures.In addition, the common reasons to participate in the study for volunteers was knowing their health status. This implies that community members are aware of the importance of undergoing a medical checkup and failing to fulfill this need recruitment and retention of volunteers in the clinical study will be a challenge.

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A PRE-CLINICAL PRIME-AND-TRAP MALARIA VACCINE BASED ON CD8$^+$ LIVER RESIDENT MEMORY T CELLS

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Tissue resident memory CD8$^+$ T cells (Trm) in the liver are critical for long-term protection against pre-erythrocytic *Plasmodium* infection. Protection can be achieved by repeated, intravenous vaccinations with radiation attenuated sporozoites (RAS). To reduce dosing frequency, we tested a DNA vaccine designed to induce potent CD8$^+$ T cell responses against the SYVPSAEQI epitope of *P. yoelli* circumsporozoite protein (CSP). In a heterologous prime-and-trap regimen, CSP-specific CD8$^+$ T cells were primed using a gene gun-delivered DNA vaccine and boosted with a single dose of RAS with or without a concurrent DNA booster. These regimens attracted expanding CD8$^+$ T cell populations to the liver where they became Trm cells. Vaccinated in this manner, BALB/c mice were completely protected against challenge, which was not reliably achieved following one dose of RAS or multiple doses of DNA-only vaccines. Our study demonstrates that potent CD8$^+$ T cell priming by DNA vaccination synergizes with naturally liver-targeted RAS vaccination and highlights a translational pathway for improving the efficacy of T cell-mediated malaria vaccines in humans.

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PLASMODIUM BERGHEI SERINE/THREONINE PROTEIN PHOSPHATASE (PP5) IS ESSENTIAL FOR GAMETOGENESIS

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Gametocytes and their following development in the mosquito vector is a complex differentiation process relies on multiply signaling transduction mediated by phosphorylation. These development steps are crucial for the parasite to complete its life cycle and transmission to a new vertebrate host. However, the functions of phosphatases involved in gametogenesis are poorly understood. Here, we present a *Plasmodium berghei* protein, Ser/Thr Protein Phosphatase 5 (*Pb*. PP5), which is highly conserved among *Plasmodium* species. Removal N-terminus TPR domains of recombinant *Pb*. PP5 protein could elevate enzyme activity by seven folds, and recombinant *Pb*. PP5^{yPP2Ac} has moderately sensitive to okadaic acid (IC_{50}=5.36 nM). Furthermore, *Pb*. PP5 is expressed in cytosol of most asexual and sexual stage parasites, except for merozoite and ookinete stages, in which some of the *Pb*. PP5 protein located beneath parasite plasma membrane. Target disruption of *pb. pp5* gene has no influence on blood stage parasite multiplication. Meanwhile, parasites lacking *Pb*. PP5 are competent for gametocytogenesis, but exflagellation of male gametocytes, following developments of ookinetes *in vitro* and oocysts *in vivo* are completely blocked. In addition, anti-*Pb*. PP5 sera moderately blocked the parasite development in the gametocytes exflagellation and ookinetes conversion. Furthermore, mosquitoes fed on mice passive transferred immunized anti-*Pb*. PP5 sera had 20 % reduction in the prevalence of infection and almost 57.6 % reduction in oocyst density, indicating blocked transmission of the parasites to mosquitoes. These results and the high degree of conservation of *Pb*. PP5 in *Plasmodium* species suggest that this protein could be an attractive target for the development of novel drugs to block the spread of malaria, and might be a potential novel transmission blocking vaccine target candidate.

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THE EFFECT OF THE RTS,S/AS01E VACCINE FOURTH DOSE ON ANTIBODY RESPONSES TO CSP AND HBSAG IN CHILDREN FROM MOZAMBIQUE

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RTS,S/AS01E is a first generation vaccine and the most advanced in development globally. However, its efficacy as given to infants and children is moderate, the duration of protection is limited, and a fourth dose, although beneficial for vaccine efficacy, seems not to induce the expected IgG levels against the immunodominant epitope of the circumsporozoite (CSP) antigen. The mode of action, immune correlates of protection and factors affecting immunogenicity and vaccine efficacy of RTS,S/AS01E are still to be elucidated. Preliminary data from our consortium measuring baseline (Month [M] 0) and peak immune responses one month after primary vaccination (M3) showed that RTS,S/AS01E induced IgM and IgG subclass responses to the CSP and hepatitis B (HBsAg) vaccine antigens, predominantly IgG1 and IgG3, with lower IgG2 and IgG4. Moreover, age, transmission intensity, prior malaria episodes, maternal IgGs, nutritional status, as well as baseline immune status had an impact on the outcome of RTS,S/AS01E vaccination. Recognizing the requirement of vaccines for induction of long-lasting immunity as well as RTS,S/AS01E limitations, we aimed to assess the immunogenicity and duration, over time, of antibodies induced with and without the fourth dose. Samples from children and infants given a forth dose of RTS,S/AS01E or a comparator (including malaria cases and controls) were analyzed. We measured IgM, IgG, IgG1, IgG2, IgG3 and IgG4 levels to RTS,S/AS01E vaccine antigens in plasma samples from M20, M21 & M32 (end-of-follow-up) from a subset of infants (aged 6-12 weeks at first vaccine dose) and children (aged 5-17 months) from the Manhiça-Mozambique trial site by quantitative suspension array technology. We will present data on the comparison between the isotypes and subclasses of antibodies to CSP (NANP, C-tem and full length) and HBsAg measured in the later time points of the trial, and relate them to baseline and peak responses to gain insight into the kinetics of responses and immunogenicity of the vaccine with and without the fourth dose.

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DEVELOPMENT OF METHODS CHARACTERIZING THE AMA1-RON2 COMPLEX

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It has previously shown that the rhoptry neck protein 2 (RON2) binds to the hydrophobic groove of the apical membrane antigen 1 (AMA1). The coupling is an essential step in the invasion of red blood cells (RBCs) by *Plasmodium falciparum* (Pf) merozoites. Vaccination with AMA1 alone induced high-titer invasion-blocking antibodies in a human challenge trial; however, the vaccine failed to provide protection even against homologous challenge. Recent study showed that immunization with an AMA1-RON2 peptide complex, but not AMA1 alone, provided complete protection against a lethal *Plasmodium yoelii* challenge in mice when Freund's adjuvant was used. This result indicates that the AMA1-RON2 complex is a promising vaccine candidate against malaria. To prepare clinical trials using AMA1-RON2 complex as a candidate vaccine, the identity and integrity of the AMA1-RON2 complex must be assessed. Due to the fact that the complex is formed by non-covalent interactions, the characterization methods must be carefully designed so that the complex is not dissociated during the evaluation. In this study, we developed a native Tris-glycine gel method to separate and identify the AMA1-RON2 complex, which was further identified and confirmed by Western blotting using anti-AMA1 monoclonal antibodies (mAbs 4G2 and 2C2) and anti-RON2 polyclonal Ab coupled with mass spectrometry. The formation of complex was also confirmed by Capillary Isoelectric Focusing (cIEF). A short term (48 h and 72 h at 4ºC) stability study of AMA1-RON2 complex was also performed. The results indicate that the complex was stable for 72 hours at 4ºC. Our research demonstrated that the native Tris-glycine gel separation/Western blotting coupled with mass spectrometry and cIEF can fully characterize the identity and integrity of the AMA1-RON2 complex and provide useful quality control data for the subsequent clinical trials.

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ANTIBODIES AGAINST ESSENTIAL MEROZOITE ANTIGENS PFRH5, CYRPA AND MSP-1 POTENTLY NEUTRALIZE ERYTHROCYTE INVASION BY *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES

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Erythrocyte invasion by *Plasmodium falciparum* is an indispensable step for its blood-stage life cycle and successful transmission to new human hosts. The invasion process is mediated by multiple ligand-receptor interactions and is an attractive step to identify critical invasion ligands that could be developed as malaria vaccine candidates. Several major ligands such as AMA-1, despite being essential, failed to elicit strain-transcending invasion inhibitory antibodies due to high antigenic polymorphisms. It is thus imperative to identify conserved vaccine targets that could generate broadly-neutralizing anti-parasitic antibodies. Here we report the vaccine potential of three essential and conserved merozoite antigens PfRH5/CyRPA/MSP-1, that induce broadly-neutralizing antibodies against diverse *P. falciparum* clinical isolates from malaria endemic regions of Mozambique, Africa. The isolates were adapted to laboratory culture and after minimal *in vitro* cultivation were characterized for their invasion phenotypes by analysing their ability to invade enzymatically-treated erythrocytes. The parasite neutralizing efficacy of antibodies targeting different merozoite ligands was evaluated using the standard *in vitro* growth inhibition assay. Majority of the isolates invaded neuraminidase-treated cells in a sialic acid independent manner and showed varied sensitivities to trypsin and chymotrypsin treatment, implying the usage of alternate invasion pathways. Invasion by few isolates was resistant to all three enzymes. All isolates irrespective of their invasion phenotype were potently neutralized by individual antibodies against RH5, CyRPA and MSP-Fu (MSP3-MSP1 fusion chimera) with 80-90% inhibition (10 mg/ml total IgG). Importantly, antibody combinations against these three antigens elicited potent strain-transcending inhibition at lower IgG concentrations in a synergistic or additive manner, thus validating the vaccine potential of PfRH5, CyRPA and MSP-1. Our study strongly suggests the inclusion of PfRH5, CyRPA and MSP-Fu in a multi-component cocktail blood-stage malaria vaccine.

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CHARACTERIZATION OF FUNCTIONAL HUMAN MONOCLONAL ANTIBODIES TO *PLASMODIUM VIVAX* RETICULOCYTE BINDING PROTEIN 2B (*PV*RBP2B) ISOLATED FROM NATURALLY EXPOSED INDIVIDUALS

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Plasmodium vivax (*Pv*) preferentially invades reticulocytes to complete their life cycle and establish infection in humans. The interactions of *P. vivax* merozoite ligand, reticulocyte binding protein 2b (*Pv*RBP2b) and Transferrin receptor 1 (TfR1) on reticulocytes, mediates the successful invasion of host cells. We isolated human monoclonal antibodies (humAbs) to *Pv*RBP2b from naturally exposed individuals and characterized whether they inhibit *Pv*RBP2b: TfR1 interaction and block *Pv* invasion of reticulocytes. *Pv*RBP2b specific single memory B cells were isolated from Cambodians using fluorescence activated cell sorting. Each cell specific cDNA library was produced and nested PCR was performed to pull out variable heavy *iggh* and light chain *iggl* genes. Paired *iggh* and *iggl* were cloned into IgG1 expression vector, expressed and purified. Inhibition of the *Pv*RBP2b-TfR1 interaction with humAbs was studied using a flow cytometry-based red blood cell binding assay. Epitope recognition by humAbs were mapped using different *Pv*RBP2b recombinant fragments. Competition ELISA was performed with a panel of well characterized inhibitory murine monoclonal antibodies to evaluate the humAbs' mode of recognition. Sequencing of 290 antibody variable heavy genes from *Pv*RBP2b- specific memory B cells identified 36 distinct clonal groups. Currently we have cloned and expressed 17 humAbs from 16 clonal groups. Eight humAbs range from partial to complete inhibition of *Pv*RBP2b binding to reticulocytes. Current epitope evaluation indicates some humAbs recognize similar epitopes to previously characterized inhibitory murine mAbs and others recognize unique epitopes. Notable four humAb are more potent inhibitory antibodies compared to any of the previously characterized murine mAbs. Isolation and characterization of humAbs to critical *Pv* invasion ligands leads to greater understanding of the mechanisms of naturally acquired immunity to *Pv* blood stage infection. The potential of passive transfer of inhibitory humAbs into non-human primates or humans can confirm their protective potential and allows novel approaches for malaria vaccine design.

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IMMUNOGENICITY AND EFFICACY OF MALARIAL CELTOS AND CSP VACCINE ANTIGENS ADMINISTERED WITH GEL-DEPOT ADJUVANT

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Half of the world's population is at risk for malaria, yet there is currently no highly effective subunit vaccine. The most promising vaccines for malaria require 3+ injections to induce modest levels of protection. Thus, there is a critical need to develop a robust vaccine that requires fewer doses. To achieve this result, the field is utilizing recent advances in vaccine adjuvant and delivery technologies. Our lab pioneered the use of a self-assembling matrix as a non-immunostimulatory vaccine adjuvant. The gel-depot adjuvant utilizes the RADA16 peptide, allowing slow release of the immunogen from the mobile phase of the matrix. This self-assembling vaccine matrix is a 3-dimensional vaccine adjuvant that increases antigen persistence, thereby enhancing immune responses to vaccine antigens. This non-reactogenic matrix adjuvant is stable without a cold-chain and eliminates local and systemic reactogenicity caused by molecular adjuvants. Here, we optimized delivery parameters for *Plasmodium berghei* (Pb) CelTOS and CSP in mice. First, we optimized the (1) injection route and (2) RADA16 concentration, which alters the kinetics of immunogen release. Then, using optimal concentration and route parameters, we optimized the (3) prime-boost interval. Compared to conventional delivery of these two antigens, we found gel-depot delivery works best via intradermal and subcutaneous routes. Evaluating time between boost, we observed that an 8 week interval is best, perhaps due to the slow release kinetics of antigen from the gel-depot. We are currently evaluating immunogenicity and efficacy of our optimized gel-depot adjuvant delivery of Pb CelTOS and CSP by sporozoite challenge. Future work includes testing *P. falciparum* CelTOS and CSP proteins in mice using optimized delivery conditions, which will include evaluations of efficacy and durability. These studies are the first phase of our long-term goal of generating a functional malaria subunit vaccine for humans. Enhancement of vaccines by non-immunostimulatory vaccine adjuvants may represent a fundamental paradigm shift in how vaccines are developed and delivered.

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A ROBUST NONHUMAN PRIMATE MODEL WITH INDUCED STERILE IMMUNITY AGAINST *PLASMODIUM VIVAX* AS A PLATFORM FOR DEVELOPING VACCINE CANDIDATES

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Malaria is caused by parasites of the genus *Plasmodium* that are transmitted to humans by the bites of *Anopheles* mosquitoes. After the elimination of *Plasmodium falciparum* it is predicted that *Plasmodium vivax* will remain a major cause of morbidity and mortality outside of Africa, stressing the importance of developing a vaccine against *P. vivax* malaria. Current studies to develop a *P. vivax* vaccine are focused on inducing stronger antibody responses. At present, there is no benchmark to assess the level of protection needed to achieve sterile protection in both *Aotus* and humans. Here we repeatedly infected six Aotus monkeys with blood stages of the homologous *P. vivax* Sal-1 and challenged them with the heterologous AMRU-1 strains. Blood samples were collected across the experiment to measure parasitemia and humoral immune responses, including correlates of protection against whole parasite and specific blood stage antigens using ELISA and a *P. vivax* protein microarray. Sterile immunity was achieved after the second homologous Sal1 infection in 4 of 4 monkeys. In these animals, antibody levels increased rapidly to 3.0 log10 after the first infection, boosting to 4.0 log10, thirty days after the second infection; by the third infection on day 166 PI, antibody levels decreased slightly to 3.5 log10 but increased to more than 4.0 log10 between 108-275 days PI. After the fourth infection with the heterologous AMRU1 strain, antibody levels reached a maximum of 4.3 log10. ELISA titers at different levels of infection showed a negative correlation vs mean (r = -0.93; Pearson correlation) and cumulative parasitemia (r = -0.90), but a positive correlation vs peak parasitemia (r = 0.74). Work linking parasite densities against antigen-specific immune responses and changes in parasite transcriptional profiles during repeated infection is ongoing. In conclusion, this study provides the first benchmark for achieving sterile immunity in the *Aotus P. vivax* animal model. These experiments provide a template for testing the efficacy of candidate blood stage *P. vivax* malaria vaccines in this non-human primate model.

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STRATEGIES FOR IMPROVING THE THROUGHPUT AND SENSITIVITY OF TESTING MALARIA T-CELL VACCINE ANTIGENS IN MICE

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Subunit T cell vaccine development for malaria is faced with a problem of too many possibilities – there are thousands of candidate antigens, yet no proven *in vitro* or *in silico* methods for efficiently selecting those that are genuinely protective. In addition, many antigens are cross-presented on APCs but are not presented by hepatocytes. Such antigens can be highly immunogenic but completely non-protective. To overcome these limitations, we have developed a novel strategy for rapidly and sensitively assessing the ***protective*** efficacy of large numbers of candidate T cell antigens. In the 'acute challenge' model, large numbers of DNA vaccines targeting individual antigens are built in parallel using codon-optimized, 500 bp synthetic DNA fragments. Coding sequences are designed to eliminate signal sequences and are cloned in-frame as ubiquitin fusion proteins to minimize the induction of antibody responses. Small groups of mice are DNA vaccinated twice against single candidate antigens using a gene gun and challenged at the peak of the immune response following the boost, a time point where 10-30% of CD8+ T cells are specific to the vaccine antigen. Protection is assessed by challenge with 2-5x10^4 wild-type, luciferase-expressing *P. yoelii* parasites and IVIS imaging 40-44 hr later. The acute challenge model allows rapid, sensitive detection of both complete and partial protection. Cryopreserved splenocytes can be quickly assessed by ELISPOT to confirm that a T cell response was generated against vaccine-encoded antigens. The extremely high T cell frequencies induced by this approach avoid miscategorizing antigens due to poor immunization (vaccination failure) or low assay sensitivity (assay failure). Importantly, evaluating protection definitively identifies liver stage antigens that are processed and presented by hepatocytes at protective time points, in contrast to immunogenicity-based assays that often detect non-protective, cross-presented antigens. This strategy should be considered as a first line approach for broadening the repertoire of liver stage vaccine antigens prior to studies focused on immune memory formation.

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MALARIA TRANSMISSION IN THE COMMUNITY AS MEASURED BY DIRECT SKIN FEEDS AND BY COLLECTION OF WILD MOSQUITOES DURING A VACCINE TRIAL IN BANCOUMANA, MALI

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Vaccines that interrupt malaria transmission such as transmission blocking vaccines (TBV) could play a key role in the elimination or eradication phase of the disease. Standard Membrane Feeding Assays and Direct Membrane Feeding Assays are used to assess the efficacy of TBV *in vitro* using a membrane feeding to allow mosquito feeding on infective blood and to assess serum transmission blocking activity. Direct Skin Feeds (DSF) are now being assessed as a closer representation of natural transmission conditions, by allowing mosquitoes to feed directly on the skin of vaccine trial subjects. However, the comparability of DSF to natural feeding of mosquitoes in the community for quantifying transmission has not been studied. Here, we measured infections in mosquitoes fed by DSF vs wild caught bloodfed mosquitoes from September to November 2017 as part of a transmission blocking vaccine study. A total of 127 healthy adult volunteers participated in DSF and mosquitoes were collected in volunteers' rooms twice a month by spray catches (SC) and by live room searching (LC). For wild caught mosquitoes, *Plasmodium* infection rates in salivary glands at time of capture were determined by ELISA for SC collected mosquitoes, and oocyst counts in midguts by microscopy 7 days post LC collection. DSFs were performed twice weekly for 12 weeks using two cups of 30 mosquitoes administered to the forearm for 15 minutes. Infection was determined by dissection and oocysts counts 7 days post feeding. Salivary gland sporozoite infection rate was 1.8% (n=731) among SC mosquitoes, and the oocyst infection rate was 1.6% (n=1,061) among LC mosquitoes. By comparison, the infection rate in DSF experiments was 0.2% (n=61,451). Survival rates were 64.0% for wild LC mosquitoes and 73.4% for mosquitoes used in DSFs. Several factors could contribute to the lower rate of infection in the DSF vs wild-caught mosquitoes, such as infectivity of human bloodmeal source (e.g., wild caught but not DSF mosquitoes may feed on children or pregnant hosts with higher parasite levels), inherent susceptibility of wild vs laboratory-maintained mosquitoes, or effects of the vaccine.

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QUALITY CONTROL OF INSECTARY REARED MOSQUITOES FOR LARGE-SCALE PRODUCTION TO SUPPORT THE ASSESSMENT OF TRANSMISSION BLOCKING VACCINES USING ARTIFICIAL FEEDS

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With the renewed interest in assessing transmission blocking vaccines using Standard Membrane Feeding Assays, Direct Membrane Feeding Assays and Direct Skin Feeding Assays, consistent, controlled laboratory mosquito production is becoming a point of interest. Mass production is needed in many cases and it is imperative to use high quality mosquitoes to ensure good entomological outcomes from the vaccine trials. To date, there are no agreed quality control procedures for large-scale production of mosquitoes in insectaries for such vaccine trials. Here, we attempt to match the performance of our laboratory colony of *Anopheles coluzzii* mosquitoes to a level that provides continued high quality entomological outcomes for malaria vaccine trials. Mosquito performance was measured by determining a number of parameters: feeding rate, proportion of blood fed females that laid eggs, larval hatching rate, pupae emergence rate, adult mosquito size, adult longevity and sex ratio of mosquitoes. In total, 50 3-day old females were used in each of 3 replicate experiments. Females were blood fed and feeding rates were determined. The females were allowed to oviposit collectively and the proportion of females that laid eggs was determined. The progeny were reared under identical conditions and all above parameters were assessed. The average feeding rate was 90% (n=150). Approximately 69% (n=150) of the blood fed females laid eggs, and the average number of eggs laid per female was 53.3. The hatching rate was 78% and the emergence rate (from pupae to adult) was 85% (n=5,073). The sex ratio was 52% males and 48% females (n=4,312). The average size of the adults was 3.15±0.03 mm for male and 3.3±0.03 mm for female (n=300). The longevity was 5 weeks for male and 6 weeks for female. Though these parameters and results can be influenced by many external factors, this study could be a basis to set quality control standards for large-scale mosquito production to support experiments that require high numbers of good quality mosquitoes.

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CAUSES OF SCREENING FAILURE DURING A TRANSMISSION BLOCKING VACCINE TRIAL AROUND DONEGUEBOUGOU, MALI

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A safe and effective malaria vaccine is a necessary tool to improve malaria control and pursue its eradication. Screening is a crucial step during a trial that relies on reference values to include or exclude volunteers in vaccine trials. We are conducting a trial of the malaria transmission blocking vaccine Pfs230D1M-EPA/AS01 in the healthy adult population in Doneguebougou and surrounding villages, 30 km north of Bamako. After obtaining informed consent, each volunteer has undergone clinical and laboratory assessments to evaluate the frequency of different diseases and as well as abnormal laboratory values to ascertain inclusion/exclusion criteria. In February 2017, we screened 169 volunteers and enrolled 110 into the trial. The eligibility rate was 78.7% (133/169). Withdrawal of consent and travel before or at the time of enrollment were 9.5% (16/169) and 4.1% (7/169) respectively. The most common causes of screening failure were due to viruses 16.6% (28/169) with 11.8% for hepatitis B, 3.6% for hepatitis C and 1.2% for HIV. Our results are consistent with those of the PfSPZ1 study of Donegeubougou in 2014 where the most common causes of screening were also due to the viruses with 21.8% for hepatitis B, 2.0% for hepatitis C and 2.0% for HIV. Their rate is slightly higher than for us and this could be explained by the fact that they did more screening than us. Biochemical abnormalities accounted for 1.2% (2/169) for both elevated creatinine and elevated alanine aminotransferase (ALT). The associated presence of hematuria and proteinuria was 1.2% (2/169). Of the 5 volunteers who failed the clinical examination, 1.8% (3/169) were due to QT_C interval> 450ms, and 0.6% (1/169) each for tachycardia and for high blood pressure. QT_C interval > 450ms and hepatitis B are the most common causes of clinical and laboratory screening failure in our vaccine study.

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HEMATOLOGICAL NORMAL RANGES IN HEALTHY CHILDREN AND ADULTS FROM BANCOUMANA, MALI, A MALARIA VACCINE TESTING SITE

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Clinical trials require the selection of healthy volunteers with normal laboratory parameters, which are defined in apparently healthy subjects from the target population stratified by sex and age. These locally normal ranges are critical in clinical trials to select healthy volunteers and assess the safety of investigational products. The Malaria Research and Training Center (MRTC) and LMIV/NIAID/NIH are monitoring the dynamics of malaria transmission in a community where transmission blocking vaccines are being tested. We enrolled 829 subjects from Bancoumana in February 2018 (before malaria transmission season) to study the dynamics of malaria transmission across the community. All volunteers underwent the following laboratory assessments: Complete Blood Count (CBC) with Hemoglobin (HGB x mg/dl), differential (White Blood Cell (WBCx10^3/µl), Absolute Neutrophil Count (ANC x 10^3/µl), and Absolute Lymphocyte Count (ALC x 10^3/µl). CBC variables in the population were described using median and percentiles (2.5 and 97.5). Here, we report the median and range values for these laboratory assessments in the following demographic categories of enrolled subjects: infants aged 0-5 years, children aged 6-14 years, and adults aged 15 and over, separated by male and females. None of the values were statistically significantly different between the sexes. In infants and children, reference values slightly differed from those found about 10 years ago in Doneguebougou by the clinical CAP laboratory. In terms of median values in adults, these results are similar to those reported previously among Malian adults. These findings add to the baseline knowledge of healthy individuals in West Africa and can be useful in the future for further studies.

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SCHISTOSOMIASIS AND HELMINTH PREVALENCE AMONG ADULTS IN A MALARIA VACCINE TESTING SITE, BANCOUMANA, MALI

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Endemic helminth infections in sub-Saharan Africa, such as helminthiasis and schistosomiasis, are known to elicit a wide range of immunomodulating responses and may impact the efficacy of vaccination. The potential impact of co-infections on vaccine immunogenicity will depend on the prevalence of local helminth and schistosomiasis infection. The aim of this study is to evaluate frequency of *Schistosoma haematobium* infection and intestinal helminths among adult volunteers involved in a phase 1 malaria transmission-blocking vaccine trial in Bancoumana, Mali. From February to March 2015, we examined the presence of *S. haematobium* and intestinal helminth co-infections in Bancoumana in adults aged from 18 to 50 years old. Bancoumana is a village located 60 km southwest of Bamako and has a population of about 10,000 people. The co-infection was assessed by testing volunteers screened to be enrolled in a malaria transmission-blocking vaccine trial. Microscopy of urine on Whatman paper and stool PCR were done respectively to detect *S. haematobium* and *S. mansoni* and 8 common gastrointestinal pathogens in each volunteer. In total, urine was collected from 359 participants in the study and the overall prevalence of *Schistosoma haematobium* infection was 6.7% (24/359). The sex distribution showed an infection frequency of 6.6% (17/257) in males and 6.8% (7/102) in females; infection frequency was not statistically different between sexes (p=0.07). Urine filtration showed that *Schistosoma mansoni* infection was not present. Subjects positive for *Schistosoma haematobium* received standard treatment using Praziquantel. Stool PCR results are pending at this time but will be discussed and compared to urine infection levels to determine overall burden of helminth prevalence.

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NUMEROUS AUTO-DISSEMINATION STATIONS ARE REQUIRED FOR THE USE OF *ANOPHELES GAMBIAE SENSU LATO* IN THE TRANSFER OF LARVICIDES TO LARVAL BREEDING HABITATS

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Larval control is an effective tool for malaria vector control in some settings. Larviciding for malaria control is limited by the difficulty to identify and treat the numerous breeding habitats of these mosquitoes. Novel strategies to apply mosquito larvicides to *Anopheles* breeding habitats are necessary to improve the cost-effectiveness. This study explored the potential of gravid *Anopheles gambiae* sensu stricto to auto-disseminate pyriproxyfen (PPF) to breeding habitats to prevent adult vector production. Cage tests were implemented to assess the best method to contaminate netting with PPF to allow the females to pick up most of the insecticide when in contact. The attractiveness of 6-day old soil infusion and cedrol to the females were compared in semi-field systems. One test and two control semi-field systems were used to investigate the transfer of PPF by females from dissemination stations to open ponds. Dissemination stations were ponds covered with netting material dusted with PPF. Transfer of PPF was assessed by observing adult emergence in larvae introduced into the three open ponds. Cage bioassays showed that gravid *An. gambiae s.s.* transferred most PPF from netting contaminated with PPF dust than when netting was contaminated with PPF formulated in oil. Ponds with water treated at 20 ppm cedrol were twice as attractive as untreated ponds, ponds treated with water treated at 5 ppm cedrol or 6-day old infusion were 1.2-1.5 times more attractive than untreated pond. Transfer of PPF by gravid females was not recorded in any of the control semi-field systems. Transfer of PPF was demonstrated in the test semi-field system, and it was dependent on the distance of the open pond from dissemination station. Overall the adult emergence rates of larvae introduced into open ponds in test semi-field system were 8-10 times reduced compared to emergence rates in the two control semi-field systems. These results provide proof of principle of the feasibility of auto-dissemination for the control of Afrotropical malaria vectors. Numerous auto-dissemination are required for effective auto-dissemination for control of Afrotropical malaria vectors.

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SPATIAL EFFECTS OF MALARIA INTERVENTIONS ON MALARIA MORBIDITY IN CHILDREN UNDER 5 YEARS IN NIGERIA

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Malaria is a significant public health problem in Nigeria. In 2014, Malaria accounted for 21% of estimated deaths in children under five, approximately 60% of outpatient care utilization, and 30% of hospitalizations among children under five. Since 2010, a substantial expansion of malaria interventions has been initiated, however, the malaria burden in Nigeria has declined disproportionately among regions nationwide. The objective of this study is to estimate the geographical variation of parasitaemia prevalence and quantify the effects of malaria interventions at the state level in Nigeria. Using the 2010 and 2015 Nigeria Malaria Indicator Survey, a spatial multilevel model will be fitted to assess the association between parasitaemia prevalence and its determinants in both years. A two-level intrinsic conditional autoregressive model was used to account for spatial correlations. State level estimates of parasitaemia prevalence were predicted and averaged by stratum-specific population estimates. Given the limited sample size in each state, the standard errors and confidence intervals for the prevalence were calculated using the weighted bootstrap method which is robust under complex survey sampling designs. The mothers' education and wealth index were identified as important predictors of parasitaemia prevalence. Population adjusted prevalence was highest in the south west regions and had varying changing pattern from 2010 to 2015. For instance, the prevalence in the state of Niger decreased from 73% (95%CI 66% - 79%) to 29% (95%CI 20%-38%) while the prevalence in the state of Taraba increased from 12% in 2010 to 41% in 2015. Artemisinin-based Combination Therapy had an overall significant effect on malaria risk after adjusting the local endemicity levels in both years and the effect became stronger in 2015. Insecticide-treated nets were shown to have a significant effect in 2010, however the effect was diminished in 2015. The predicted results and maps in this study provide visual aids for decision-makers to access the effects of interventions and identify the priority area where the targeted strategy and resources were needed.

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ENTOMOLOGICAL IMPACT OF INDOOR RESIDUAL SPRAYING WITH PIRIMIPHOS-METHYL: A PILOT STUDY IN AN AREA OF LOW MALARIA TRANSMISSION IN SENEGAL

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Scaling-up of effective anti-malarial control strategies in Central-West region of Senegal has resulted in the sharp decline in malaria prevalence in this area. However, despite these strategies, residual malaria transmission has been observed in some villages (hot spots). The objective of this study

was to assess the impact of indoor residual spraying (IRS) with pirimiphos-methyl on malaria transmission in hot spot areas. The malaria vector population dynamics were monitored in each of the six selected villages (4 of which used IRS, 2 were unsprayed control areas) using overnight human landing catches (HLC) and pyrethrum spray catches (PSC). The host source of blood meals from freshly fed females collected using PSC was identified using the direct ELISA method. Females caught through HLC were tested by ELISA for the detection of *Plasmodium falciparum* circumsporozoite protein (CSP) and *Anopheles gambiae* complex was identified using PCR. Preliminary data shown that the densities of *Anopheles* populations were significantly lower in the sprayed areas (179/702) compared to the control. Overall, malaria transmission risk was 14 times lower in the intervention zone (0.94) compared to the control zone (12.7). In the control areas, three *Anopheles* species belonging to the Gambiae complex (*Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles melas*) maintained the transmission, while only *An. coluzzii* was infective in the sprayed areas. In conclusionThe preliminary data from this pilot study showed that IRS with the CS formulation of pirimiphos-methyl is likely very effective in reducing malaria transmission risk. However, additional studies including further longitudinal entomological surveys as well as ecological and ethological and genetical characterization of vectors species and their populations are needed to better characterize the entomological impact of indoor residual spraying with pirimiphos-methyl in the residual transmission areas of Senegal.

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EX-POST RICE FARMERS WILLINGNESS TO PAY FOR MALARIA LARVAL SOURCE MANAGEMENT: THE CASE OF RUHUHA COMMUNITY IN EASTERN RWANDA

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With the aim to ensure sustainability and integrate this Bti intervention in rice fields to complement the existing malaria control strategies, we assessed the change in rice farmers' WTP levels after the experience of Bti intervention and perceived benefits, as well as its effectiveness in reducing malaria risk. The WTP was assessed through the contingent valuation method using a bidding game procedure and the impact of Bti spraying on the maximum WTP for that intervention was estimated using difference-in-differences technique, combined with propensity score matching. This evaluation conducted in 2017 (*ex post*) among 288 rice farmers made use of mixed methods, a quantitative panel study (longitudinal) comparing with data collected in 2015 (*ex ante*) and two focus group discussions. After two Bti experiments conducted in a period of two years, the mean *lumpsum* WTP after Bti spraying was significantly higher in the self-organized group (treated without supervision) than in the comparison group and the treated group with supervision ($p<0.05$). The mean *progressive* WTP after Bti spraying was significantly higher in the treated group with supervision than in the comparison group ($p<0.001$) and lower in the self-organized group (treated without supervision) than in the treated group with supervision ($p<0.001$). The size of land significantly increased between the two experiments ($p<0.001$), making higher the total contributions using the *progressive* option than *lumpsum*. In both experiments, all participants were still willing to financially contribute any amount of money for Bti, except one farmer who was not able but would wish so. Our study findings suggest that the *ex post* WTP was higher than the *ex ante,* especially for treatments involving self-organization of the farmers whose fields were sprayed without external supervision. This self-organization aspect confirms the community empowerment, ownership and participation; which is a key success factor for sustainability of the intervention. The average maximum WTP in both experiments is far lower than the actual cost of Bti (less than 25%).

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BLOOD FEEDING BEHAVIOR OF MALARIA VECTORS IN THE ERA OF INTENSIVE VECTOR CONTROL EFFORTS IN WESTERN KENYA

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Regular monitoring of the feeding behaviors of malaria vectors is crucial to determine the frequency of human-vector contact and implement effective vector control interventions. This study was conducted to assess the feeding preferences of malaria vectors in western Kenya. *Anopheles* mosquito collections were done from September 2015 to April 2016 in Ahero and Iguhu sites using CDC light traps (indoor and outdoor), pyrethrum spray catches (indoor) and pit-shelters (outdoor). Data on insecticide-treated nets (ITN) ownership and the number of potential blood-meal hosts available in the area were collected using questionnaires. Polymerase chain reaction (PCR) was used to identify *Anopheles gambiae* species complex. Mosquito blood-meal sources were determined using ELISA. The proportion of households owning at least one ITN was 88.5% and 80.5% in Ahero and Iguhu, respectively. A total of 10,864 *Anopheles* mosquitoes comprising *An. gambiae* s.l. (71.4%), *An. funestus* s.l. (12.3%), *An. coustani* (9.2%) and *An. pharoensis* (7.1%) were collected. Most (61.8%) of the anophelines were collected outdoors. PCR result (n = 581) revealed that 98.9% *An. arabiensis* and 1.1% *An. gambiae* s.s. constituted *An. gambiae* s.l. in Ahero while this was 87% *An. gambiae* s.s. and 13% *An. arabiensis* in Iguhu. The human blood index (HBI) and bovine blood index (BBI) of *An. arabiensis* was 2.5 and 73.1%, respectively. *Anopheles gambiae* s.s. had HBI and BBI of 50 and 28%, respectively. The HBI and BBI of *An. funestus* was 60 and 22.3%, respectively. Forage ratio estimate revealed that *An. arabiensis* preferred to feed on cattle, *An. gambiae* s.s. showed preference for both human and cattle, while *An. funestus* preferred human over other hosts. Compared with data collected two decades ago before the scale-up of ITNs, the HBI of *An. gambiae* s.s. has decreased by 20% with proportionate increment in its BBI. This suggests an increasing tendency of *An. gambiae s.s.* to feed on cattle. *Anopheles arabiensis* was highly zoophagic while *An. funestus* still showed anthropophagic behavior. Additional control tools that complement the existing interventions are required to control zoophagic vectors.

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EVALUATION OF TOXICITY AND CROSS-RESISTANCE OF NOVEL CANDIDATE INSECTICIDES CLOTHIANIDIN, NEONICOTINOID, AND CHLORFENAPYR, PYRROLE, AGAINST WILD AND LABORATORY *ANOPHELES ARABIENSIS* IN JIMMA, ETHIOPIA

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Insecticide-based preventative measures are an integral part of malaria control programs. However, the evolution of resistance threatens to undermine progress. Chlorfenapyr and clothianidin are two insecticides included in control products because of their unique modes of action. Prior to their use in any program, it is important to establish toxicity towards local vector populations and assess cross-resistance with other insecticides commonly used for vector management. To investigate the effectiveness of these insecticides against the predominant Ethiopian malaria vector species, *Anopheles arabiensis*, diagnostic doses and cross-resistance

were tested, using standard intensity resistance bioassays, against both a laboratory-reared susceptible strain and a wild, multi-insecticide resistant population of *An. arabiensis*, from Asendabo, Oromia Region. A range of doses was tested for chlorfenapyr and clothianidin and the identified diagnostic dose was used to assess cross-resistance to deltamethrin, permethrin, malathion, propoxur, and bendiocarb. Complete mortality was observed with the laboratory strain using the recommended diagnostic doses for chlorfenapyr (100 µg/bottle) and clothianidin (2%/filter paper). Field populations were resistant to the organophosphate, malathion (83% mortality), capable of surviving 2X, 5X and 10X the diagnostic dose of both deltamethrin and permethrin, but susceptible to carbamates bendiocarb and propoxur. Interestingly, field populations of *An. arabiensis* were significantly more susceptible to clothianidin, reaching 100% mortality by day 2 compared to the laboratory strain (100% mortality by day 3). This study demonstrated that the putative diagnostic doses of chlorfenapyr and clothianidin are appropriate for monitoring resistance within *An. arabiensis.* The unique mode of action leading to an absence of cross-resistance makes chlorfenapyr and clothianidin potential candidates for inclusion in national vector control programs, particularly in areas with high pre-existing or emergent resistance to multiple insecticide classes.

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THE IMPACT OF PERIODIC DISTRIBUTION CAMPAIGNS OF LONG LASTING INSECTICIDAL TREATED BED NETS ON MALARIA VECTOR DYNAMICS AND HUMAN EXPOSURE IN DIELMO, SENEGA

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The implementation of long-lasting insecticidal-treated bed nets (LLINs) have contributed to halving the mortality rate due to malaria since 2000 in sub-Saharan Africa. However, some resurgences of malaria transmission have occurred in some areas following the introduction of indoor interventions, underscoring the fragility of these strategies. Thus, to achieve the World Health Assembly's new target to reduce the burden of malaria over the next fifteen years by 90%, it is necessary to understand how the spatiotemporal dynamics of malaria vectors and human exposure to bites are modified in the context of scaling-up global efforts to control malaria transmission. This study was conducted in Dielmo, Senegal, before the introduction of LLINs, Period 1 (P1), following the introduction of LLINs (P2) and two rounds of LLINs renewals (P3, P4). Mosquitoes were sampled indoors and outdoors using human landing catches. Variations in mosquitoes' biting rates and human exposure were analyzed using a General Linear Mixed Model with the response variable, the mosquito bites and the fixed factors being, the species, the month, the collection site and the periods (P1, P2, P3 or P4). Data analysis showed that the implementation of LLINs is correlated to a significant decrease in the biting densities of the main malaria vectors *An. gambiae* s.l and *An. funestus*. The bulk of bites occurred during sleeping hours, but the residual vector populations had a higher propensity to bite outdoors after the renewal of LLINs in P3 and P4. The shift of outdoor biting could be due to a phenotypic plasticity or the occurrence of several species which exhibits different behaviors in their responses to vector control tools. In this study, the relative protection provided by LLINs to malaria exposure in users relative to non-users (P*f) decreased from 63% in P1 to 45% in P4, due to the increased proportion of outdoor biting after the renewals of LLINs. In addition, the resurgence of malaria in villagers not using bed nets and staying outdoors showed the need to combine LLINs with complementary control measures against residual exposure to achieve the goal of eliminating malaria transmission.

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MODELLING ALLEE EFFECTS IN A TRANSGENIC MOSQUITO POPULATION DURING RANGE EXPANSION

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As mosquito populations expand their range, they may undergo mate-finding Allee effects such that their ability to successfully reproduce becomes difficult at low population density. With new technology, creating target specific gene modification may be a viable method for mosquito population control. We develop a mathematical model to investigate the effects of releasing transgenic mosquitoes into newly established, low-density mosquito populations. Our model consists of two life stages (aquatic and adults), which are divided into three genetically distinct groups: heterogeneous and homogeneous transgenic that cause female infertility and a homogeneous wild type. We perform analytical and numerical analyses on the equilibria to determine the level of saturation needed to eliminate mosquitoes in a given area. This model demonstrates the potential for a gene drive system to reduce the spread of invading mosquito populations. The impact of mosquito population size on the transmission of infectious diseases such as malaria and dengue has been well established. We use our model to determine how the basic reproductive number, R_0, will change with the introduction of transgenic populations during range expansion.

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GENOMIC AND MOLECULAR CHARACTERIZATION OF THE BACTERIAL POPULATIONS OBSERVED IN THE WATER OF BREEDING SITES AND IN LARVAE OF *ANOPHELES COLUZZII* AND *ANOPHELES GAMBIAE* IN NANGUILABOUGOU AND KOUROUBABOUGOU

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The microbiota composition of mosquitoes is influenced by their aquatic breeding environment. The exact factors that define the structure of the mosquitos' microbiota including malaria vectors are currently unknown. From June 2014 to November 2015 we conducted genomic and molecular characterization study of the bacterial populations observed in the breeding sites water and larvae of *Anopheles gambiae and An. coluzzii* the two majors vectors of malaria in Kouroubabougou and Nanguilabougou. The samples were collected from two breeding sites with different ecological settings. The inventory of the bacterial populations, was conducted to better understand their frequencies distribution in larvae and in their respective breeding sites water. Frequencies of the identified bacteria were associated with the allelic polymorphism of the *TEP-1* gene in *An. coluzzii* and *An. gambiae*. We collected 149 samples in which we identified 11 species, 49 genera and 82 strains. The bacterial species populations were composed of *Bacillus sp*. The species *Bacillus anthracis* and *Bacillus thuringiensis* were present only in *An. coluzzii* in Nanguilabougou and Kouroubabougou. *Bacillus cereus, Bacillus amyloliquefacins* and *Bacillus subtilus* were observed only in Nanguilabougou and in *An. coluzzii*. *Enterobacter cloacae* and *Enterobacter ludwigii* were present in *An. gambiae* with the *S1/S1* TEP1 genoptype in Nanguilabougou. *Bacillus cereus, Bacillus amyloliquefaciens* and *Bacillus subtilis* were more associated with the TEP1 genotype of *S1/S1* in *An. gambiae* in the village of Kouroubabougou. In this study we used the classical method of bacteria culture and the associated

molecular, genomics and bioinformatics tools to identify and characterize the bacterial populations. An optimization of the method for future studies would involve the metagenomics analysis approaches to increase the maximum size of identified bacteria species and strains. This will help to determine, the factors that might promote a specific microbiome associated with the population-dynamics of *An. coluzzii* and *An. gambiae* in Mali.

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THE IMPLEMENTATION AND THE IMPACT OF ADAPTING TECHNOLOGIES TO SUPPORT RESIDUAL MALARIA TRANSMISSION: A CASE STUDY IN UNGUJA, ZANZIBAR

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A recent project was carried out in Unguja, Zanzibar to understand mosquito and human behaviours that may allow malaria vectors to avoid contact with insecticide treated nets (ITNs) and indoor residual spraying (IRS) thereby maintaining malaria transmission. The project adapted a secured web-based applicationknown as Ifakara Entomology Bioinformatics System(IEBS) to ensure a collection of high quality mosquito data with capability to link mosquito and human behaviour data. The development of IEBS and its functionality has been described elsewhere, here, we present the implementation and the impact of IEBS in supporting a residual malaria transmission study in Zanzibar. The system was adapted to collect data in standardized formats, link field data with laboratory results, label samples properly, and provide quality data control. Also, to produce a cleaned data file with a data dictionary and meaningful reports containing key entomological indicators on time. The system has contributed to the successful completion of the project by overcoming data management challenges facing most entomological studies. High-quality data is available to address the project's objectives and key information such as species composition and their host preference, infection status, biting and resting behaviours are readily accessible in IEBS's dashboard. The information on where, how, and when each mosquito sample was collected including their storage information is also available in the system. In addition, IEBS's capability to link mosquito and human behaviours data to a household level that can also be referenced to existing malaria cases information is very important in addressing critical questions requiring datasets from these sources. A system such as IEBS can contribute toward periodic collection of quality mosquito-based data which is essential to inform vector control strategies in eliminating malaria transmission.

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FIRST REPORT ON EFFICACY OF REPELLENT-IMPREGNATED FOOTWEAR FOR PERSONAL PROTECTION AGAINST MALARIA MOSQUITOES

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Despite significant impact achieved by long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), these tools are insufficient for complete disruption of malaria transmission across endemic regions. This study investigated the hypothesis that commonly used foot-wear, if impregnated with effective repellents, could complement the gains achieved by the existing interventions. We developed low-cost sandals fitted with transfluthrin-impregnated hessian bands, and assessed their protective efficacy against outdoor-biting mosquitoes. Semi-field experiments were conducted with sandals affixed with hessian bands measuring 48cm^2 or 240cm^2, and either size treated with technical grade transfluthrin solution at 3%, 5% and 8%. Untreated sandals with similar hessian surface areas were used as controls. Mosquito landings on volunteers were compared to assess protective efficacy afforded by the treatments, relative to control. In semi-field tests, sandals fitted with 48cm^2 hessian bands impregnated with 8%, 5% and 3% transfluthrin reduced mosquito landings by 68.87%, Incidence rate ratio (IRR)=0.31 (95% C.I. 0.25-0.38), p<0.0001), 62.52%, IRR=0.37 (0.28-0.50), p<0.0001) and 22.95% IRR=0.77 (0.62-1.96), p=0.018) respectively. Sandals affixed 240cm^2 bands and treated with 8% transfluthrin reduced landings by 57.91%, IRR=0.42 (0.29-0.61, p<0.0001) while bands measuring 48cm^2 and treated with 8% transfluthrin reduced landings by 54.44%, IRR=0.45 (0.32-0.65, p<0.0001). In field experiments, the 48cm^2 band sandals treated with 8% transfluthrin conferred 77.63%, IRR=0.22 (0.14-0.37, p=<0.0001) and 68.32%, IRR=0.32 (0.25-0.41, p<0.0001) protection against all mosquitoes relative to controls. In conclusion, transfluthrin-impregnated footwear conferred significant protection against mosquito bites in semi-field and field settings. However, Various improvements, optimization and validation of this approach is required. Nonetheless, this approach could potentially constitute a complimentary tool against mosquito-borne diseases.

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CHARACTERIZATION OF OUTDOOR MOSQUITO BITING AND RESTING BEHAVIORS AS ONE OF THE DRIVERS OF ONGOING MALARIA TRANSMISSION IN ZANZIBAR

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Current vector control interventions, notably long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) have contributed significantly to the reduction in malaria burden. However, in certain settings, residual transmission persists, despite over 80% coverage with LLINs and IRS. We conducted a study in Unguja island of Zanzibar to determine the magnitude and drivers of residual malaria transmission by assessing when and where it occurs. Six sites from three districts with greater than 5/1000 annual parasite incidence and which received IRS in 2016 were selected by using a stratified random sampling approach. A total of 135 households were chosen ranging from 20-25 households per site and consented for the study. Mosquito vector surveillance was carried out indoors and outdoors from 6:00PM-7:00AM using human-baited double net (HDN) trapping, a standardized exposure-free method. Additional collections were done indoors using mouth aspirators to retrieve resting mosquitoes from wall and ceiling surfaces, and outdoors using resting bucket traps and pit traps. Out of 689 malaria vectors collected, PCR analysis shows that 98.4% were *Anopheles arabiensis*, 1% *Anopheles merus* and 0.6% *Anopheles gambiae s.s.* Sporozoite ELISA analysis indicates that all mosquitoes were negative for the malaria parasite. The results show more *An. arabiensis* were collected outdoors (~85%) compared to indoors (~15%) indicating a high proportion of outdoor biting, which could be one factor contributing to ongoing residual transmission in Zanzibar. Furthermore, results show that large numbers of *An. arabiensis* were caught in outdoor resting sites, where the pit trap (67.2%) collected more mosquitoes compared to the outdoor HDN trap (32.8%). Therefore, testing of novel techniques targeting outdoor biting and/or resting mosquitoes, is warranted in these settings. If found to be effective, these new approaches would complement the existing interventions against mosquitoes that bite and/or rest outdoors, hence contributing toward malaria elimination.

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FITNESS COST OF L119F-GSTE2 METABOLIC RESISTANCE ON THE LIFE TRAITS OF *ANOPHELES FUNESTUS* FIELD POPULATION FROM MIBELLON, CAMEROON

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Poor understanding of the fitness cost of resistance on mosquito field populations is a serious threat for implementation of successful resistance management strategies. To better inform resistance management, we evaluated the fitness cost of L119F-GSTe2 (first DNA-based molecular marker of metabolic resistance in *Anopheles funestus*) related to distinct aspects of development and reproduction of natural *An. funestus* s.s. populations. *An. funestus* s.s. field mosquitoes were collected in Mibellon (Cameroon). A cocktail polymerase chain reaction (PCR) was used for molecular identification and a new allele-specific PCR (AS-PCR) performed to genotype the L119F-GSTe2 mutation (associated with DDT and permethrin resistance). We compared the fecundity, fertility, larval and pupal developmental time and adult longevity as well of L119F-RR homozygous resistant mosquitoes to that of the L119F-RS heterozygotes and L119F-SS homozygous susceptible mosquitoes. In this *An. funestus* s.s. field population, L119F-SS mosquitoes had more chance to lay eggs compared to L119F-RS and L119F-RR, interestly, L119F-RR mosquitoes produced significantl lower number of eggs compared to L119F-RS and L119F-SS ($P = 0.003$) suggesting a fitness cost of L119F mutation on fecundity of females mosquitoes. Otherwise, assessment of the odd ratio for pupae formation showed that L119F-RS developed significantly faster than L119F-RR ($1.04< OR <5.26$; $0.0001<P<0.42$) and slightly faster than L119F-SS ($1.38< OR <1.39$; $0.03<P<0.08$). However, L119F-RR showed higher longevity compared to other genotypes ($2.2< OR<7.5$; IC 95%: 1.04 - 21.28; $0.001< P< 0.050$). These results suggest that L119F-GSTe2 mutation have a detrimental impact on some life-traits of *Anopheles funestus* field mosquitoes indicating that resistance management strategies such as insecticide rotation could help reverse the DDT/ pyrethroids resistance. However, increase longevity observed in resistant mosquitoes could be problematic if not well managed.

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INHIBITION OF *PLASMODIUM* INFECTION IN *ANOPHELES* MOSQUITOES BY GENETICALLY ENGINEERED *ASAIA SP.* BACTERIA

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Plasmodium sp., the parasite that causes malaria, is transmitted by *Anopheles sp.* mosquito vectors. The symbiotic bacteria within the mosquito midgut can be transgenically modified to affect the mosquito's phenotype, otherwise known as *paratransgenesis*; this strategy can be used to engineer the bacteria to secrete anti-*Plasmodial* effector molecules outside of the cell and into the mosquito midgut to combat the parasite. One such bacterial candidate is *Asaia sp.*, a Gram-negative, rod-shaped bacterium that has been shown to colonize the midgut, ovaries, and salivary glands within the *Anopheles* mosquito. However, common secretion signals, such as the *E. coli* Type II OmpA and TolB leader peptides, and signals from closely-related species do not function in *Asaia*. Also, a genetic library screen had found only one native secretion signal that provided sufficient secretion of protein into the supernatant. Therefore, the *Asaia sp. SF2.1* genome sequence was used to identify Type II secreted proteins, and further processed using SignalP4.1 to identify the leader signals. The top 20 of these signals were cloned into the plasmid pNB92, which contains a constitutive promotor and the C-terminal domain of *E. coli* alkaline phosphatase. Of these constructs, 13 were stable in the *Asaia sp. SF2.1* lab strain. Strains were grown overnight to log phase and separated into the supernatant, lysate, and cell surface fractions. Secretion of alkaline phosphatase was tested by ELISA and Western blot assays for the abundance of protein into the different fractions. Of the 13 signals, five mediated very efficient secretion. To ensure alkaline phosphatase is active when secreted, another multiwell plate assay using PNPP substrate was used. This is important because some of the anti-malarial effector molecules being used contain disulphide bonds, which are formed in the periplasm and are important for proper protein folding and function. Alkaline phosphatase was active when secreted with all five signals. *Asaia* strains with these signals expressing scorpine, a strong anti-*Plasmodial*, have shown significant oocyst reduction in *in vivo Plasmodium* inhibition experiments.

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PURSUING EFFECTIVE LLIN COVERAGE AND UTILIZATION IN NEW AREAS OF NORTHERN RAKHINE STATE, MYANMAR

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The PMI-supported Defeat Malaria project works with communities, partners and the National Malaria Program to optimize access to effective malaria interventions based on situational analyses and ongoing monitoring. Long lasting insecticidal nets (LLINs) continue to play a critical role in vector control for reducing malaria transmission. A baseline assessment to plan effective LLIN distribution in Northern Rakhine State (NRS) was conducted in June 2017. The assessment comprised of quantitative interviews with 422 household representatives selected by multi-stage sampling (random selection of villages, systematic sampling of households) from 6 new townships assisted by Defeat Malaria in NRS (Kyauktaw, Minbya, Myebon, Pauktaw, Ponnagyun, Sittwe). Bed-net data were collected at the household level using a bed-net characteristics assessment tool, with observation of available nets. Only 6.6% and 2.1% of respondents previously received malaria related messages about the importance of sleeping under LLINs and that mosquitos spread malaria, respectively. The survey revealed that 91% of households owned at least one LLIN, 62% owned at least one LLIN for every two persons, and 67% of household members slept under a LLIN the previous night. Of households possessing at least one bed-net, they comprised LLINs (78%), ordinary nets (21%), and ordinary nets treated with insecticide (<1%). Nearly 4% owning LLINs reported they washed them more than 5 times within the previous 3 months due to bedwetting of children. More than 90% reported LLINs were dried under the sun after washing. In Minbya Township, anecdotal reports showed that several people preferred ordinary nets to LLINs as the hole size of LLINs permits the entry of ticks. Distribution of LLINs should be coupled with appropriate informational materials and inter-personal communication promoting continuous and correct use by all household members. LLIN monitoring should be conducted through observation of available bed-nets with enquiry of quality aspects of LLIN and participatory learning with communities for better action to achieve and maintain LLIN coverage and utilization targets.

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STANDARDIZED MONITORING OF DURABILITY OF LONG-LASTING INSECTICIDAL NETS IN FIVE COUNTRIES IN AFRICA AND ASIA

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Following the 2014 WHO recommendation on measurement of durability of Long-lasting Insecticidal Nets (LLIN) the US President's Malaria Initiative (PMI) has developed a standardized protocol and tools to support countries to carry out monitoring of LLIN durability following their mass distributions (see www.durabilitymonitoring.org). The basic design is that of a representative cohort of 345 campaign LLINs per site which is established within six months after distribution and followed with annual assessment of attrition, net integrity, and insecticidal effectiveness for 36 months. Primary outcomes are the proportion of cohort nets surviving in serviceable condition at each time point and the median survival in serviceable condition in years. The USAID/PMI funded VectorWorks project is supporting five countries in durability monitoring with a total of 12 sites: Mozambique, Nigeria, Tanzania/Zanzibar, Democratic Republic of Congo (DRC) and Myanmar. In Mozambique (3 sites) and Nigeria (3 sites) the same or a very similar brand of LLIN is compared between areas with different environment and net use, care and repair behavior while in Zanzibar, DRC and Myanmar different LLIN brands are compared between two similar sites. To date, 5 sites have completed the 24 months and 7 sites the 12 months data collection and all LLIN brands have shown sufficient insecticidal effectiveness, i.e. >80% of samples passed the WHO criteria for optimal effectiveness. After 12 months the proportion of campaign LLIN surviving in serviceable condition varied between 69% and 98% corresponding to a median survival estimate between 1.5 and 5.6 years. The biggest differences were found in the comparisons of the same LLIN brand between different sites after 24 months: Nigeria 2.7 to 5.6 years and Mozambique 2.7 to 3.7. Among the comparisons of two different LLIN a significant difference was found in Zanzibar after 12 months (2.3 vs. 3.5 years, p=0.02) and DRC (1.5 vs. 2.4 years, p=0.0001) while in Myanmar the difference (3.5 vs. 4.2 years) was not statistically significant. Complete 24 month data and 36 months data from 5 sites will be available in September 2018.

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OXIDATIVE STRESS-INDUCED DIFFERENTIAL PACKAGING OF PROTEINS IN ENTEROTOXIGENIC *ESCHERICHIA COLI* OUTER MEMBRANE VESICLES AND ITS IMPACT ON HOST-PATHOGEN INTERACTIONS

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Enterotoxigenic *Escherichia coli* is the leading cause of traveler's diarrhea worldwide and it remains an important causal agent of diarrheal disease, especially among children of lower income countries. Because it must adjust to different ecological niches such as fruit and vegetable surfaces, water, and its animal or human host; its cell envelope must undergo remodeling. In Gram-negative bacteria, the cell envelope is the first barrier against environmental stress and studies have shown that the process of outer membrane vesiculation is essential for bacterial growth and survival during stressful conditions. Recent studies in our lab revealed that outer membrane vesicles (OMVs) can contain varying ratios of lipopolysaccharide (LPS) types during cell envelope remodeling under stress in *Salmonella enterica*, suggesting a role for vesicles in membrane remodeling. In this study, outer membrane vesicles and outer membrane fractions from ETEC were isolated from bacterial cultures, purified via density centrifugation, and submitted for proteomic analysis using quantitative one-dimensional liquid chromatography, tandem mass spectrometry (1D-LC-MS/MS) to quantify differences in protein expression in vesicles and membranes during oxidizing and non-oxidizing conditions (plus or minus Hydrogen Peroxide). Protein packaging into vesicles was determined by calculating the $\log_2$ of the ratio of protein expression in OMVs/outer membranes for both treatment conditions. Results indicated that two distinct sets of proteins were shown to be differentially packaged into OMVs as a function of peroxide treatment. Upon further analysis implementing a Bayesian hierarchical model, lipoproteins were observed to be preferentially exported during stress, in contrast to integral proteins, which were preferentially retained in the outer membrane. These results suggest possible roles for previously uncharacterized lipoproteins in the oxidative stress pathway and allow us to speculate about potential roles for OMVs in membrane remodeling of pathogenic bacteria undergoing environmental shifts both outside and inside the human host.

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BURDEN OF ACUTE GASTROINTESTINAL INFECTIONS IN OUAGADOUGOU, BURKINA FASO

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Gastrointestinal infections are one of the major health problems in developing countries. The present study aims to estimate the prevalence of gastrointestinal infections in Ouagadougou, the capital of Burkina Faso. A door-to-door survey of selected residents in Ouagadougou city was conducted. Of the Ouagadougou's 30 districts, nine most populated ones were selected to the study. The residents of these districts have middle incomes as those of the secondary cite of Burkina Faso. The overall prevalence of gastrointestinal infections in the 30 days prior to the interview was 77/491 (15.7%): among children 44/223 (19.7%) and among adults 33/268 (12.3%). Diarrhea and abdominal pain were the most common symptoms among 33 adult cases while diarrhea and vomiting were the most common among children. None of the cases were hospitalized and a stool sample was taken in three of 77 cases. Medication for gastrointestinal infections was received by 55% percent of adults and 77% of children. Our results shown that antibiotics with and without prescription were the most common medicine used. Washing hands before meals and boiling milk before drinking had a protective effect against gastrointestinal infections.

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A RANDOMIZED, OBSERVER-BLINDED, PHASE I STUDY TO ASSESS THE SAFETY AND IMMUNOGENICITY OF VI-DT CONJUGATE VACCINE COMPARED TO VI-POLYSACCHARIDE (TYPHIM VI®, SANOFI PASTEUR) TYPHOID VACCINE IN HEALTHY FILIPINO ADULTS AND CHILDREN

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As the currently licensed typhoid vaccines have limitations and cannot be administered in children less than 2 years of age, the International vaccine Institute developed a typhoid conjugate vaccine (Vi-Polysaccharide conjugated to diphtheria toxoid (Vi-DT)) which was transferred to SK Chemicals in Korea. After completing the preclinical study phase I clinical

trial was conducted in the Philippines. In this randomized, observer-blinded Phase I study to assess the safety and immunogenicity of Vi-DT compared to Vi-Polysaccharide vaccine, 2-45-year old participants (stratified into 3 age cohorts) were randomized to Test (Vi-DT) and Comparator (Vi-Polysaccharide) vaccine administered at 0 and 4 weeks. The objectives were to evaluate the safety and immunogenicity (anti-Vi IgG and serum bactericidal antibody, SBA) of 25 µg of Vi-DT comparatively to Vi-Polysaccharide (Typhim Vi®, Sanofi Pasteur) vaccine. 48 participants in each age cohort for a total of 144 participants were enrolled and randomized to Vi-DT vs. Comparator equally. No SAE was reported in either group. No subject was discontinued from the study due to AE. All solicited and unsolicited AEs were mild or moderate in both groups with the exception of a 4-year old girl in Test group with severe (grade 3) fever that resolved without sequela. All subjects (100%) in Test group showed seroconversion after 1st and 2nd doses while 97.1% and 97.2%, respectively in Comparator group. Vi-DT showed 4-fold higher GMT of anti-Vi IgG than Comparator. No further increase of GMT was observed after the 2nd dose. SBA seroconversion rate in Test group was higher than Comparator group after first dose (71% vs. 52.2%) and second dose (70.4% vs. 51.4%). SBA GMT showed similar pattern post first and second doses. Anti-DT responses post first dose in the test group were higher, 26-fold rise compared to baseline value, while a 0.93-rise in the Comparator group. The results of this first-in-human Phase I trial of Vi-DT show that the vaccine is safe, generally well-tolerated and immunogenic in participants aged 2-45 years. These results allow pursuing the clinical development of Vi-DT in children 6 to 23 months of age.

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"CHOLERA CONFERENCE": OUTBREAK DURING AN INTERNATIONAL SCIENTIFIC CONFERENCE, KENYA, 2017

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Cholera, pervasive in Kenya since 2015, has typically affected informal settlements and other low income communities. On 20 June 2017, officials were alerted to a suspected cholera outbreak among attendees of an international scientific conference with >400 participants, in Nairobi. The Kenya Ministry of Health investigated from June 21 to identify cause of the outbreak. We carried out a retrospective cohort study; a cohort member was any conference attendee. A suspected case had acute watery diarrhea (AWD) within 20-28 June 2017; confirmed case was culture-positive. We administered a structured questionnaire by telephone, email and online survey, collecting syndromic and exposure information. We calculated food-specific attack rates, risk ratios and in a nested case-control analysis (non-cases as controls) calculated adjusted odds ratios (aOR) to identify factors independently related to being a case. There were 456 registered participants: 88% from Nairobi County, 10% from other Kenyan Counties and 2% international. We interviewed 55% (249/456) of participants (70% by telephone, 28% online survey and 2% by email). Of those interviewed, 55% (137/249) had AWD; of these, 36% were hospitalized (median duration 3 days, range 2-11 days), 13% were culture-confirmed for cholera, 2 were international participants. Attack rates were 41% females, 50% males; 44% age 20-39 years and 50% ≥40 years. RDT positivity was 67% (8/12). Most cases presented with AWD (95%) and abdominal pain (73%). Cases peaked on conference day two, with a point-source pattern. Average incubation period was 35(11-59) hours. Eating chicken (aOR 2.49, 95% CI 1.22-5.06) on 20 June was associated with illness; drinking soda was protective (aOR 0.20, 95% CI 0.08-0.52). Chicken served over lunch on 20 June was the most probable source; however we were not able to identify exact contamination point. Improved food industry/health sector collaborations can allow trace-back during food-borne outbreaks for cholera and other pathogens.

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LONGITUDINAL ASSESSMENT OF ENTERIC PATHOGEN EXPOSURE IN MALAWI CHILDREN

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Enteric pathogens are the main cause of diarrhea infection although a high burden of enteric pathogens has also been reported in the absence of diarrhea. Diarrhea is the leading cause of morbidity and mortality in under five children. In Africa, about 440million cases and 350000 deaths are reported annually. Children in developing countries like Malawi are exposed to risk factors that predispose them to increased enteric infection burden. Sixty healthy children were recruited at 6months and followed up once every month until 18 months. Stool samples were collected every month. Stool samples were tested using Enteric TaqMan array card assay, which uses real-time polymerase chain reaction for the simultaneous detection of multiple enteropathogen. Analyses to determine pathogen exposure events at different time points were performed. A total of 1526 targets (grouped depending on pathogen) were detected from 442 samples tested from all time points. E. coli (EAEC, EPEC and ETEC) and Enterovirus constituted 60% of the positive targets. There was a very high rate of multiple pathogen detection per sample with 1.6%, 10.7% and 87% of samples having 0, 1 and >2 positive detection. Longitudinal analysis shows continuous exposure to E. coli and Enterovirus with no significant difference at different time points. Giardia infection was associated with children greater than 15 months while cryptosporidium was common in 7 to 12 months olds. A high rate of of individual variability was observed in pathogen detection at different time points. Malawian children are exposed to multiple pathogens very early in life. E. coli which may have adapted to the gut microbiota of Malawian children may not be clinically significant but need to be further studied for the immunological implication it may have in vaccine response and also its role in other enteric pathogen infections.

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GUT MICROBIOTA PROFILES OF CHILDREN BEFORE AND AFTER DRINKING CHLORINATED WATER

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Household members of cholera patients are at elevated risk of cholera infections during the first one week period than the general population as they live in close association sharing food and drinking water that are often found contaminated. We investigated the gut microbiota of household children before and after drinking chlorinated water, which was part of a randomized control trail of cholera hospital based intervention for 7 Days (CHoBI7), to see whether drinking chlorinated water had any effect on their intestinal microbiota. For this, bacterial community DNA was extracted from fecal samples of five children (age 3-7 yrs) living in urban slum of Dhaka, at day 0 (before intervention) and at day 8 of drinking chlorinated water. Illumina MiSeq sequencing of the microbial community DNA in stool samples revealed the presence of bacteria belonging to family Vibrionaceae at day0, but not at day 8. A total of 65 bacterial families were observed at day 0, which reduced to 60 with 6 families that were unique at day 8. Eleven families were unique at day 0 of which 6 belonged to the phylum Proteobacteria that included mostly pathogenic bacteria, and 5 of these families belonging to Proteobacteria were lost due to drinking chlorinated water, as observed at day 8. None of these five children developed cholera or any other diarrheal illness during this most risky one week period. While the phyla Bacteroidetes,

Firmicutes, Proteobacteria and Actinobacteria were predominant with the relative abundance of (mean ± SEM %) 62 ± 6, 32 ± 7, 4 ± 1 and 1 ± 0.7 at day 0, respectively, the proportion changed at day 8, when bacteria belonging to the phylum Firmicutes increased [49% ± 12 (p=0.057)] over Bacteroidetes [39% ± 12 (p=0.034)] as observed for all five children at day 8. Furthermore, the proportion of beneficial Firmicutes belonging to the family Lachnospiraceae, Bifidobacteriaceae, Clostridiaceae, Ruminococcaceae, increased due to chlorination of drinking water. The observed decrease of harmful Proteobacteria and increase of beneficial Firmicutes suggest chlorination to be a means to prevent environmental enteropathy-related gut microbiota dysbiosis and ill health.

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CLINICAL PREDICTORS OF BACTERIAL DIARRHEA AMONG INTERNATIONAL TRAVELERS TO NEPAL

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Acute diarrhea is a common illness among international travelers, affecting approximately 20% of individuals traveling to at-risk countries and contributing to approximately one-third of post-travel medical visits. Clinicians and travelers have limited tools to differentiate bacterial from non-bacterial causes of traveler's diarrhea (TD). Therefore, TD is typically treated empirically with antibiotics based on symptom severity, regardless of etiology. Development of a clinical prediction rule to assess the etiology of TD may aid clinicians in correctly identifying episodes of bacterial diarrhea as well as limiting inappropriate use of antibiotics. We collected de-identified clinical data from 284 international travelers who presented with acute diarrhea to the CIWEC Travel Medicine Center in Kathmandu, Nepal. Both conventional and molecular methods were used to determine diarrheal etiology from stool samples. Logistic regression and machine learning algorithms were used to identify predictors of bacterial diarrhea based on clinical and demographic variables. Categories of variables included age, gender, nationality, purpose of travel, presenting symptoms, symptom duration, temperature, stool grade, and stool microscopy results. Diarrheal etiologies among the 284 cases were identified as follows: 98 bacterial, 51 viral, 73 mixed pathogens, 6 protozoal/parasite, 55 no pathogen detected, and one unknown. Random forest and logistic regression analyses indicated that the strongest predictors of bacterial over viral or non-detected etiologies included red blood cells noted on stool microscopy, a non-Asian country of origin, temperature greater than 38°C, number of diarrheal episodes in the last 8 hours, and mucous noted on stool microscopy. Our preliminary analysis demonstrated multiple promising variables for the clinical prediction of bacterial diarrhea. By the time of presentation, internal cross-validation of a clinical prediction rule and external validation against an independent data set will be completed.

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DETECTION OF *TROPHERYMA WHIPPLEI* IN CAMBODIA FROM PATHOGEN-NEGATIVE STOOL SAMPLES

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Tropheryma whipplei is a rod-shape Gram-positive bacterium responsible for a disease called Whipple's Disease (WD). There are five common clinical features of WD including classic, localized, acute, acute asymptomatic, and opportunistic (associated with immunosuppression) infections. The most prominent symptom of WD is gastroenteritis, but endocarditis, encephalitis, pneumonia, and bacteremia may also occur. Diarrhea, abdominal pain, and steatorrhea are among the most frequently observed symptoms in classic WD. The majority of WD patients are male Caucasians, although this disease rarely occurs in Asian and African patients, this population may be carriers. There has only been one report of *T. whipplei* in Southeast Asia (SEA) from stool samples of healthy Laotian kindergarteners. *T. whipplei*, however, was detected in saliva, urine, blood, stool, and lymph nodes, etc. Detection of *T. whipplei* was previously based on two rounds of real-time PCR. In this study, a duplex real-time PCR detection was developed and optimized for the detection of *T. whipplei* and applied to a set of pathogen-negative stool samples previously negative for any enteric pathogens routinely tested. Samples positive for *T. whipplei* were further confirmed by sequencing of PCR products. Preliminary, 88 pathogen-negative cases and 83 controls stool samples from a diarrhea surveillance study among local population in Battambang, Cambodia, which included children aged 3 months to 5 years and active military personnel aged 18-60 years, were selected for testing. Two cases (one adult and one child) and 3 controls (all children) were positive for *T. whipplei*. This accounts for 3% detection. Detection efforts are ongoing and additional samples will be tested. These results add to the general epidemiology of *T. whipplei* in the SEA region, specifically in Cambodia, in addition to those previously reported in Laos.

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IMPACT OF ROTAVIRUS VACCINATION ON DIARRHEA RATES ACROSS PROVINCES OF PERU WITH VARYING PIPED WATER ACCESS, PIPED SEWER CONNECTION ACCESS, AND POVERTY LEVELS

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Peru has undergone rapid economic growth over the past decade, accompanied by declines in poverty and increases in access to piped water and sewer connections. Rotavirus vaccination was added to the national immunization program in 2009. Little is known about the impact of recent health/infrastructure developments on diarrhea rates in Peru. We analyzed spatially detailed data on clinic visits for diarrhea from 2005-2015 and on infant rotavirus vaccinations provided by The Peruvian Ministry of Health. Household data on access to piped water/sewer connections and poverty levels were obtained from the Peruvian National Institute of Statistics and Informatics. We examined rates of childhood diarrhea in the provinces of Peru by fitting three Poisson mixed models to investigate the impact of rotavirus vaccination on diarrhea rates, controlling separately for access to piped water, access to a sewer connection, and the percent of the population living in poverty. The rate of clinic visits for diarrhea in children under 5 years old in Peru decreased by approximately one third from 2005 to 2015. Across all models, there was a significantly lower rate of childhood diarrhea when provinces were vaccinating at least 50% of infants, controlling for each covariable of interest and accounting for long-term secular trends. Diarrhea reductions were similar to those published for other countries in Latin America after rotavirus vaccine introduction. This is the first nationwide analysis of the impact of rotavirus vaccination on diarrhea in Peru; our approach is unique in accounting for other factors that may contribute to long-term diarrhea trend.

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SUSCEPTIBILITY TO SYMPTOMATIC ENTEROTOXIGENIC *ESCHERICHIA COLI* INFECTIONS IN NON-SECRETOR NICARAGUAN CHILDREN

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Enterotoxigenic *Escherichia coli* (ETEC) is an important causative agent of Diarrhea in Children and adults from all over the world, including

Nicaragua. A study conducted in Bangladesh have suggested that children with Lewis blood group "a" antigen (Le^a) have more often symptomatic than asymptomatic Enterotoxigenic *Escherichia coli* (ETEC) infections (*P<0.001*). Furthermore, another study carried out with the same population showed that two non-synonymous *FUT2* single nucleotide polymorphisms (rs200157007-TT and rs601338-AA) are associated with symptomatic but not asymptomatic ETEC infection irrespective of the child's Lewis secretor status. Based on the above observations, we conducted the present study in order to investigate if a Non Secretor Status makes also Nicaraguan children more susceptible to symptomatic ETEC infections. A total of 234 children ≤5 years of age (91 symptomatic and 143 asymptomatic) participated in a community- and hospital-based study of acute diarrhea in León, Nicaragua, during 2014–2017. In brief, clinical cases were evaluated according to the World Health Organization strategy for diarrhea management and fecal and saliva samples were collected from each child. PCR was used to detect the ETEC pathotype. The Lewis phenotype and Secretor status was determined by an ELISA based assay. In general ETEC was detected in 14.1% (33/234) of the children, with14.3% ETEC positive cases in the symptomatic and 14.0% of the asymptomatic children. We found no association between the Lewis phenotype and symptomatic ETEC infection. However, we could see a higher proportion of symptomatic than asymptomatic ETEC infections in Non-secretors Children (23.1% symptomatic and 12.0% asymptomatic), and similar proportions of ETEC infections in Secretors children (12.5% symptomatic and 14.7% asymptomatic). In conclusions, our results also suggest that a Non secretor status is a host feature affecting susceptibility to ETEC infection and have implications in the current vaccine efforts.

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PREVALENCE AND PHENOTYPES OF ANTIBIOTIC RESISTANCE IN *E. COLI* ISOLATED FROM THE MAL-ED BIRTH COHORT STUDY IN RURAL TANZANIA

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The emergence and spread of antimicrobial resistance is a serious global public health crisis. Drug-resistant gram-negative bacteria, like *Escherichia coli*, are particularly concerning given their significant morbidity and mortality. Despite the increasing prevalence of drug-resistant gram-negative bacteria worldwide, there are significant knowledge gaps in low resource countries. We aimed to characterize the prevalence and phenotypes of drug-resistant *E. coli* carriage in children up to age 5 from stool collected in the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) birth cohort study in rural Tanzania. 262 children were enrolled in the MAL-ED Tanzania site. We randomly selected 100 children who had *E. coli* specimens archived every 6 months through 60 months. Up to 5 lactose-fermenting colonies were selected from growth on MacConkey agar. Drug susceptibility testing of 18 antibiotics was performed by disk diffusion. CLSI interpretive criteria were used for determination of resistance. Multi-drug resistance (MDR) was defined as non-susceptible to at least 1 antibiotic in at least 3 antimicrobial categories. 823 *E. coli* specimens were available for testing. Antibiotic resistance was identified in some samples for all drugs except ertapenem, which was 100% susceptible. The highest rate of resistance was for ampicillin (93%) followed by cefazolin (92%) and cotrimoxazole (90%). 15 (1.8%) specimens met criteria for extended-spectrum beta-lactamase (ESBL) based on combination disk testing with cefotaxime/cefotaxime-clavulanate and ceftazidime/ceftazidime-clavulanate. 696 (84.6%) specimens met criteria for MDR. In conclusion, while ESBL *E. coli* carriage appears to be rare in this community, the prevalence of MDR *E. coli* carriage is high. Drug-resistance is common at 6 months of age.

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AR-12 AND SILDENAFIL COMBINATION THERAPY FOR THE TREATMENT OF INTRACELLULAR *S. TYPHIMURIUM* INFECTIONS

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Salmonella enterica is a gram-negative bacterium and a major contributor to bacterial foodborne illness which, according to The World Health Organization, infects over 550 million people worldwide and results in 230,000 deaths each year. Importantly, multi-drug resistant (MDR) strains of *S. enterica* are continually increasing, sparking significant health concerns globally. To overcome antibiotic-resistance, host-directed therapies, which act on the host cell rather than on the pathogen, have been developed to circumvent resistance mechanisms by reducing or eliminating selective pressure on the pathogen. AR-12 (OSU-03012), a celecoxib derivative that has completed Phase I clinical trials for its anti-tumor activity, has been identified as a host-directed therapy against a number of intracellular pathogens. Previous work in our lab has shown that AR-12 reduces *S. enterica* serovar Typhimurium bacterial burden *in vitro* and *in vivo*; however, AR-12 is toxic to the host cell, resulting in a narrow therapeutic window. Furthermore, it has been reported that treatment with AR-12 in combination with FDA-approved Sildenafil, indicated for treatment of erectile dysfunction and pulmonary arterial hypertension, results in decreased bacterial viability compared to AR-12 treatment alone in various extracellular bacteria strains. In the current study, we evaluated the anti-infective properties of AR-12 in combination with Sildenafil as a potential host-directed therapy against intracellular S. Typhimurium infection. A synergistic response was observed following treatment of sub-optimal doses of AR-12 and Sildenafil to clear intracellular *S.* Typhimurium infections *in vitro*, as defined by the Chou-Talalay Combination Index. These results suggest that a combination of two host-directed therapeutics is a promising approach in the treatment of intracellular *S.* Typhimurium by mitigating cellular toxicity associated with treatment by therapeutic doses of AR-12 alone. Furthermore, this treatment strategy may prevent the emergence of resistant strains by reducing selective pressure on the pathogen.

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EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *KLEBSIELLA PNEUMONIAE* BACTERAEMIA AND REDUCED SUSCEPTIBILITY TO CARBAPENEM IN LAGOS, NIGERIA

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Klebsiella. pneumoniae exhibiting extended spectrum beta -lactamase (ESBL) production is increasing worldwide and mechanism is associated with the rapid dissemination of the genes under the selective pressure of antibiotic. Carbapenem usage has increased in clinical practice as a result of expanding resistance to other β-lactam antibiotics but its use in Nigeria is usually reserved for live threatening gram negative infections. The observed treatment failures associated with *Klebsiella*-associated bacteremia in recent times necessitated the investigation of the burden of ESBL producing *K. pneumoniae* and carbapenem resistant strains. A total of 127 patients with pyrexia of unknown origin attending referral public hospitals between April and September 2015 were recruited. Samples were cultured, isolates were identified and antimicrobial susceptibility test was performed on all the isolates .Detection of carbapenemase enzyme activity was carried out. Isolates were screened for ESBL production by the Double Disc Synergy test (DDST).Three gene makers; *bla* CTX-M1, *bla*SHV and *bla*TEM were used to characterize ESBL. Clonal relatedness

of the isolates was assessed by Random Amplified Polymorphism DNA (RAPD)- PCR typing technique. Specifically, 34% (43/127) *K. pneumoniae* bacteremia was identified. The overall prevalence of, ESBL and carbapenem resistant was 69.8% and 7.0% respectively. Of the ESBL producing isolates, 14.7% contained either of *bla*CTX-M1 or *bla*SHV gene makers. None of the three markers were detected in carbapenem resistant isolates. Four distinct RAPD types were exhibited by test isolates. There was no correlation between antibiotic resistance phenotype and RAPD fingerprints. This study revealed circulation of ESBL producing bloodstream *K. pneumoniae* and strains carrying *bla*CTX-M-1 and *bla*SHV genes in our environment. The observed emerging carbapenem resistance strains potent an early warning signal for the prudent use carbapenem antibiotic to prevent wide spread resistant. Further studies on the application of modern molecular techniques to assess the diversity and clonal relatedness of strains are in sight.

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ESTABLISHING NEONATAL AND PEDIATRIC INTENSIVE CARE UNITS, OUTREACH EDUCATION TO LOCAL HEALTH FACILITIES, AND IMPLEMENTATION OF NEONATAL RESUSCITATION PROGRAM: IMPACT ON NEONATAL AND CHILD MORTALITY & MORBIDITY: MENDEFERA REGIONAL REFERRAL HOSPITAL, SOUTHERN REGION, ERITREA, A 6 YEARS EXPERIENCE

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The United Nations' Millennium Development Goal 4 was to reduce the global under-five mortality rate by two-thirds by 2015. While Eritrea did achieve this goal, disparities between rural and urban areas remain with regard to infant and child survival. Mendefera Regional Referral Hospital serves a population of 800,000, receives referral from 65 different level health facilities. It is where interns, midwives and nurses do their rotations. With only one pediatrician to oversee the pediatric care of the hospital and the southern region and after conducting outreach education program to referring health facilities, establishing neonatal & pediatric intensive care units and Implementing " Helping Babies Breathe"(HBB) program; Decreased in-hospital stay (from 5.4 days in 2009 to 3.3 days in 2016), drop in hospital admission rate (from 1846 in 2009 to 440 in 2015), less referral-outs to regional hospital from local health facilities (from 614 in 2009 to 113 in 2015) were seen. Deaths related to pneumonia, gastroenteritis and malnutrition deceased with overall child mortality going down (from 92 deaths in 2009 to 20 in 2016). Neonatal mortality decreased by 51% (term by 45% & Preterm by 55% from 2012 to 2016), as well as incidents of perinatal asphyxia (by 45%) and meconium aspiration syndrome (by 70%). Overall, neonatal mortality decreased (by 40%) in the region. Establishing hospital systems and regional health activities that prioritize the neonate and the critically ill child with a reliable standardized protocol that strengthens local health facilities results in significant decrement in neonatal/child morbidity and mortality. It is not resource intensive. High-tech machines are not needed to improve pediatric care in Eritrea. Most of the childhood killer diseases can be managed using basic caring units (NICU & PICUs) and regular training of the staff. Intensive care unit is not a luxury in an African set up but a necessity and is feasible, cost effective produces highly motivated staff with improved skill of management. Conducting basic neonatal resuscitation program brings about significant change.

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RISK FACTORS FOR DIPHTHERIA DURING THE OUTBREAK IN INDONESIA

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Diphtheria outbreak in East Java Indonesia has been noted since 2011. Most of the patients were children. Some risk factors were presumed as having the influential contribution. The objective of this study was to evaluate the risk factors for diphtheria in children during the East Java outbreak 2011-2015 All children aged under 18 years who were recorded during the outbreak with proven culture results of toxigenic *C. diphtheriae* were recruited as participants. The control group was healthy children that have close contact with diphtheria cases but with negative culture results. All participants were reviewed, visited, interviewed, and underwent physical examinations. Immunization cards were checked. The house and surrounding environment were observed. Pearson chi-square and logistic regression tests were used ($p<0.05$ considered as significant). There were 97 patients and 194 controls in the study, mostly lived in the northern and eastern part of the province (86.6%). Most parental education was the elementary school. Incomplete primary diphtheria immunization was significantly found in the case group (68.0 vs 44.3%, $p<0.001$). Most parents did not have sufficient knowledge about diphtheria and its prevention (83.5 vs 73.2%, $p=0.005$). Significant risk factors in multivariate analysis were incomplete primary immunization, contacts with seasonal workers, and pesantren (traditional school) dormitory inhabitants. Significant risk factors for incomplete primary diphtheria immunization was the inadequacy of parental knowledge (OR 2.756; 95% CI 1.551-4.897). As the conclusions, the significant risk factors for this outbreak were incomplete primary diphtheria immunization, contact with seasonal workers, and traditional school dormitory status. This data and result finding were not available in this area previously and would become the basis for better outbreak management.

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THE THERAPEUTIC POTENTIAL OF ANTIBIOTICS AND VITAMIN A IN TREATING MULTIDRUG RESISTANT INVASIVE NON-TYPHOIDAL *SALMONELLA* INFECTION

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There are approximately 3.4 million cases and 680,000 deaths globally each year due to invasive non-typhoidal *Salmonella* (iNTS) infection, a severe septic manifestation of a common cause of gastroenteritis. Children in sub-Saharan Africa are disproportionately affected, as the risk factors for iNTS include young age, malnutrition and concurrent malaria infection. The mortality rate from iNTS is 20-25% and this is in part due to the emergence of multidrug resistant strains. The *Salmonella* clinical isolate D23580 has an integron that confers resistance to five different antibiotics and is more prone to lead to invasive disease. Better therapeutics are needed to alleviate iNTS disease in vulnerable populations. Vitamin A is an important micronutrient in immune cell development and has been successfully used to prevent diarrhea when given prophylactically in global health interventions. However, there is yet to be a study assessing vitamin A as treatment with antibiotics once a patient presents to the hospital with iNTS. Our study seeks to assess the therapeutic potential of vitamin A with antibiotics in a mouse model of iNTS, utilizing the multidrug resistant clinical isolate D23580. Our results will inform the efficacy of a clinical trial

assessing routine care of antibiotics alone to co-treatment of antibiotics with vitamin A with the goals of decreasing mortality rate and improving human health outcomes globally.

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PASTEURELLA MULTOCIDA INFECTIONS IN DOG OWNERS WITH OPEN WOUNDS

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Pasteurella multocida (PM) infections in humans usually result from cat or dog bites. PM is a gram-negative coccobacillus, which is part of normal flora in the upper respiratory tract of mammals.Rare infections without bites have been reported. PM infections could be life threatening. Serious complications include septic arthritis, tenosynovitis, osteomyelitis, bacteremia, meningitis, brain abscess, spontaneous bacterial peritonitis and brain abscess. We present the cases of two dog owners seen within the last 6 months, with unsuspected infections caused by PM. The first patient, owner of three dogs with a small toe ulcer and a chronic shin ulcer presented with sepsis: Fever (T 101.2F}, chills, tachycardia (101/bpm), Leukocytosis (WBC 18,900 with 14% bands). The patient had a history of prior wound infections with MRSA, MSSA and *Enterobacter.* Based on prior history, he was treated with meropenem and vancomycin. Blood cultures on second day grew gram-negative bacilli that were later identified as PM. The patient was penicillin allergic. His isolate was susceptible to doxycycline. The patient was treated with doxycycline. He recalled one of the dogs licking the toe ulcer. The second patient with a small submetatarsal ulcer of the right hallux and diabetes mellitus type II, presented with a diabetic foot infection. The wound cultures grew PM and MSSA. She owned 2 dogs.She admitted to walking bare-footed at home and suspected "dog drool" on the floor may have contaminated her ulcer. She was treated with intravenous ceftriaxone. The take home message is that dog owners with open wounds must protect their wounds to prevent contamination with dog saliva. In dog owners, especially with open wounds, possibility of PM infections should be considered. The organism in both cases was susceptible to penicillin, ceftriaxone and doxycycline. Some PM infections are life threatening and these cases should serve as a warning to dog owners.

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INCREASING ELECTIVE *HAEMOPHILUS INFLUENZA* TYPE B VACCINE COVERAGE IN THAILAND

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Thailand is one of only three countries not to include the *Haemophilus influenza* type b (Hib) vaccine as part of the childhood vaccination program. Few reports have been published on Hib vaccine coverage in Thailand. We compared patient demographics and medical history to identify factors associated with elective Hib vaccination. Our study included pediatric patients (age < 18 years) hospitalized with influenza-like illness at a public hospital in Bangkok, Thailand between 2009 and 2015. Records from 139 children were included in the analysis. A retrospective review was conducted to analyze characteristics associated with increased likelihood of Hib vaccination. 46 patients (33%) had received at least 1 dose of Hib vaccine. Females composed 44.6% of the total population, and 47.8% of the vaccinated population. Patients who received PCV vaccination were more likely (OR 16.1; 95% CI, 3.4-75.5) to receive Hib vaccination, as were patients who had ever received influenza vaccination (OR 6.1; 95% CI, 2.6-14.4). A significant trend towards increased vaccine coverage over time was found. Children born between years 1994-1998, 1999-2003, 2004-2008, 2009-2013, and 2014-2015 had 6.6%, 25.5%, 39.6%, and 60% Hib coverage respectively (p=0.004). No significant difference in vaccine coverage was found between public and private insurance. Children from outside of Bangkok were equally likely to be vaccinated as those from the Bangkok-metro area. Patients with asthma, neurological disease, metabolic disorders, or developmental delay showed no increased likelihood of vaccination. Hib is not part of the childhood vaccine schedule in Thailand, and recipients must pay out of pocket. We report an increasing trend towards voluntary vaccination over time, as well as increased likelihood of receiving other elective vaccines not included in the national vaccine program, such as PCV and influenza. This trend was consistent across all insurance types, cash payees, medical comorbidities, and place of residence. The results suggest increased interest in elective vaccines to prevent childhood illness across all socioeconomic status despite financial burden.

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GEOGRAPHICAL DISTRIBUTION AND ASSOCIATED SEROVARS OF LEPTOSPIROSIS IN INDIA

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Leptospirosis is prevalent worldwide and endemic in India. Outbreaks of leptospirosis have been reported from the coastal regions of India causing significant morbidity and mortality. However, the available data are mostly from tertiary health-care settings. In 2014, as part of Global Health Security Agenda, we initiated hospital-based acute febrile illness (AFI) surveillance in 32 district/sub-district hospitals in 10 states of India. We defined AFI as documented or reported fever <15 days duration; we enrolled and collected demographic, clinical and epidemiological data using a questionnaire, along with blood samples from all eligible admitted AFI cases. A positive real-time PCR and/or a positive anti-leptospira IgM ELISA in serum with an index value of ≥1.5 was considered positive for leptospirosis. A randomly selected sample of 100 IgM ELISA positive samples were subjected to microscopic agglutination test. We enrolled 27,431 AFI patients from June 2014 to July 2017; of these, 14,134 (51.5%) were positive for any pathogen. The major etiologies were influenza (16.6%), dengue (9.7%), scrub typhus (8.6%), leptospirosis (8.1%), malaria (4.7%) and KFD (2.2%). Percentage positivity of leptospirosis varied by state from 3.1 - 14.5 and was highest in Tripura (14.5%), Kerala (13%), Assam (9.9%), and Karnataka (9.2%) The median age of cases was 34 years (IQR: 22, 45); 53% were male. Predominant clinical symptoms were headache (84.44%), vomiting (36.23%), abdominal pain (30.72%), and redeye (10.49%). A significant proportion of cases were icteric leptospirosis (60%). Major leptospira serovars identified included bratislava, autumnalis, georgia, mankarso, pomona, pyrogenes, canicola and alexi. While 63 (3.1%) cases were referred to higher centers, only two cases died. AFI surveillance has identified leptospirosis as the fourth most common pathogen causing AFI in India and has revealed wide geographic spread. This study underscores the importance of introducing routine diagnostic testing for leptospirosis at sub district and district level hospitals for rapid case detection and appropriate clinical management to reduce morbidity.

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WITHIN-HOST MODELING OF *SALMONELLA TYPHIMURIUM* GROWTH DYNAMICS USING TIMER DATA

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Fluorescence-based assays enable the measurement of biological parameters at the resolution of a single cell, marking an advance beyond previous, population-level techniques to study within-host bacterial infection. One such method, known as TIMER, uses the ratio of two fluorophores to measure the rate of most recent division in *S.* Typhimurium infection, providing insight into the role of division rate heterogeneity in bacterial persistence. Nevertheless, quantifying the contribution of heterogeneity in the biological and assay parameters to the measured fluorescence intensities is necessary for proper inference of within-host dynamics. To provide such a capability, we developed an individual-based model that infers biological and assay parameters using Approximate Bayesian computation with Sequential Monte Carlo simulation. When applied to simulated data, our model infers these parameters with a high level of accuracy and appropriate levels of uncertainty. Moreover, variance-based global sensitivity analysis suggests that TIMER can measure differences in the rates of most recent division, though variability in assay parameters may confound these estimates. Finally, applying our approach to experimental data, we evaluate three hypotheses regarding the relationship between heterogeneity in division rates and the emergence of tolerance during antibiotic treatment: (i) the level of antibiotic tolerance correlates with the division rate, (ii) the existence of a non-dividing subpopulation with high antibiotic tolerance drives persistence, and (iii) tolerance emerges through an alternative, growth-independent mechanism.

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CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF DIPHTHERIA: A PRIMER FOR THE MODERN WORLD

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Diphtheria, once a major cause of childhood morbidity and mortality, has all but disappeared as a public health threat since the discovery and widespread use of diphtheria toxoid vaccine. However, recent outbreaks such as those in Venezuela and Yemen, and among Rohingya refugees, highlight the risk from this disease when unrest, infrastructure failure, or marginalization interrupt routine vaccination services and healthcare access. Diphtheria has been rarely studied for nearly a century, leading to gaps in knowledge about its transmission and control. Misconceptions about the epidemiology of diphtheria, including the impact of vaccination and treatment, can hamper control efforts. We reviewed 82 published studies of diphtheria and re-analyzed their data to characterize key aspects of the natural history and transmission of diphtheria and the implications for control. We estimate that diphtheria has a mean incubation period of 1.7 (95% CI, 1.0-3.0) days, and those developing symptoms remain infectious for 17 (95% CI, 16-18) days. In an unvaccinated population, the reproductive number for symptomatic diphtheria is 1.4-4.0. Asymptomatic cases remain colonized for an average of 18 (95% CI, 13-25) days and infect 40% as many people as symptomatic ones. Three doses of DTP vaccine are 82% (95% CI, 77-86) effective against symptomatic disease, but vaccinated individuals can remain colonized and transmit *Corynebacterium diphtheriae*. Although protection from vaccination is incomplete, given the range of reproductive numbers, vaccination coverage of 56-92% with three doses should be adequate to eliminate transmission. Given the mechanisms of vaccine protection, antibiotics are critical to reduce the infectious reservoir and control outbreaks. Detection and treatment of approximately three-quarters of all asymptomatic cases is important for rapid containment.

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PROGRESS TOWARD GLOBAL ADOPTION OF THE WORLD HEALTH ORGANIZATION STANDARDIZED ULTRASOUND CLASSIFICATION OF CYSTIC ECHINOCOCCOSIS

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Cystic echinococcosis (CE) is a parasitic zoonosis for which ultrasound (US) is the gold standard modality for diagnosis. In 2003 the WHO published a standardized US classification of CE, which became the basis for WHO treatment guidelines. Use of a standardized classification also facilitates global comparisons of treatment outcomes and epidemiological studies. In 2014, however, adoption of the classification was called into question by a publication indicating that, between 2004 and 2014, only half of studies utilizing a classification used the WHO classification. More recent studies have demonstrated that the WHO classification best reflects the natural history of CE and is used with high reliability by experts in the field; despite these attributes, the classification's impact is ultimately limited by the extent of its adoption. A PubMed search using the terms "Echinococcus granulosus ultrasound," "Echinococcus granulosus classification," "cystic echinococcosis ultrasound," and "cystic echinococcosus classification" revealed publications on human CE using a US classification (as identified in an available abstract or full text). Classification(s) used, year of publication and country of first author's institution were recorded. From 2004 to 2010, the WHO classification was used in 50% or fewer of included publications for 6 of the 7 years. After 2011, it appeared in a low of 75% (2013) to a high of 96% (2017) of included publications. Of all included studies published from 2004 to 2017, the WHO classification was referenced in 18% (3/17) from Africa, 64% (32/50) from Asia, 79% (89/113) from Europe, 89% (8/9) from North America and 100% (9/9) from South America. These findings suggest that the WHO classification has been progressively taking preference internationally as the US classification of choice for staging CE. Continued use of the classification developed by Dr. Hassen Gharbi of Tunisia in 1982, used widely prior to development of the WHO classification (which reversed two stages in order to more closely reflect the natural history of CE), suggests that adoption of a new classification takes time and varies regionally.

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HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS - A CROSS-SECTIONAL STUDY

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The Alveolar echinococcosis (AE) is a rare zoonosis caused by the parasite Echinococcus multilocularis. To date, nothing is known about the health-related quality of life (HRQoL) in patients with AE. The aim of the study was to evaluate the HRQoL in patients with AE in comparison of the healthy population. We used the SF-36 questionnaire to evaluate the HRQoL in patients with AE. The SF-36 scale scores were obtained according to the developers algorithms. SAS Version 9.2 was used for the statistical analysis of AE-cases (n=30) and the healthy control group (n=35). The analysis showed that the HRQoL in people with AE is reduced in comparison with the control population. The study group consisted of 15 (50,0%) men and 15 (50,0%) women; the control group of 16 (45.7%) men and 19 (54.3%) women. The mean age of the patients was 55.73±16.65 years, while that of the control group was 54.57±15.34 years. The physical quality of life in patients with AE (45.21±11.42) was not significantly less than that of the control group (50.54±10.52); p=0.0568. Nevertheless, AE-patients show lower SF-36 scores for the physical quality of life. For the mental quality of life, patients with AE had a significantly lower score (45.46±10.57) than the control group (51.57±9.04); p=0.0154. The HRQoL in people with AE is reduced in

comparison with a control population. Assessment of the physical and mental quality of life in patients with AE may help to evaluate the patient outcome.

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CHARACTERIZATION OF THE HU DENSITY OF CYSTOID LESIONS OR CYSTOID AREAS WITHIN LESIONS DUE TO HEPATIC ALVEOLAR ECHINOCOCCOSIS USING THE EMUC-CT CLASSIFICATION

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The EMUC-CT classification for the diagnosis of hepatic alveolar echinococcosis describes cystoid portions as a subcriteria for primary morphology Type I and II. Type IIIa/b are "Primarily cystoid" lesions and Type IV lesions are designated as "Small cystoid/metastasis-like" ones. Aim of the study was, to evaluate CT-scans regarding the density of cystoid lesions or cystoid portions of AE-lesions classified after EMUC-CT by measuring the Hounsfield Units (HU). This shall help to differentiate such regions against normal cysts and may show differences between the types concerning their cystoid structures. The HU density of cystoid areas of n=170 AE-lesions of different patients classified after EMUC-CT was measured. Patients were chosen who had not yet or had just shortly been treated by benzimidazoles. Measurements were performed by two raters independently within the same transversal section of a CT-scan showing cystoid regions. The measurements were performed in two different places which the raters subjectively regarded as to be the most hypodense ones by using a defined sized Region of Interest (ROI). The statistical analysis was performed using SAS V.9.2. HU determination of all cystoid areas, evaluated in Type I-IV lesions showed a mean value of 23,13±16,83 and thus differed clearly from normal cysts (HU~0). HU values of Type I, II and IIIa/b each showed significant differences versus Type IV (p<.0001). Measurements in Type IV show the highest mean value. So HU measurement of the density of cystoid regions in hepatic AE lesions offer a good differentiation against normal cysts. Thus, AE lesions with very hypodense strutures were correctly named "cystoid" and not "cystic" within EMUC-CT. The range of the detected values can be explained by the fact that cystoid areas at times derive from necrosis and sometimes from alveolar conglomerates. The highest mean value of Type IV is result of the fact that some of those lesions still show a tiny central alveola with surrounding hypodense fibrotic tissue and some of them lost the central alveola. This circumstance is depicted in the designation of Type IV as "small-cystoid/metastasis-like" by EMUC-CT.

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EVALUATION OF THE INTERRATER RELIABILITY IN USING THE EMUC-CT CLASSIFICATION IN A GREAT COLLECTIVE OF PATIENTS WITH HEPATIC ALVEOLAR ECHINOCOCCOSIS

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Computed tomography, mostly combined with PET, provides one of the most important diagnostic tools in suspected alveolar echinococcosis (AE). The recently established "Echinococcus multilocularis Ulm classification for computed tomography" (EMUC-CT) depicts the various morphologies of liver lesions due to AE. The classification scheme assigns morphological criteria of hepatic AE to five groups of primary morphologies (type I-V), some of them including subcriteria, and to six calcification patterns. The aim of the actual study was, to evaluate the interrater reliability in using the EMUC-CT classification in a great collective of patients with hepatic AE. Retrospectively the contrast enhanced CT scans in venous phase of n=121 different Patients with hepatic AE were evaluated. The cases were at first classified by the reference reader who at the same time taught the second reader how to use the classification. The reference reader was an experienced consultant in radiology and the second reader a resident with more than three years of experience in radiology. At least one week after the reference reader had classified the collective, the second reader began to re-classify all the cases separately from the reference reader. Calculation of Interrater-Reliability (Cohens-Kappa) was performed with SAS V. 9.2. The interrater reliability of the primary morphologies showed 0.8268 (95% KI: 0.7453-0.9084) with p<.0001. Concerning the subcriteria, the accordance was 0.8940 (95% KI: 0.8225-0.9656) with p<.0001. For the patterns of calcification, the interrater reliability showed a concensus of 0.6603 (95% KI: 0.5602-0.7604) with p<.0001. Further sub-analysis were performed. In summary the interrater reliability in using the EMUC-CT classification was high. The actual study could show this in a great collective of AE cases for the first time. The results encourage to use the classification in diagnosis of hepatic AE. For this, in order to use it properly, it certainly is crucial to be closely familiar with the application of the very detailed classification scheme which comprehensively covers the complex CT-morphological appearance of AE.

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CYSTIC AND ALVEOLAR ECHINOCOCCOSIS A ZOONOTIC INFECTIONS WITH DIFFERENT EPIDEMIOLOGY AND CLINICAL COURSE

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Human echinococcosis is caused by larval stages of several *Echinococcus* species, small intestinal flatworms of canines. Two most important species are *E. granulosus* causing cystic echinococcosis (CE) with nearly worldwide distribution, and *E. multilocularis* causing alveolar echinococcosis (AE), an emerging infection in the western, central and eastern Europe. The human diseases are rare in the Czech Republic (CZ). CE is relatively benign, however, AE is potentially life-threatening due to progressive tumor-like growth in liver and risk of metastatic dissemination. There have been treated total 12 patients (4 women, 8 men) with CE since 2005, and 13 patients (9 women, 4 men) with AE since 2012 at our departments. CE seems not to be more endemic in the CZ. Ten CE cases were diagnosed in migrants from endemic regions: Bulgaria (3x), Romania, Macedonia, Montenegro, Russia, Kazakhstan, Tajikistan, and Uzbekistan. Two patients are Czech citizens: a 68-year old female acquired infection in the CZ in past, probably, a 36-year old man is living in the CZ, but he travelled to eastern Europe and Asia for many times. The age of CE patients was between 28 and 68 years at the time of diagnosis. 10 patients had cysts in liver only, 1 patient in lung only and 1 patient in both liver and lung. Serology for echinococcosis was positive in all patients. The treatment involved surgery and temporary albendazole in 10 patients, temporary albendazole only in 1 patient and "wait and watch" in 1 patient. All AE cases were acquired in the CZ (12 patients) or in Slovakia (1 patient). The age of AE patients was between 7 and 70 years at the time of diagnosis. 12 patients had lesions in liver only, 1 patient had the main mass in liver and multiple metastasis in lung. Serology for echinococcosis was borderline or positive at all patients. The treatment is based on long-term continuous albendazole administration, which is not tolerated in 1 patient (treated with mebendazole). In addition, 7 patients underwent liver resection, 2 patients liver transplantation and one patient died from AE unrelated reason.

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CELL-FREE DNA (CFDNA) IN URINE AS A NOVEL DIAGNOSIS FOR HUMAN NEUROCYSTICERCOSIS

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Diagnosis of Neurocysticercosis (NCC) is difficult because it is based on a combination of clinical, epidemiological, radiological and immunological findings. Most commonly diagnosis for this condition is Western Blot (WB) which recognizes antibodies in serum samples, however it does not a direct test, is not quantitative and cannot distinguish between a past and current infection. As is important to recognize active infections, current researches are focused on the detection of antigen; furthermore, molecular approaches targeting products of the parasite have been reported in cerebroespinal fluid (CSF), but this process is invasive. Nevertheless, parasite fragments from various parasitic infections have been detected in urine, seeing that, this report aim to demonstrate that this is also the case with *Taenia solium*. In our work, we collected 25 urine samples of positive patients confirmed by WB and radiology from the "Instituto Nacional de Ciencias Neurológicas" in Peru, 50 ml of urine were filtered through Whatman N°3 filter paper, DNA was extracted with QIAamp DNA mini kit (QIAGEN) and the product was amplified using conventional PCR with two different sets of primers which recognize highly repetitive sequences in *T solium* genome. The PCR products were visualized in agarose gel and positive samples were confirmed by sequencing and by BLAST analysis. Our results report that we detected at least 9 positive of the 25 urine samples. Thus, in this study we demonstrate, for the first time, that cfDNA of *T. solium* is present in the urine of patients, this detection using non-invasive clinical samples offers significant benefits for the accurate diagnosis of NCC.

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TOWARDS THE DESIGN AND OPTIMIZATION OF INTERVENTION STRATEGIES AGAINST *TAENIA SOLIUM* TAENIOSIS/CYSTICERCOSIS BY MULTI-MODEL COMPARISON

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The cestode *Taenia solium* causes the neglected tropical disease (NTD) neurocysticercosis, the leading cause of preventable epilepsy in *T. solium* endemic low and middle-income countries. The 2012 World Health Organization's roadmap on NTDs had proposed that, by 2015, a validated strategy should be available for the control and elimination of *T. solium* taeniosis/cysticercosis and that, by 2020, appropriate control and elimination interventions should be scaled up in selected countries. With 2020 on the horizon, mathematical or computational models of *T. solium* transmission are urgently needed to inform and optimize the design of intervention strategies targeting control and elimination. Here we present the findings of a systematic review to identify and compare existing *T. solium* transmission models, and related transmission models of other Taeniidae family infections. We compare approaches to model design, the capacity of different models to project the impact of different intervention strategies and identify gaps requiring further model development. We also provide a comparative simulation analysis of two recent *T. solium* transmission models: the deterministic, population-based, model EPICYST and the individual-based, stochastic model cystiSim. We highlight areas of concordance and disparity, and discuss our findings in the context of epidemiological, clinical, economic, and longitudinal intervention data needs, and future developments of the *T. solium* transmission models. We argue that this work provides a foundation for enhanced future collaboration and partnership between epidemiologists, modellers, implementers and stakeholders invested in cysticercosis control and elimination.

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TREATMENT OF INDIVIDUALS LIVING WITH NEUROCYSTICERCOSIS AND HIV/AIDS: A SCOPING REVIEW

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Neurocysticercosis (NCC), due to infection with cysts of *Taenia solium*, is the single most important risk factor for acquired epilepsy globally. Many regions endemic for NCC are also endemic for HIV, yet literature on HIV and NCC co-infection is sparse and no current treatment guidance exists for this patient group. This study aims to scope the currently available literature on NCC and HIV co-infection, with focus on clinical characteristics, diagnostics and treatment outcomes. The scoping literature review methodological framework and PRISMA guidelines were followed. A total of 13,777 records identified through database searching and 45 additional records from other sources, were reduced to 57 included studies after a standardised selection process. Included studies were analysed for data relating to the study aims. The one identified experimental study demonstrated poor outcomes in surgical treatment (ventriculoperitoneal shunt insertion) of intraventricular NCC in HIV-positive patients. Twelve observational studies were identified, in which prevalence of NCC was shown to be similar in HIV-positive and -negative populations, with no significant association with CD4 count. Of the 26 cases of HIV and NCC co-infection extracted from 21 case series/reports, 15 suffered with seizures (58%) and 14 with headaches (54%). Fourteen (54%) cases had positive serum serology (7 ELISA-Ab +ve, 5 EITB-Ab +ve) and two case studies reported negative serum serology. Four (14%) were treated surgically, 15 (58%) received albendazole, three (12%) praziquantel and 13 received adjuvant steroid. Fifteen patients were reported to have clinically improved, and two died, one due to an adverse response after starting albendazole, dexamethasone and anti-retroviral therapy. Nine patients received at least two of either anti-helmintic, anti-epileptic and/or anti-retroviral medications. This review demonstrates that evidence to guide treatment of NCC and HIV co-infection is lacking. We will discuss issues relating to T. solium-NCC diagnosis and use of multiple medications in this patient group, and we will highlight pressing research needs.

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ASSOCIATION BETWEEN CLINICAL-RADIOLOGICAL CHARACTERISTICS AND SUICIDAL IDEATION IN PATIENTS WITH CALCIFIED NCC AND DEPRESSION

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Neurocysticercosis (NCC) is an infection of the central nervous system caused by *Taenia solium*. It is the first cause of seizures in underdeveloped countries and there is evidence linking NCC with neuropsychiatric disorders. Depression is one of the most common mental disorders in the world and a major cause of disability, contributing to the overall global burden of disease. The objective of this study was to evaluate the association between the presumptive diagnosis of suicide attempts or suicidal ideation and the clinical-radiological characteristics of calcified NCC and depression. A total of 136 patients were included. Calcified NCC was diagnosed by Computed axial tomography (CT). Depression and suicide attempts or suicidal ideation were evaluated through International Neuropsychiatric Interview (MINI). Multivariate methods of analysis was performed with generalized linear models, poisson family and log link function.The frequency of suicidal ideation was 52.6% in the study population with depression ($p < 0.01$), this is a statistically higher frequency than that reported for the general population. Patients with seizures had a slightly higher frequency of suicidal ideation (53.8%) compared to patients without seizures (46.2%), this association was not significant (p 0.399). Occupations in the home are specifically related to the group of women where an association is seen. Regarding occupation, there was an association between being a housewife and having suicidal ideation ($p < 0.05$). The number and location of the calcifications did not present associations with statistical significance with suicidal ideation. We have found a high frequency of suicidal ideation in patients with depression and Calcified NCC. There was no association between clinical-radiological characteristics and suicidal ideation. We suggest the development of future studies because we could promote assessment of suicidal behaviors and its evaluation in early stages of the disease.

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EVALUATION OF CROSS-REACTIVITY OF *TAENIA HYDATIGENA* AND *ECHINOCOCCUS GRANULOSUS* IN THE ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT ASSAY IN PIGS

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Taenia solium is an important zoonosis that infects humans as the definitive host (taeniasis) and pigs as the intermediate host (cysticercosis). Serologic diagnosis of porcine cysticercosis is limited to antigen detection using ELISA, which is known to cross-react with other *Taenia* species, and antibody detection using the lentil lectin glycoproteins Western blot test (LLGP-EITB), which has not been adequately evaluated for cross-reactivity to other parasites of pigs. Recent findings from field studies suggest that the GP50 antigen band of the LLGP-EITB may cross-react to *Taenia hydatigena*, a common and similar parasitic infection of pigs. The objective of this study was to evaluate the specificity of the LLGP-EITB assay in pigs infected experimentally with *T. hydatigena* and *Echinococcus granulosus*. A total of 12 three month-old Landrace pigs were obtained from a commercial grange in an area where *T. solium* is not endemic. All animals were confirmed to be negative at baseline on the LLGP-EITB. The pigs were divided into two equal groups; 6 pigs were each given an oral challenge with a single gravid proglottid of *T. hydatigena*, while the other 6 pigs were each given an oral challenge with 50 gravid proglottids of *E. granulosus*. Serum samples were collected on day 0 and every 2 weeks after challenge. At 12 weeks post-challenge all pigs underwent a detailed necropsy. *T. hydatigena* cysticerci were found in 2 of 6 pigs from the first group, while hepatic echinococcosis was observed in all 6 pigs from the second group. Four of the *T. hydatigena* exposed pigs were positive on EITB, with the positive reaction limited to the GP50 diagnostic band only, and two of these pigs had cysts after necropsy. On the other hand, only one *E. granulosus* exposed pig was positive to EITB, the reaction again limited to GP50 only, and only on the week 12 blood sample, despite the fact that all 6 of these pigs formed hepatic echinococcosis. These results provide definitive evidence that the GP50 diagnostic band in pigs cross-reacts against *Taenia hydatigena*.

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TO REALLY KNOW THE PARASITE: EVIDENCE WORKSHOP OF CYSTICERCOSIS-TAENIASIS TO PROMOTE CYSTICERCOSIS KNOWLEDGE, PREVENTION, AND SURVEILLANCE IN NORTHERN PERU

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Taenia solium cysticercosis is a common cause of epilepsy in developing nations. We created a 4-hour workshop to promote knowledge, prevention, and control in four villages (pop. 838) in Perú as part of an ongoing study of community-engaged surveillance and control. Adolescents and adults were invited to a "Healthy Pig Fair." Children were invited to an age-adapted "Tapeworm Birthday Party." We promoted the events via door-do-door visits and posters highlighting the opportunity to view the parasite in a microscope. The workshop included dialogue on economic losses due to pig infection using data from participating villages, followed by dissection of larval cysts from a recently-slaughtered heavily infected pig, evagination of cysts, and viewing of nascent tapeworms with light microscopes. Participants recorded observations of larvae on worksheets and learned about microscopic eggs and the *T. solium* life cycle. To measure knowledge change, we analyzed data from a community survey of adult heads of households, comparing individual knowledge 2 months pre-workshop (baseline) to knowledge 2-3 months post-workshop. We also gathered data from workshop participants in a post-workshop survey and focus group. Knowledge of the three *T. solium* life cycle stages increased significantly in the villages. Comparing knowledge gains for survey respondents who attended the workshop (n=52) to non-attendees (n=138) revealed both groups' knowledge increased, with greater gains for attendees. Knowledge of human-to-pig transmission increased by 39% and 22%, respectively, and knowledge of pig-to-human transmission increased by 43% and 9%. Only workshop attendees had significant gains in knowledge of human-to-human transmission (13% vs. 5%). Increased knowledge and participant willingness to publicly share experiences with *T. solium* in focus groups support the workshop's effectiveness. The use of local evidence, experiential learning, and community participation created a space to decrease stigma and influence knowledge and risk perception, all of which are barriers to behavior change in community interventions to prevent cysticercosis.

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DETECTION OF *TAENIA* EGGS IN SOIL AFTER MASS ANTI-HELMINTHIC TREATMENT: RESULTS FROM A COMMUNITY-WIDE SOIL SAMPLING IN NORTHERN PERU

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Taenia solium, the cestode parasite that causes cysticercosis, is transmitted from humans to pigs when carriers of the adult intestinal tapeworm defecate outside and pigs consume the infected feces. Lack of sanitation and open-field defecation are important risk factors for *T. solium* transmission, however, the role of contaminated soil in this transmission is unclear. Specifically, do *T. solium* eggs persist in soil, how long do they persist, and what concentration of eggs can be found in naturally infected soil? In this study, we sought to determine the proportion of households with *T. solium* eggs present in the soil in a village in northern Peru (pop. ~1,000). Five months prior to soil collection, all inhabitants of the study village were offered niclosamide for treatment of intestinal tapeworms. We then collected soil samples from the reported defecation sites of participating households, either from within the open defecation area or in front of the latrine if used. To do this, we removed visible fecal material from the indicated area, and placed 200 grams of topsoil in a plastic container for transport to the Center for Global Health. Each soil sample was evaluated for the presence of *Taenia spp.* eggs and other soil-transmitted helminths using light microscopy and a sugar-Percoll sedimentation technique previously validated by our group. A total of 336 soil samples were collected from 317 households. We found that 2.9% (10/336) of soil samples contained *Taenia spp.* eggs, and the average number of eggs detected per positive sample was 4. The proportion of households with contaminated soil was greater for open defecation sites (5.5%, 3/51) compared to soil collected from outside the latrine (2.7%, 7/255), although this difference was not statistically significant (p=0.27). Other parasites detected included *Strongyloides spp.* (52%), *Ascaris spp.* (39%), *Enterobius spp.* (16%), and *Trichuris spp.* (6%). Definitive speciation of *Taenia spp.* and other parasites with PCR is pending. These results show that *Taenia spp.* eggs are prevalent in the soil of endemic communities, and that contaminated soil could play a role in ongoing transmission of the parasite.

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METHODOLOGY OF AN OPEN CLINICAL COHORT OF INDIVIDUALS WITH EPILEPSY IN THE CONTEXT OF A CYSTICERCOSIS ELIMINATION DEMONSTRATION PROGRAM: TUMBES PROJECT

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Epilepsy is a neurological condition characterized by recurrent and unpredictable interruptions of normal brain function. Aleading cause of epilepsy in LMIC countries isneurocysticercosis (NCC), a helminthicinfection of the central nervous system. In 2006, we opened a primary-care health center staffed with general practitioners under the supervision of a neurologist, to attend people with epilepsy (PWE) identified during a large-scale cysticercosis elimination demonstration program for cysticercosis in Tumbes, Peru. The main objectives of the cohort werea) to evaluate incidence of epilepsy and epilepsy due to NCC in the context of an ongoing elimination program, b) to determine mortality of PWE, c) to assess the feasibility of managing PWE in a primary-care setting where neurology specialists are limited, d) to assess treatment compliance of PWE in this setting, and e) to assess withdrawal of antiepileptic treatment. Between 2006-2017, we enrolled 1813 PWE at the clinic in an open cohort; 586(32.3%) were identified through population-based screening while the remaining 1227 (67.7%) were referred to the clinic by others. 930 (51.3 %) were women, the mean age was 35.9(SD 17.6) ,1493 (82.4 %)were from Tumbes and 320 (17.6%) from neighboring regions; 1038 (57.3%) resided in rural areas.1523(84.0%) had active epilepsy at enrollment. Main seizure types by individual included 1311(72.3%) focal, 496 (27.4%) generalized, and 6 (0.3%) indeterminate. Etiology was symptomatic epilepsy in 905(49.9%), of which 556 (61.4%) had NCC and 349 (38.6% had other etiologies. Those without symptomatic epilepsy included 818 (45.1%) with idiopathic epilepsy, 73 (4.0%) withcryptogenic epilepsy and 17 (0.9%) with undetermined epilepsy. Antiepileptic drug therapy was started in 1463 (80.7%) upon enrollment, the majority (89%) with monotherapy (carbamazepine or phenytoin). The clinic has successfully established a large, representative cohort of PWE from the community setting. Data from this cohort is being analyzed to characterize epilepsy in our region. NCC is an important cause of epilepsy in Tumbes, found in 556/1813 (30.7%) of PWE.

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VALIDATION OF A LOW-COST SEDIMENTATION TECHNIQUE FOR THE DETECTION OF *TAENIA* SPP. EGGS IN SOIL

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Cysticercosis, a cystic larval infection in humans and pigs, is caused by ingestion of eggs from the adult *Taenia solium* tapeworm. Open defecation by human carriers, and resulting contamination of soil with *T. solium* eggs, may contribute to transmission among pigs and other humans, however, an acceptable and inexpensive method for detecting *Taenia spp.* eggs in soil has not yet been developed. In this study, we validated a new sedimentation technique for detecting *Taenia* spp. eggs in soil that uses low-cost materials available in Peru. For this validation, we used soil collected from the grounds of Center for Global Health in Tumbes, Peru. Three 200g samples of soil were seeded with 200, 100, and 50 *T. solium* eggs each, representing concentrations of 1, 0.5, and 0.25 eggs per gram of soil (EPG), respectively. Each sample was homogenized with a 10% solution of commercially available dish-soap, and filtered through tri-folded surgical gauze. Three 10mL samples of each filtrate were then collected and centrifuged with 10ml of 1.5X sucrose. The interphase of each solution was collected, suspended in saline, and aggregated into a single tube containing 5mL of Percoll. After centrifugation, a 1mL pellet containing the parasitic eggs was recovered and examined microscopically (10X) to determine the number of *T. solium*

eggs recovered from each sample. For each seeded 200g sampled of soil, 30mL (7% of total volume) of filtrate were analyzed for *T. solium* eggs. For soil seeded at 1 EPG (200 eggs total), 11 *T. solium* eggs were recovered, representing 83% of the eggs expected in 30mL of filtrate. For soil seeded at 0.5 EPG (100 eggs) and 0.25 EPG (50 eggs), recovery rates were each 30%. Based on these results, we calculated that this sedimentation technique provides adequate power (>80%) for detecting egg contamination if present at 1 EPG in soil collected from natural conditions. This technique was recently used to detect *T. solium* eggs in the soil of an endemic Peruvian village, and may lead to more opportunities in the future to study the role of soil as a vehicle for *T. solium* transmission.

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THE CASE OF ERADICATION OF SICKLE CELL ANEMIA DEATHS IN AFRICA

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Sickle Cell Disease (SCD) causes the greatest burden to both survival of children under five years (U5Y) and to the Public Health Management (PHM) in the endemic areas of Africa, India and other developing areas of the world. It is the most dangerous - in terms of rate of morbidity and mortality, of all known hemoglobinopathies. Several confounding factors (both natural and inflicted), in the milieu of 'hypoxia' contribute to the expression of symptoms in SCD patients."In the late 1960s and early 1970s, as sickle cell anemia was caught up in the torrent of U.S. congressional and presidential politics, the malady became widely characterized as a "neglected disease," a disease of a people whose "pain and suffering" had been ignored for too long, and a disease finally achieving its moment of national recognition". [1], [3].A neglectful healthcare policy gave way to present epidemic proportions, with an annual rate of 150,000 babies born with SCD in Nigeria - the highest recorded incidence of SCD in the world. [5]. SCD patients have compromised immunity, with increased incidence of meningitis, septicemia and a high mortality. [6]. WHO indices for Nigeria showed she suffers a 10 to 40% carrier state, with a prevalence of 2% [9]. A further 75% infant cases, and 80% share of the mortality in the whole of Africa. [11].A cure for SCD is possible with gene therapy, but this is technologically complex and expensive especially in low resource settings. It is nearly impossible to eradicate SCD because of its pathophysiology, but we can ameliorate the rate of death due to complications of stroke during the crisis moments. Transcranial Doppler (TCD) scanning, is the technique of choice to evaluate and diagnose the probable onset of stroke in SCD patients. Unlike Ebola - a deadly disease with a quick swift decimating rocket style, Malaria and SCD are silent killers with a 'chameleon' tactics and seeking the most vulnerable of all - our children, especially the U5Y age group. Peter Piot, the Belgian microbiologist who discovered Ebola in 1976 said: "we shouldn't forget that this is a disease of poverty, of dysfunctional health systems - and of distrust." [17].

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U.S. TRAVELERS' CONCERN ABOUT ZIKA INFECTION AND WILLINGNESS TO RECEIVE A HYPOTHETICAL ZIKA VACCINE

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The ongoing Zika pandemic has affected many countries that are common travel destinations. We assessed willingness to receive a prophylactic Zika virus (ZIKV) vaccine, currently under development, among travelers to areas with reported autochthonous ZIKV transmission. We surveyed United States (U.S.) residents aged 18-44 years who had ever heard of ZIKV and planned to travel to Florida and/or Texas (n=420) or a U.S. territory or foreign country (n=415) in 2017, using a nationally representative internet panel. Travelers to Florida and/or Texas reported less concern about ZIKV infection than travelers to other destinations (27.4% vs. 35.5%, p=0.01). Female sex, Hispanic ethnicity, discussing ZIKV with medical professionals, ZIKV risk perception, and self-efficacy for ZIKV prevention predicted concern about ZIKV infection in both groups. Travelers to Florida and/or Texas (43.4%) and other destinations (44.1%) were equally willing to receive a ZIKV vaccine. Hispanic ethnicity, discussing ZIKV with medical professionals, and concern about ZIKV infection predicted vaccine willingness in both groups. Likelihood of using existing ZIKV prevention methods, confidence in the U.S. government to prevent ZIKV spread, self-efficacy for ZIKV prevention, and knowledge about ZIKV symptoms further predicted vaccine willingness in travelers to other destinations. In multivariable analyses, only concern about ZIKV infection was associated with vaccine willingness in both groups (Prevalence Ratio [95% Confidence Interval]: Florida and/or Texas: 1.34 [1.06, 1.69]; Other: 1.82 [1.44, 2.29]). Targeted communications can educate travelers about ZIKV risk to generate ZIKV vaccine demand regardless of intended destinations, and targeting travelers who are pregnant or planning pregnancy.

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REDUCING ANTIBIOTIC USE FOR TRAVELERS' DIARRHEA

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Travelers often self-treat mild or moderate diarrhea with antibiotics (abx), even though it is seldom required, and may lead to bacterial resistance and other serious adverse effects. We studied the effect of providing a novel anti-diarrheal product (DiaResQ®, PanTheryx, Inc) to clients at 7 travel clinics in Colorado on reducing abx self-treatment by international travelers. Pre-travel, clinicians discussed with clients that this product was the preferred option to for diarrhea instead of abx; and either, both or neither were provided on client request. We conducted a telephone survey of returned travelers >18 years of age to assess occurrence of diarrhea and self-treatments. Clinic records of these travelers were reviewed for demographics, travel details, and products provided or prescribed. Out of 3005 answered calls, 448/598 (79.1%) respondents participated in the survey. For calls not answered, 33/359 (9.2%) responded to a follow-up email, for a total of 481 responses, of which 158 (32.8%) reported having diarrhea. The traveler's mean age was 43.9 years (+/- 15.1), 54.3% female, mean trip length 18.4 days (+/- 20.7) with 59.8% ≤ to 14 days. Pre-travel, 42.3% received abx and 35.5% received the product, with 12.7% receiving both. Receiving the product and reported incidence of diarrhea did not differ by age, gender, length or trip or travel destination. During travel, among those who reported diarrhea, 31.1% used abx and 43.1% used the product. Receiving the product was associated with reducing abx use from 58.3% to 22.2% (61.7% reduction, RR=.54, p<.01) while among those using the product abx use fell from 57.5% to 25.0% (56.5% reduction, RR=.57, p<.01) and loperamide use fell from 22.1% to 7 .7% (65.2% reduction, RR=.85, p< .01). Combining previously reported changes in prescribing practices with these changes in client use gives an overall 76.0% reduction in self-treatment with abx by these travelers. For each 1000 travelers with diarrhea who received the product there would be 239 fewer self-treatments with abx. This non-drug option substantially reduces travelers' self-treatment of diarrhea with abx and loperamide.

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WHO STAYS AND WHO GOES: PREDICTORS OF HOSPITAL ADMISSION AMONG PATIENTS PRESENTING WITH FEBRILE ILLNESS IN A RURAL UGANDAN HEALTH CENTER

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Fever is a common reason for presentation to care in rural, low-resource settings. However, not much is known about clinical decision making in such settings. In this prospective, observational, single-center cohort study of patients presenting with fever to a health center in rural western Uganda, we examined demographic and clinical factors predictive of an initial disposition of inpatient admission after clinical evaluation, but before laboratory testing. We then assessed the impact of laboratory results and system factors associated with a change between initial and final disposition plans. 4,924 patients with suspected febrile illness were included in the primary analysis. The strongest predictors for an initial disposition of admission after clinical examination were impaired consciousness (adjusted risk ratio [aRR], 3.18; 95% confidence interval [CI], 2.41 to 4.20) and documented fever (aRR, 2.29; 95% CI, 1.81 to 2.88). Providers initially planned to discharge many patients with significant vital sign abnormalities, including tachypnea (8.0%) and hypotension (1.3%). Anemia strongly predicted a final disposition of admission after an initial disposition of discharge (aRR, 35.16; 95% CI, 18.74 to 65.96); however, other laboratory abnormalities including hypoglycemia, acidosis, and hyperlactatemia did not impact disposition planning (all $P \geq 0.20$). In those with an initial disposition of admission, living farther than the two neighboring villages was associated with a final disposition of discharge (aRR, 2.14; 95% CI, 1.10-4.18). In conclusion, a significant number of patients with abnormal vital signs and laboratory results are not admitted for inpatient care. Geographic factors may influence a patient's final disposition contrary to a provider's initial disposition plan. Future work should assess the longer-term repercussions of discharge with severe illness and a broader study population.

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INVESTIGATION OF DROUGHT ASSOCIATED SCABIES OUTBREAK IN ETHIOPIA

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The impact of the severe drought in Ethiopia attributed to El Niño weather conditions has led to high levels of malnutrition that increased the potential for diseases outbreak. Currently Ethiopia is experiencing a scabies outbreak in drought-affected areas where there is shortage of safe water for drinking and personal hygiene as a direct result of the drought. Following a house to house census to assess the prevalence of scabies a detailed study has been done looking at the disease burden. Following the outbreak report in September 2015 we undertook "training of trainers" to relevant health workers on scabies. The training was cascaded to the health extension workers in the affected areas. Screening and management guideline and protocol were also distributed. A house to house data collection was undertaken to assess the prevalence of scabies. 1125770 people in the 68 districts in Amhara Region were surveyed using a simplified tool. A subsequent detailed data were collected from two zones and six woredas from 474 participants who had been diagnosed with scabies. This looked at the specificity of scabies diagnosis, age distribution, severity, duration of illness, super infection and other socio-demographic variables. The house to house census has registered 1125770 populations. 379,000 confirmed cases of scabies have been identified, with mean prevalence of 35.3% (0.2 to 60.7%). The detailed study has revealed that the specificity of scabies diagnosis by the health extention workers was 98.3%, mean duration of illness was 5 months with SD of +/- 2.8. 42% of patients were recorded to have severe illness. 75.1% of cases have affected family members. 39% of cases are school aged children and 30 % of them were noted to have bacterial super infection. 11% of the students have dropout from school because of scabies or/and drought. We concluded that the scabies burden in the region is enormous complicated by the nutritional shortage emergency and water scarcity. A coordinated response in case identification and intensified case management is mandatory to control the epidemic.

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CERVICAL CANCER SCREENING AND TREATMENT ON THE DOMINICAN REPUBLIC/HAITIAN BORDER

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Cancer mortality is a major global health problem, with cervical cancer (CC) the third most prevalent cancer worldwide, 75% of associated deaths occurring in LMICs. In 2009 our NGO instituted a first world CC screening and treatment program on the LMIC Haitian/Dominican Republic border. Haiti, with a CC incidence of 94/100,000 women, lacks a national CC screening program, and the Dominican Republic, with a CC incidence of 37/100,000 women, has such a program, but with suboptimal coverage and costly, difficult to access referral care. We are based in the Dominican border town of La Descubierta and four surrounding villages, available for some 4,000 unselected Dominican and Haitian women, many never pap screened. Donated supplies and equipment are obtained, and ThinPrep paps, HPV reflex testing and biopsy specimens are collected and shipped to the US for pathology services. Volunteer gynecologists and staff pursue precancerous results with colposcopy, biopsies, LEEPs and cryotherapy, care not available publicly or privately on the frontier. Patient records, photos and path reports are entered into an electronic database, and patients are found and encouraged to return for follow up with free transport, a meal and a gift bag of toiletries. Over nine years, through March 2018 (still ongoing,) 1413 women are enrolled, with: 354 abnormal (ASCUS or higher) paps, 25% of all women; a positive HPV rate of 20.3% (estimated 15.9% in Caribbean region); 19 cancers (5 deceased), a yearly CC incidence rate of 149/100,000 women; and prior abnormal results - 3 Ca in situ, 1 AGCUS, 29 ASCUS, 39 LGSIL and 15 HGSIL - now negative after treatment and repeated testing. To date, 2459 visits, 2063 paps, 273 colposcopies, 238 biopsies, 92 ECCs, 44 LEEPs, 2 conizations and 8 cryosurgeries have been done. Thus, on the LMIC Haitian/Dominican Republic border, deficient in resources, health infrastructure and often official and cultural beneficence, we are able to sustain an effective first world, tissue-based (not "See and Treat") CC screening and treatment program for poor Dominican and migrant Haitian women.

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RAPID DETECTION OF *MYCOBACTERIUM ULCERANS* INFECTION BY RECOMBINASE POLYMERASE AMPLIFICATION

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The current control strategy for Buruli ulcer emphasizes early diagnosis and prompt treatment, with the goal of avoiding the complications associated with advanced stages of the disease. There is no diagnostic test for the disease appropriate for use at the primary health care level where

most cases are detected and treated. Diagnosis based on clinical signs is unrealiable in inexperienced hands and complicated by infections that have similar presentations. A diagnostic test for the early detection of Buruli ulcer in symptomatic patients with sufficient positive predictive value to put patients on appropriate treatment is a priority according to the WHO. The aim of this study was to develop and evaluate the use of RPA for the detection of *Mycobacterium ulcerans* (*M. ulcerans*) the causative agent of Buruli ulcer at the point of patient care. Specific fragment of IS2404 of *M. ulcerans* was amplified in 15 minutes at a constant 42ºC using the RPA method. The amplification product was analyzed on a real-time portable fluorometer. The method was tested for sensitivity and specificity with molecular standard of IS2404 DNA fragment, various *M. ulcerans* strains, other mycobacteria and environmentally associated bacteria. Additional investigation was done to assess the assay performance as a diagnostic tool using archived DNA from clinical samples of symptomatic patients. All results were compared with that of a highly sensitive IS2404 PCR. The detection limit was 10 copies of IS2404 in 15 minutes using plasmid standard and 125 fg with genomic *M. ulcerans* DNA equivalent 25 genomic copies. The assay was highly specific in detecting all 7 strains of *M. ulcerans* from different part of the globe with no observed cross reactivity with other mycobacteria and clinically relevant bacteria species. The clinical specificity of the BU-RPA assay was 100%, whiles the sensitivity was 86%. We have developed a real-time isothermal RPA assay for the detection of *M. ulcerans* as a cheaper alternative to PCR. Combining this assay with a simple extraction protocol will maximize its use as point of care test for confirming suspected cases of Buruli ulcer.

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NUTRITIONAL AND ENVIRONMENTAL FACTORS ASSOCIATED WITH SPECIFIC DIARRHEAL PATHOGENS AMONG GUATEMALAN CHILDREN

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Description of prevalence and diversity of diarrheal pathogens in children in low and middle income countries (LMICs), as well as nutritional and environmental risk factors associated with pathogen acquisition, will inform preventative public health measures. From 4/2015 to 3/2016, subjects 6—35 months old with acute, non-bloody diarrhea were enrolled in a diarrheal treatment trial at rural (N = 172) and urban (N = 144) sites in Guatemala. Baseline stool samples were tested for 23 diarrheal pathogens (bacterial, parasitic, and viral) by multiplex PCR (FilmArray GIP® Biofire). Associations between nutritional and environmental characteristics and specific pathogen infection were tested using multivariable generalized linear models. Simultaneous infection with multiple pathogens was common (mean per child: 4.8 rural, 2.7 urban). The most prevalent pathogens were campylobacter, toxigenic *C. difficile*, *E. coli* pathotypes (EAEC, EPEC, ETEC, STEC O157:H7), shigella, cryptosporidium, giardia, adenovirus, norovirus, and sapovirus. In multivariable models adjusted for rurality (proxy for poverty and sanitation), acute malnutrition was associated with toxigenic *C. difficile*, shigella, cryptosporidium and norovirus. Chronic malnutrition was associated with shigella and giardia. Presence of domestic animals (pigs, fowl, dogs, cats) in the house did not increase risk of putative animal-associated pathogens (campylobacter, *C. difficile*, ETEC, STEC O157:H7, shigella, and cryptosporidium). Age, gender, household crowding and number of children under age 5 did not confound these associations. Guatemalan children with acute or chronic malnutrition were more susceptible to certain pathogens, suggesting that both preventative efforts and therapeutic interventions for diarrheal illness should prioritize malnourished children, and account for different pathogen spectrums between acute and chronic malnourishment. The presence of domestic animals was not associated with any specific etiology, suggesting that modes and frequency of diarrheal pathogen transmission in LMIC households is complex and incompletely understood.

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SYSTEMATIC REVIEW AND META-ANALYSIS OF THE DIAGNOSTIC ACCURACY OF LEPTOSPIROSIS LATERAL FLOW IGM POINT-OF-CARE TESTS

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Leptospirosis is underdiagnosed by clinicians in low- and middle-income countries, as traditional laboratory methods for diagnosis are unavailable. Lateral flow assays that use antigen derived from heat-treated whole cell *Leptospira* serovar Patoc strain Patoc 1 (LFA) are among the most promising point-of-care assays. We aimed to estimate the sensitivity and specificity of LFA by conducting a systematic review and meta-analysis. On 4 July 2017 we searched three medical databases for relevant articles. Two reviewers determined article relevance using pre-determined inclusion/exclusion criteria. For included articles we assessed study quality using quality assessment of diagnostic accuracy studies 2 (QUADAS-2) domains, characteristics of participants and testing methods. We estimated sensitivity and specificity for each study against the study-defined case definition as the reference standard, and performed a meta-analysis using a random-effects bivariate model. We assessed for publication bias using Deeks' funnel plot assymetry test. Our search identified 225 unique reports, of which we included 11 (4.9%). We considered one (9.1%) study to be high quality. Case definitions of leptospirosis were consistent with World Health Organization or Centers for Disease Control and Prevention definitions in one (9.1%) study. We did not find evidence of publication bias (p=0.63). Our estimate of pooled sensitivity and specificity was 80.1% (95% confidence intervals [CI] 71.5%) and 89.5% (CI 80.0-94.8%), respectively. The evidence base for determining the sensitivity and specificity of LFA is small. Most published studies are at moderate or high risk of bias, and case definitions vary markedly. Further well-conducted studies that use robust case definitions may alter estimates of diagnostic accuracy.

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USE OF ELECTRONIC HEALTH RECORDS TO IMPROVE PATIENT OUTCOMES IN RESOURCE CONSTRAINED SETTINGS

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On April 30, 2011, the Health Frontiers in Tijuana, Mexico (HFiT) student-run free clinic was established in Tijuana's *Zona Norte*. HFiT serves vulnerable populations, including substance users, sex workers, the homeless, deportees, and indigent patients. HFiT patients often reside within the *Zona Norte*, although persons from more than 60 miles away have also received care at HFiT. HFiT was founded as a partnership between the University of California, San Diego School of Medicine (UCSD) and Tijuana's Autonomous University of Baja California School of Medicine (UABC), the only publicly funded medical school in Tijuana. The great need for healthcare for marginalized populations, combined with the desire of both UCSD and UABC to impact local communities, led to the planning and founding of the HFiT clinic. HFiT is the product of a true, binational partnership between the UCSD School of Medicine, the Universidad Autonoma de Baja California School of Medicine (UABC), and Desayunador Salesiano "Padre Chava", a community grass roots organization in Tijuana. HFiT provides accessible, quality healthcare for the

underserved in Tijuana's Zona Norte in a respectful environment where students, health professionals, patients, and community members from the border region learn from one another. The HFiT clinic trains future clinicians about global health and health care for the underserved and at the same time brings essential clinical services to a large number of vulnerable individuals living in our border region. In an effort to improve patient care, data collection, and outcome management, the HFiT provider community began utilizing an electronic medical record (EMR) developed by NotesFirst. NotesFirst has developed an EHR platform specifically in support of doctors and healthcare providers in lower-middle income regions. This technology has specific application both for electronic patient documentation at the point-of-care, and also in settings where the patient population is in flux, such as following a public health emergency, natural disaster, or political turmoil.

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FIVE YEAR MORTALITY AMONG CHILDREN AGED 0 TO 14 YEARS IN UNIVERSITY COLLEGE HOSPITAL, SOUTHWEST NIGERIA

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Childhood mortality statistics is a crucial health index of a population, a reflection of the health system and health seeking behaviour of the population. Causes of childhood mortality are usually from preventable diseases, especially in low and middle-income countries like Nigeria. It understanding will enhance formulation and implementation of strategic policies to reduce childhood mortality.This study seeks to determine the causes of mortality among children aged 0 to 14 years admitted in University College Hospital, Southwest Nigeria from January 2012 to December 2016. It was a cross-sectional trend analysis of secondary data analysis of mortality statistics of children aged 0 to 14 years admitted in University College Hospital, Ibadan from January 2012 to December 2016 using extracted data from the department of Medical Records. The health data was extracted from the hospital's statistic register and patients case note. Annual number of children aged 0 to 14 years admitted along with recorded deaths during the five years period were extracted from the statistic register. Mortality rates was calculated for the five years. Cause of deaths were also extracted from the register and socio-demographics of the patients and parents were extracted from the patients case notes. The median age of participants was 2.55 ± 0.0years, with 56.7% being under-1 year. There was male preponderance of 55.1% with the majority being of Yoruba tribe (88.2%) and 80.9% living in urban areas. Mortality was highest among the under-1 with 60.4%, 66.0%, 57.6%, 59.6% and 64.6% for years 2012 through 2016. The overall mortality rate was highest in 2012 (12.1%) and lowest in 2015 (6.9%). Overall causes of childhood mortality show congenital diseases 41.4%, infectious diseases 29%, non-infectious diseases 17.4% and other causes of deaths 12.2%. Other causes of mortality include malnutrition, eye diseases, heart diseases, gun shot and kerosene ingestion. The major cause of childhood mortality in this study was congenital diseases, followed by infectious diseases. Sepsis, respiratory diseases and malaria rank highest among the infectious group for the five years.

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COST-EFFECTIVENESS OF PODOCONIOSIS LYMPHOEDEMA TREATMENT IN NORTHERN ETHIOPIA: RESULTS FROM THE GOLBET TRIAL

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Podoconiosis is a non-filarial geochemical lymphoedema that occurs in the tropical volcanic highlands and causes life-long disability and reduced labour productivity among subsistence farmers. We conducted a pragmatic, randomised controlled trial of a hygiene and foot-care intervention for people with podoconiosis in the East Gojjam zone, northern Ethiopia. Participants were allocated to the immediate intervention group or the delayed intervention group (control). The intervention included training in foot hygiene, skin care, bandaging, exercises, and use of socks and shoes, and was supported by lay community assistants. Cost-effectiveness analysis was conducted using the cost of productivity loss due to acute dermatolymphangioadenitis (ADLA), a complication characterised by fever, rigors, and a rapid increase in pain and swelling of the leg. The health outcome in the cost-effectiveness analysis was quality of life measured using the Dermatology Life Quality Index II (DLQI). The cost of the foot hygiene and lymphoedema management kit was 550 EBT ($66) per person per year. The cost of delivery of the intervention, including transportation, storage, training of lay community assistants and administering the intervention was 1355 ETB ($163) per person. Participants who received the intervention spent on average 42 (SD=30) days off economic activity due to ADLA, resulting in a productivity loss of 1704 ETB (SD=1232). Participants from the control group lost on average 55 (SD=31) days per year due to ADLA, costing 2237 ETB (SD=1253). Dermatological quality of life measured using DLQI was higher (scores were lower) in the intervention group (9.35, SD=4.23) compared to the control group (11.3, SD=3.7). Subgroup analyses demonstrated that participants with a monthly family income <1000 ETB ($119) had lower productivity loss and higher dermatological quality of life as a result of the intervention, compared to participants with an income >1000 ETB. Results of our analyses suggest that the proposed lymphoedema management strategy is more effective and less costly for people from the poorest families.

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GUINEA-WORM DISEASE. A SYSTEMATIC REVIEW OF CASE REPORTS ABOUT THE FIRST NEGLECTED TROPICAL DISEASE TO BE ERADICATED

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Guinea-worm disease is a parasitic disease caused by *Dracunculus medinensis*. It affects people living in rural and isolated communities who depend mainly on open surface water sources for drinking water. Although it mortality is low, morbidity is considerably high and may cause huge disabilities which are devastating. Diagnosis is clinically performed, and no specific treatment nor vaccines are available. The objective of our review was to describe the epidemiology of Guinea-worm disease published cases. A systematic review was conducted following the "*PRISMA*" guideline. PubMed database was searched using the search terms "*Dracunculiasis*" or "*Guinea-worm disease*" and limited to case reports published in English or Spanish, but without time frame. Full text articles were assessed for relevance and data extraction was performed as an iterative process. During the initial search 26 articles were obtained, from which 19, containing 25 cases were included. Females were more affected than males (13 vs 12) and the medium age of presentation was of 25 years (+/-SD 16.69). The 67% of the patients came from Africa and Nigeria was the country which reported the most (36%). Pain (60%), swelling (36%) and fever (16%) were the most common initial clinical manifestations, and the presence of an ulcer containing the Guinea-

worm was found during physical examination in only 40% of the cases. Diagnosis was most commonly done through histopathology (56%). Surgery was performed in 48% of the cases and mechanical extraction in 36%. The decision to do not perform no treatment at all was taken in 16% of the cases. Mortality rate was of 4%. Guinea-worm disease has been set as the first neglected tropical disease to be eradicated, and as it seems to come to an end, there are few case reports published in the literature. We consider that the trends presented in our systematic review, rather than being analyzed from a historic point of view, should be taken as a lesson by health professionals to enhance the active surveillance of the remaining cases. No patient should be left behind and reemergence must be prevented.

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SURGICAL SITE INFECTION BY MYCOBACTERIUM ABSCESSUS: LESSONS LEARNED FROM AN OUTBREAK INVESTIGATION IN BANGALORE, INDIA

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Mycobacterium abscessus, a rapidly growing NTM (Non-Tuberculous Mycobacteria) has been implicated in Surgical Site infection outbreaks lately. We investigated an outbreak of delayed surgical site infection in a 50 bedded Mission Hospital in Bangalore. We identified these cases by a retrospective-prospective study using the case definition of delayed onset nodule/sinus development +/- discharge at the surgical site that had initially fully healed. 18 tissue/discharge samples were sent for microbiological isolation. Environmental sampling was done including water from various inlets for source identification. 42 cases (40 Females) presented with symptoms, 46 days post surgery (range 9 to 134 days), among a total of 373 cases operated from January to June 2017. Cases were 35 Caesarean Section (19 Emergency), 2 hysterectomy, 3 hernia repair, 1 Laparoscopic removal of IUCD and 1 Tubal Ligation. None had fever or loss of weight/appetite. All tested negative for HIV, 5 had Gestational diabetes mellitus. All were prescribed a combination of Levofloxacin, Clarithromycin and Injection Amikacin, pending cultures. 4 cases that did not respond to first 2 weeks of antibiotics underwent Surgical debridement. 10 out of 18 samples were smear positive for Acid Fast Bacilli and 2 grew mycobacteria sensitive to all antibiotics except Tetracycline and Ofloxacin. Species was identified through Line Probe Assay. Cultures of water samples grew an array of microorganisms making Mycobacterial isolation difficult. Corrective measures taken included- setting up an HIC (Hospital Infection Control) Team to ensure compliance to infection control policies, changing the Theatre layout and installing a Reverse osmosis water plant. At 9 month follow up, 26 patients were healed, 12 were lost to follow up. 4 patients who refused treatment persisted to have sinuses. Unlike other outbreaks, follow up was challenging with majority being healthy nursing mothers. This study highlights that a ubiquitously present organism like NTM can cause outbreaks even among routine surgeries, thus emphasising the role of HIC and importance of sterile precautions like water quality.

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HIGHLIGHTING THE NEED FOR APPROPRIATE CASE-MANAGEMENT OF UNDER-FIVE DIARRHEA AND PNEUMONIA IN PUBLIC HEALTH FACILITIES OF UTTAR PRADESH AND BIHAR, INDIA

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Under-five mortality rate is high in India; majority of these deaths are due to pneumonia (29%) and diarrhea (21%). This multi-faceted study was undertaken to understand case-management of childhood diarrhea and pneumonia. Across ten districts in Uttar Pradesh and Bihar, we interviewed 1283 caregivers, 817 Accredited Social Health Activists (ASHAs), and 769 informal private providers. Additionally, in 64 public health facilities, we observed the treatment given to 423 under-five children diagnosed with pneumonia and 891 with diarrhea, interviewed 232 facility based providers, and performed a supply audit. We found that more than 95% of the caregivers (95% CI: 94%-97%) went to private providers, and rest visited public providers. Only around 6% (4%-7%) visited ASHAs as majority did not consider them as a health provider. Knowledge of pneumonia and diarrhea was poor across all provider types. Skills, measured using video vignettes for ASHAs and informal private providers, and by observing the treatment provided by facility based providers, was even lower. Counting respiratory rate for one minute, the most important diagnostic tool for pneumonia, was done for only 2% of pneumonia cases (1%-4%). 13% (10%-15%) of diarrhea cases were assessed for dehydration using the skin pinch test. 12% (9%-15%) of pneumonia cases and 17% (14%-19%) of diarrhea cases were given appropriate treatment. Of all severe pneumonia (291) and severe diarrhea (39) cases, only 3% (1%-5%) were admitted, and 4% (2%-6%) got appropriate treatment. We developed a mathematical model (ADAPT) to examine the potential impact of increasing evidence-based interventions to prevent, diagnose, and treat pneumonia and diarrhea, and that of changes in the mix of providers from whom patients seek care. Results showed that with a 20-percentage point improvement in quality of care within facilities, and diversion of traffic to public facilities from 1% to 41%, more than 36,000 lives could be saved annually in these two states. There is an urgent need for improved quality of care for children in India and we have provided information to help stakeholders design the appropriate strategies.

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MICRONUTRIENT LEVELS IN A PEDIATRIC UGANDAN COHORT WITH SICKLE CELL ANEMIA

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Micronutrient deficiency has been associated with morbidity in children with sickle cell anemia (SCA) living in the US and Europe, but there is limited information on micronutrient deficiency in children with SCA living in Africa. We assessed baseline micronutrient levels in Ugandan children age 1-4 years enrolled in the Novel use Of Hydroxyurea in an African Region with Malaria (NOHARM) study, a randomized controlled trial that compared hydroxyurea therapy vs. placebo. We measured

serum concentrations of vitamin A (HPLC), vitamin B12 (microbiological assay), vitamin D (mass spectrometry), vitamin E (HPLC), and plasma concentrations of folate (chemiluminescence assay) in 99 randomly selected children in the NOHARM study using blood collected prior to the start of study treatment. Median (IQR) concentrations were: vitamin A (retinol), 26.6µg/dL (20.8-30.4); vitamin B12, 622.0pg/mL (461.3-870.3); vitamin D (25OH-D), 29.0ng/mL (25-35ng/mL), and vitamin E (α-tocopherol), 20.6µmol/L (17.1-25.1). Median folate level was beyond the upper limit of detection (>52.1nmol/L). Prevalence of vitamin deficiency was: vitamin A (retinol <20µg/dL), 18.2%; vitamin B12 (<200 pg/ml), 3.0%; vitamin D (25OH-D <20ng/mL), 6.1%, and vitamin E (α-tocopherol <11.6µmol/L), 1.0%. No folate-deficiency (folate <10nmol/L) was present, likely due to routine folic acid supplementation for chronic hemolysis in this population. 52.5% of children were vitamin D-insufficient (25OHD <30ng/mL). C-reactive protein (CRP) was negatively correlated with retinol (Spearman rho=-0.240, p=0.02) but not with other micronutrients. Inflammation and hemolysis may alter interpretation of some nutritional biomarkers in children with SCA. However, vitamin D insufficiency was common in children with SCA and unrelated to inflammation. Future studies will assess whether baseline micronutrient deficiencies are associated with subsequent SCA-related morbidity.

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INTERVENTIONS TO REDUCE PEDIATRIC TUBERCULOSIS MORTALITY IN INDIA AND NIGERIA USING MAP-IT: A MATHEMATICAL MODEL TO ESTIMATE INTERVENTION IMPACTS ON PEDIATRIC TUBERCULOSIS MORTALITY

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Tuberculosis (TB) is a leading cause of pediatric morbidity and mortality in low income countries. Mathematical models, such as the Model for Assessment of Pediatric Interventions for TB (MAP-IT), a user-friendly, web-based, non-stochastic model, can be used to estimate the impact of TB interventions on pediatric TB-associated mortality. We used MAP-IT to model scenarios of improved availability and utilization of single and packaged TB preventive, diagnostic, and treatment interventions to estimate the impact on pediatric TB-associated mortality in India and Nigeria, two high-burden countries, from 2017-2021. The current-care scenario estimated 276,800 and 82,500 TB-associated pediatric deaths from 2017-2021 in India and Nigeria, respectively. In India, interventions the greatest impact on pediatric mortality were improved pediatric fixed dose combination (FDC) regimes (15.2%); improved clinical diagnosis (12.6%); and improved contract tracing (2%). In Nigeria, improved pediatric FDCs and clinical diagnosis had the greatest individual impact on pediatric mortality, with 8.7% and 8.6% reductions in pediatric mortality, respectively. Packaging TB interventions maximized the impact on pediatric TB mortality, with three-quarters of mortality prevented in both India and Nigeria. \ Improved pediatric FDCs and clinical diagnosis had the greatest individual impact on pediatric TB mortality. However, packaged TB interventions have the greatest impact on the number of lives saved. Intervention strategies must address obstacles to implementation, including service access, financing, availability of interventions, and education. In conclusion, MAP-IT estimates indicate packaged interventions including improved diagnostics and child-friendly FDCs regimens had the greatest impact on pediatric TB mortality in two high-burden countries.

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PEDIATRIC DISCHARGE FROM HOSPITAL: KENYAN HEALTHCARE WORKERS' PERCEPTIONS AND REPORTED PRACTICES

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Children discharged from hospital in low- and middle- income countries (LMICs) are at increased risk of mortality during the subsequent 6 months compared to community peers. Outcomes may be improved by quality care at discharge, yet little is known about factors influencing its provision. This study aims to assess healthcare worker (HCW) attitudes, perceptions, and reported practices regarding discharge care of inpatient children. All clinicians (doctors, clinical officers), nurses, and nurse and clinical officer trainees involved in pediatric discharge care at 10 Western Kenyan hospitals are recruited to complete a 28-question survey. A subset will be invited for in-depth interviews. To date, 43 HCWs from three hospitals have completed the survey. Interim results indicate that 72% believe that children discharged from hospital are less likely to die compared to community children, although 60% reported that >90% of readmissions could be prevented with better discharge and follow-up care. Most respondents (97%) report discharge care to be very important, and as consequential as inpatient tasks, to patient outcomes. Participants

almost unanimously indicated that the range of discharge care tasks are implemented at their facility, and any single task is performed by diverse cadres. Only 35% of HCWs indicated that their hospital delivers pediatric discharge care "very well" compared to ideal practices. We plan for 60 additional surveys and 30 interviews to be completed by July 2018. Interim survey results suggest HCWs underestimate post-discharge mortality burden, but are able to identify several important and potentially modifiable barriers to effective care such as take-home medication stock-outs, provider-caregiver miscommunication, and lack of family valuation of and adherence to discharge care instructions. Although participants indicated that currently available guidelines are "very informative" for discharge care delivery, these resources are heavily inpatient management focused and provide limited discharge care guidance. Additional research is needed to understand how to improve pediatric discharge care in LMICs.

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THE IMPACT OF POLYPARASITISM ASSOCIATED WITH PREGNANCY IN LAMBARÉNÉ AND SURROUNDINGS AREAS IN GABON

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The burden of parasites, particularly soil-transmitted helminths, schistosomiasis and *malaria*, is often very high in pregnant women in Sub-Saharan African countries such as Gabon. Having multiple parasite infections in these endemic areas is common because of the coexistence of their vectors. Thus, anemia associated with parasites is a major factor in women's health, especially during pregnancy and is an important contributory factor in poor health outcomes for newborns. We aimed to investigate the impact of polyparasitism among pregnant women attending antenatal care in Lambaréné. Pregnant women in their third trimester were recruited in two cross-sectional studies. The diagnosis of helminthiasis and malaria were determined by microscopy using stool and urines as well as blood and tissue specimens respectively. Logistic regression models were applied to assess the association between the infection status of mothers and pregnancy outcome variables (low birth weight, *In utero* growth retardation, maternal anemia and prematurity). A total of 927 pregnant women were enrolled in this analysis with a median age of 24 years. Parasites such as *Plasmodium falciparum* detected in mother peripheral blood and placenta tissue were respectively 30.5% (255/836) and 16.5% (70/424), Soil-transmitted helminths and *Schistosoma haematobium* were 37.6% (311/827) and 24%(199/830). At least 65.6 % (438/668) of the pregnant women had one parasite, polyparasitism was found with two, three and more than four parasites in respectively 21.6% (157/726), 3.3% (24/731) and 0.7% (5/668). The mean birth weight was 2950g and 19% (170/893) had low birth weight. The pregnancy outcomes observed in this analysis were all associated with malaria and polyparasitism with four parasites. Polyparasitism increased the risk of having low birth weight in our study. The prevalence of polyparasitism with more than three parasites was low in our study, but highly associated with the poor pregnancy outcomes. Therefore, public health measures and intensive antenatal care services are vital to promoting safe pregnancy in resource-limited settings.

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MALNUTRITION AMONG RESIDENTS OF THE VELLORE DEMOGRAPHIC SURVEILLANCE SYSTEM, SOUTH INDIA

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Malnutrition, which includes obesity and under-nutrition, has significant public health impact in terms of morbidity, mortality, and economic loss. Anthropometric measurements of population in a Demographic Surveillance System (DSS) give us a chance to describe the distribution of malnutrition in different age groups. In 2015, the Vellore Demographic Surveillance System (VDSS), which covers a total population of 154,452 residents, collected anthropometric measurements of all available individuals in 22 urban wards and 21 rural villages in Vellore, Tamil Nadu, India. Z scores were calculated based on the World Health Organisation (WHO) growth standards using the WHO Anthro plus software. Anthropometric measurements were obtained for 89,308 (58%) residents of which 9,916 (11%) were children less than 5 years of age, 17,736 (20%) were 5-19 years of age and 61,656 (69%) were adults. Among children <5 years, stunting (HAZ SD<-2) was seen in 2,489 (25%; 95% CI 24.25-25.96), underweight (WAZ SD<-2) in 2,343 (24%; 95% CI 22.8-24.47) and wasting (WHZ SD<-2) in 1,072 children (11%; 95% CI 10.21-11.43). Multivariate regression analysis showed that children residing in urban areas (adjusted odds ratio (AOR) 1.16; 95% CI 1.03-1.30), overcrowded dwellings (AOR 1.25; 1.06-1.46) and houses using solid fuel (firewood) for cooking (AOR 1.36; 95% CI 1.13-1.63) were significantly at higher odds of being stunted. In the age group of 5-19 years, thinness (Body mass index, BMI, for age (BAZ) SD <-2) was seen in 4,081 (23%; 95% CI 22.39-23.63), overweight (BAZ SD >1) in 1,657 (9%; 95% CI 8.92-9.77) and obesity (BAZ SD >2) in 700 individuals (4%; 95% CI 3.66-4.24). Among adults (>19 years), undernutrition (BMI <18.5) was present in 7,796 (13%; 95% CI 12.38-12.90), overweight (BMI 25-29.9) in 16,771(27%; 95% CI 26.85-27.55) and obesity (BMI ≥30) in 8,138 individuals (13%; 95% CI 12.93-13.46). The burden of under-nutrition is high in children and the prevalence of overweight and obesity is high among adults indicating the need for strategies to handle the dual challenges in southern India.

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PITFALLS OF PREDICTING LEPTOSPIROSIS BY COMMON CLINICAL AND BIOCHEMICAL MARKERS

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Blood biochemistry, together with clinical parameters is being used to diagnose leptospirosis and differentiates it from dengue in resource poor setting, due to lack of point of care diagnostic methods. The objective of this study was to investigate the usability of commonly use predictors of leptospirosis. We carried out a prospective study from April 2016 to January 2018 at four major leptospirosis endemic areas in Sri Lanka. All undifferentiated afebrile patients were recruited as "possible" cases of leptospirosis and the clinical features and blood biochemistry and symptoms was monitored daily until the 10th day of illness. We specifically

looked at WBC, platelets and heamatocrit, which have been reported in literature as markers to differentiate leptospirosis from dengue. In addition, the clinical symptoms listed in leptospirosis case definition were also monitored. Case confirmation was done using a real time PCR & previously validated lateral flow immune assay (both done on all cases). Of the 624 screened, 269 confirmed as leptospirosis. At least one WBC count was more than 11000/mcL in 55(27.8%) patients while 47(23.7%) had at least one count less than 4000/mcL. At least one platelet count was less than 150000/mcL in 159(82%) confirmed cases. More than 20% increased in heamatocrit from baseline was observed in 23.3%(n=44). Fever was not an admission symptom in 28(13.1%) patients, while headache, myalgia, conjunctival suffusion and jaundice were not evident at the hospital admission among 46(21.4%), 45(20.9%), 171(79.5%), 177(82.7%) respectively. Among patients who were positive only for antibodies, WBC count was higher through out the illness compared to the patients diagnosed using PCR while the platelet count had gradual drop with the disease progression in antibody positive patients. The common clinical and biochemical markers are misleading and differentiation with conditions like dengue is extremely difficult. Immune response associated changes in blood biochemistry may be explaining the previous observations among leptospirosis patients diagnosed primarily with antibodies.

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THE EFFECT OF OBSTETRIC ULTRASOUND DURING THE FIRST STAGE OF LABOR ON TIME TO DECISION MAKING ON DEFINITIVE MODE OF DELIVERY AT MBARARA REGIONAL REFERRAL HOSPITAL IN WESTERN UGANDA

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Mbarara Regional Referral Hospital (MRRH) is a tertiary care hospital that serves the western region of Uganda and neighboring countries. MRRH delivers an average of 12,000 pregnancies per year. Only a small fraction of these pregnancies are ever evaluated by prenatal ultrasound. Ultrasound has been shown previously in African settings to predict successful vaginal births, to detect the need for operative vaginal delivery and to alter management plans. A program recently introduced to MRRH for training the resident doctors in the basics of obstetric ultrasound aims to increase the use of prenatal ultrasound near the time of delivery. We are interested in assessing the impact of this program. We have designed an open label randomized controlled trial of the impact of ultrasound during the first stage of labor on time to decision making for the definitive mode of delivery. At the time of admission mothers in the first stage of labor with no particular indication for ultrasound will be consented and randomized either to be evaluated by ultrasound, or to not be evaluated by ultrasound (the standard of care in this setting). These women will be followed until discharge. We will collect data on time to decision on delivery mode, time to delivery, adverse maternal and fetal outcomes and maternal satisfaction with care. Data analysis will be done both by intention to treat and per protocol. We anticipate that obstetric ultrasound utilized during the first stage of labor will reduce time to decision making for mode of delivery. This is expected to improve maternal and fetal outcomes as well as maternal satisfaction. Monitoring the impact of new programs is critical for evidence-based medicine. While ultrasound is periodically used in high risk pregnancies at MRRH, it has never been introduced to the general obstetric population. This study will inform hospital policy with regards to the optimal use of obstetric ultrasound during the first stage of labor and will provide evidence to advocate for the expansion of this technology to other regional referral hospitals in Uganda, aiming ultimately to achieve sustainable improvements in maternal and child health.

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FEMALES OF HBAS GENOTYPE HAVE REDUCED CONCENTRATION OF THE MALARIA PROTECTIVE *DEOXYHEMOGLOBIN S* THAN MALES

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The quantity of the intra-erythrocytic deoxyhemoglobin S (Hb S) affects the level of protection against malaria and also the sickling phenomenon. This study reports on significantly lower concentration of Hb S in females than males. Data came from 350 children, aged 12-47 months who participated in a phase 2b malaria vaccine trial. Hemoglobinopathy and G6PD deficiency typing was necessary to ascertain equal representation of these malaria protective traits across the vaccine cohorts. Hemoglobin types (HbAA, HbAS) and % Hb S were evaluated by HPLC. Alpha thalassemia (alpha-thal) and G6PD genotypes were evaluated by PCR. The overall prevalence for HbAS was 20%, 46% for 3 alpha genes and 10% for 2 alpha genes and 14% for G6PD A-. More females of HbAS/ $\alpha\alpha/\alpha\alpha$ genotype had low Hb S than males and had mean % Hb S of 37.5 % + 5.4 SD, compared to 42.0% + 2.5 SD in males of same genotype (P=0.0415). Consistent with reduction of the malaria protective Hb S in females, parasite load was three times higher in females of HbAS without alpha gene deletion (geo mean 3,118, 95% CI: 1779-5465) than males (1,676, 95% CI: 752-3733). The X-chromosome linked G6PD deficiency did not influence the level of Hb S. We conclude that, the low Hb S in these females explains the resultant higher malaria parasite load. We speculate that the low Hb S in females could also explain observations suggesting that the sickling phenomenon tends to be less severe in females than males.

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CLINICAL IMPACT OF MERCURY TOXICITY AMONG INDIGENOUS PEOPLES OF THE PERUVIAN AMAZON

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An estimated 10,000 indigenous peoples live in Madre de Dios, located in the Southern Peruvian Amazon Basin, a region in a state of emergency concerning mercury levels in the region's rivers, some attributed to illicit gold mining but some unexplained. Measurements in 2014 found a mean hair total mercury concentration of 26.3±12.8 µg/g with a range of 1.5 to 53.1 µg/g (WHO safe level of 6 µg Hg/g). Mercury, in different forms, can result in varied presenting toxicities. Because the source and impact of the mercury in these indigenous communities is as of yet undetermined, this pilot study aimed to assess possible mercury-related toxicities as part of an integrated environmental and health assessment. Full physical exams with a focus on nervous system function were conducted on 35 individuals in the isolated Matsigenka community of Maizal. Neurologic assessments were modified for participants older or younger than 10 years. Additionally, urine and blood samples were taken to assess for proteinuria and anemia. Hair samples were obtained to measure mercury levels. Surveys were conducted to determine possible mercury exposure. Environmental samples were also collected. The median age for 13 adults was 29 (22 - 65) and for 22 children was 5 (1 - 11). Physical exam findings were largely normal excepting decreased head circumference in children (69.6% below the 3rd percentile for age). Neurologic assessment among adults revealed normal executive function (79%), possible diminished short-term memory (36.7%), and abnormal cerebellar function (61.6%).

Neurocognitive assessment among children revealed no appreciable deficits but was complicated by language and cultural barriers. Mild anemia was detected in 62.2% (84.6% of adults 45.5% of children) and 21% had at least trace proteinuria. Mercury levels are pending at time of submission. Preliminary analysis shows no overt signs of chronic or acute mercury toxicity in this population. Abnormal findings in neurologic functioning need to be correlated with pending mercury levels. More studies are needed to develop and validate neurocognitive assessment tools for isolated, indigenous populations.

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NATURAL HISTORY OF SAPOVIRUS INFECTION IN A NICARAGUAN BIRTH COHORT: THE SAPOVIRUS-ASSOCIATED GASTROENTERITIS [SAGE] STUDY

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With the advent of rotavirus vaccines, norovirus and sapovirus are now the main viral causes of acute gastroenteritis (AGE) in children. Unlike for norovirus, data on the natural history, development of immunity, and transmission modes for sapovirus are scarce, as most epidemiological AGE studies do not test for sapovirus. We launched a birth cohort and nested case-control study in León, Nicaragua to understand the burden of sapovirus-associated gastroenteritis. We will recruit 400 mother-infant pairs from antenatal care patients in local health posts. Trained field workers recruit mothers aged ≥15 years at home within 15 days of birth. Enrolled mothers provide monthly AGE risk factor data and report weekly if children had AGE in the last week. Mother-infant pairs provide the following routine clinical specimens: breastmilk (baseline, monthly); blood (mother: baseline; child: baseline, 6 weeks, 5 months, semi-annually thereafter); saliva and stool (child: baseline, monthly). During AGE episodes, we collect stool to test for sapovirus in addition to rotavirus and norovirus using real-time polymerase chain reaction. When sapovirus is detected, field workers collect risk factor data from all household contacts of the case. To assess transmission dynamics and duration of viral shedding, a nurse collects one stool sample from each household contact and follow-up stool samples from the case until sapovirus-negative. A trained lab technician collects environmental swab samples from the case household including toilet handle, kitchen faucet, kitchen knife, child's toy, and the mother's hands. We interview two age-matched control households to collect risk factor data; one also provides stool and environmental samples. Enrollment began June 12, 2017. As of March 15, 2018, 357 of 870 eligible mother-infant pairs have been enrolled. 109 stool samples were collected from 138 AGE cases, of which 2 were sapovirus-positive. Weekly surveillance continues until children reach 3 years. This study will yield critical data on sapovirus natural history, immunity, and transmission in children.

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RELATIONSHIP OF DEMOGRAPHIC VARIABLES AND CLASSIFICATION OF LEPROSY CASES IN GEORGIA SINCE THE EARLY 1900S

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Leprosy (also known as Hansen's Disease) is a disease that is diagnosed in approximately 160 people in the USA each year. Recent studies have shown different forms of transmission in the southern US (zoonotic versus person-to-person), but epidemiologic data are lacking about factors associated with multibacillary infection, the most infectious form. Additionally, no work has been done to evaluate if country of origin, between domestic and international birth locations, has a relationship with the type of leprosy diagnosed. We collected data from patient files and surveillance reports on 123 leprosy patients from the National Hansen's Disease Program who had been reported by or lived in the state of Georgia since the early 1900's. Of the 123 patients, 31.71% were born in the USA and 68.29% were born in other countries. Most patients had multibacillary leprosy for both domestic (69.23%) and foreign born (63.10%). Patients born domestically were on average younger than those foreign born, but both had more male patients. A logistic model was built to examine the relationship between country of origin and type of leprosy, based on the World Health Organization's classifications of multibacillary and paucibacillary. We controlled for age, sex, and ethnicity. While the model showed no significant relationship between country of origin and type of leprosy, being Asian or Pacific Islander (97.36% of which were foreign born) was associated with a higher risk of multibacillary infection when controlled with the other variables (aOR = 5.714; 95% CI: 1.254 - 26.287). Leprosy is known to be highly endemic in Micronesia and other areas in Asia. There have been several articles describing the epidemiological data of Asians and pacific islanders in the USA, such as the Marshallese in Arkansas and Micronesians in Hawaii. However, more research should be done to see if these population is at greater risk for multibacillary leprosy. With the global community dedicated to the elimination of leprosy, understanding which populations are at greater risk for this more infectious type of leprosy is important to create targeted interventions and prevention strategies.

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ARBOVIRUS AND MALARIA CO-INFECTIONS AMONG FEBRILE KENYAN CHILDREN

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Although malaria parasites and arboviruses circulate in Kenya with overlapping endemicity, estimates of febrile illness among children due to arboviral infections remain limited. The effects of malaria and arbovirus co-infection on childhood morbidity is even less studied. From an ongoing febrile cohort in Western and Coastal Kenya (NIH R01 AI102918), we recruited pediatric patients presenting to outpatient care in four varied clinical sites. Using multiplexed real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays, we studied the rates of arbovirus viremia and malaria parasitemia among febrile children under the age of 17 years presenting with lymphadenopathy and joint pain. Specifically, 105 complementary DNA (cDNA) samples were tested for dengue (DENV), chikungunya (CHIKV), Zika (ZIKV), West Nile (WNV), Yellow Fever (YFV), Rift Valley Fever (RVFV), and O'nyong-nyong (ONNV) viruses, and pan-*Plasmodium* parasites. Overall, 65/105 (62%) of febrile children were parasitemic by rRT-PCR. 35/105 (33%) of patients were viremic with DENV. 23/105 (22%) presented with DENV-malaria co-infection. 9/105 (9%) presented with CHIKV viremia, 3/105 (3%) of which were co-infected with malaria. There were 2 children with malaria parasites, DENV viremia, and CHIKV viremia. There were no significant differences in age, gender, reported symptoms, bednet use, or hospitalization rates between patients presenting with arbovirus-malaria coinfections and those with solo malaria

parasitemia. Socioeconomic status was similar between the two groups with the exception that co-infected children were more likely to live in a home with dirt floor rather than concrete floor. There were no cases of WNV, YFV, ZIKV, RVFV, or ONNV detected. Given the large number of co-infections detected, we plan to analyze a total of 1255 pediatric cDNA samples to further investigate differences in disease severity and risk factors for arbovirus and malaria co-infections. This study will inform interventions to better care for febrile children visiting outpatient clinics in Kenya.

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THE INTRODUCTION OF INFANT FORMULA AMONG CHILDREN FROM PERI-URBAN SHANTYTOWNS OF LIMA, PERU (1995 - 2016)

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Exclusive breastfeeding (EBF) during the first 6 months of life is considered the first line of nutritional child protection. In Peru, breastfeeding rates have historically been high [66%, Demographic and Health Survey (DHS) 2017]. However, in recent years there has been a reported decline in EBF rates, and presumably increased in the introduction of infant formula (IF) at earlier ages. Surveys such as the DHS are limited to characterize the age of the introduction of IF due to their cross-sectional nature. Over the past three decades, we have carried out three cohort studies in two peri-urban communities in Lima, with daily active surveillance of gastroenteritis and child feeding practices via verbal report from the mother. This offers an excellent opportunity to compare breastfeeding practices among three birth cohorts during other decades (1995-1997, 2007-2010, 2016-2018). We conducted secondary data analysis of the rates of exclusive or predominant breastfeeding (in which some water is allowed), and conducted a survival analysis to calculate the age of introduction of infant formula. A total of 304, 289 and 345 children were recruited in each cohort during the first two months of life. The percentage of children who received exclusive or predominant breastfeeding during the surveillance period was 13.48%, 5.19% and 9.57% [95%Confidence Interval (CI):10.07—17.83%; 3.14—8.45%; 6.87—13.17%] during 1995-1997, 2007-2010 and 2016-2018, respectively. The percentage of children who received IF or mixed feeding during the first 6 months of life was 27.6%, 86.2% and 58.6% (CI:22.9—33.0%; 81.7—89.7%; 53.3—63.7%), respectively. The percentage of children with EBF among our current study (2016-2018) was lower than that reported by DHS. Finally, the average age at which IF was introduced has decreased over the last two decades, and was calculated to be 4 months, 3 months, and 1 month for each cohort, respectively.

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ETIOLOGIES OF GASTRO-INTESTINAL PERFORATIONS IN KILIMANJARO, TANZANIA. PROSPECTIVE HOSPITAL-BASED SENTINEL SURVEILLANCE

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Gastro-intestinal (GI) perforations are highly morbid and potentially fatal events, especially in low income countries where rapid access to surgical interventions and access to adequate post-operative services remain limited. Establishing the etiologies of GI perforation events is fundamental to prioritizing disease prevention efforts. As part of a study to assess the incidence in our catchment of typhoid fever complicated by intestinal perforations, we have conducted two years of prospective sentinel surveillance at two referral hospitals in the Kilimanjaro Region, Tanzania. From March 2017-March 2018, we screened 2,339 surgical admissions, and captured 23 GI perforation events-- 10 gastric, 5 duodenal, 5 ileal, and 3 appendiceal. Of those with perforation, the median age was 47 (range 3-82) years, 20 (87%) were males, and 3 (13%) died in-hospital. Of 23 perforations with samples submitted for histopathology, all had intra-operative tissue or peritoneal fluid submitted for aerobic culture. The histopathologic diagnoses were peptic ulcer disease (n=10), typhoid perforation (n=4), ruptured appendix (n=3), and ulceration or fibrous reaction not otherwise specified (n=3). Microbiologically, 16 had polymicrobial infections, with a total of 40 isolates. *E. coli* (n=10 cases) and *Klebsiella* spp. (n=7 cases) were the most common isolates. *Salmonella enterica* were not recovered from any of the surgical samples. Further histologic confirmation, including immunohistochemistry for *H. pylori* and *S. enterica* serovar Typhi, is in progress. Based on these interim results, peptic ulcer disease and typhoid fever are the most common causes of GI perforation in Kilimanjaro, Tanzania. As both diseases are preventable and treatments are readily available to prevent progression to these severe complications, our interim results have direct implications for health policy and clinical practice in northern Tanzania.

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UNPRECEDENTED HUMAN INFECTION WITH *RICKETTSIA PARKERI* STRAIN ATLANTIC RAINFOREST IN NORTHWESTERN COLOMBIA: CASE REPORT

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Ticks are, along with mosquitos, one of the main vectors of infectious diseases worldwide. In Colombia, there are several reports of Rocky Mountain spotted fever (RMSF) caused by *Rickettsia rickettsii*, but other rickettsial diseases have also been suspected. The goal of this study was to describe the clinical, serological and molecular findings of a new human rickettsiosis in our country. The study patient was a 47-years-old male, previously healthy, and resident in a rural area of the municipality of Turbo (Antioquia), that worked as a farmer. He sought health care because a history of fever, chills, weakness, nausea and hiporexia, after a tick-bite on the abdomen at the level of left iliac spine. When the patient consulted, appear to be normal at the physical examination, without fever and showing just the scar at the inoculation site. The lesion was accompanied by adenomegaly and lymphangitis in the inguinal area. The physician suspected rickettsiosis and sent samples for hematological, biochemical, molecular and serological tests with acute and convalescent phase sera. At the same time, the physician started treatment with doxycycline 200 mg/day. The hemathological and biochemical test were normal, but the creatinine phosphokinase was slightly high. Through

Indirect Immunofluorescence (IFI) using six rickettsia species, we found antibodies against Spotted Fever Group (SFG) with titers between 256 and 1024 but without quadruple difference among the different antigens. PCR showed amplification of genes *sca4*, *gltA* and *ompB* for *Rickettsia* spp. that after being sequenced revealed close homology to *Rickettsia parkeri* strain Atlantic rainforest. These are the first evidences of a new tick-borne rickettsiosis, of minor virulence, in our country; that could explain some of the acute undifferentiated febrile diseases in some Colombian regions.

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CHIKUNGUNYA VIRUS OUTBREAK AND MALARIA CO-INFECTION: IMPACT ON CLINICAL MANIFESTATIONS AND DISEASE SEVERITY IN THE SOUTHERN COAST OF KENYA

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Chikungunya virus (CHIKV), an arbovirus endemic and underdiagnosed in East Africa, causes acute febrile illness and long-term sequelae. In late 2017, an outbreak of CHIKV was documented in Mombasa, Kenya, close to our coastal Kenya study sites. The objective of this study was to determine the impact and severity of this CHIKV outbreak (November 2017 to present date (March 2018)) on the Southern Kenyan Coast and document co-infection with malaria. Our ongoing study (NIH R01AI102918) recruits acutely febrile children aged 1 to 17 years in Ukunda (urban) and Msambweni (rural) health facilities. At the acute visit, physical examination, pediatric quality of life surveys (pedsQL), household demographic and behavioral questionnaires were administered and blood was drawn for CHIKV (RT-PCR) and malaria (microscopy or *Pf* rapid diagnostic test) testing. Of the 259 acute-febrile participants tested, 171 (74.7%) had CHIKV viremia, 87 (33.6%) had malaria parasitemia (98.5% *pf*) and 58 (22.4%) were co-infected with both CHIKV and malaria. Co-infections reported headache more frequently (50%). Those with CHIKV viremia showed more severe clinical manifestations (PedsQL score 77±0.9) compared to other cause febrile illness (PedsQL score 85±1.4). 20 (8.7%) reported local travel, with only 4 (1.7%) reporting travel to Mombasa (epicenter of the ongoing outbreak), suggesting local transmission on the South Coast. These results indicate that CHIKV transmission and co-circulation with plasmodium are on-going in coastal Kenya and that CHIKV is more clinically severe compared to other cause-febrile illness. There is need for robust vector control interventions and ongoing vector and human surveillance in the area to detect and respond rapidly to future outbreaks.

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GROUP B STREPTOCOCCUS IN PREGNANCY AND NEONATAL COLONIZATION AT PRIMARY HEALTH CARE INSTITUTION NIGERIA

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Studies in some sub-Saharan African countries like Zimbabwe, Malawi, Kenya, and the Gambia revealed that Group B Streptococcus (GBS) is emerging as the main cause of neonatal sepsis and meningitis. However, in Nigeria, information on GBS disease prevalence remains sparse. We sourced to isolate GBS from the rectovaginal and neonatal samples that were obtained from a tertiary hospital in a populated area of Osun state and give an updated information on the antibiotic susceptibility patterns, using demographic and clinical parameters. One hundred and seventy samples were collected from consenting mothers and neonate from June 2016 to January 2017. Ninety-Eight (98) GBS isolates were recovered from vaginal, rectal of the pregnant woman at the point of labour and Umbilical cord of the neonate within 24hrs of birth. cultures for the isolation and identification of Group B Streptococcus (GBS) were carried out using the CDC recommended microbiological methods. The presence of resistant genes was examined using PCR . The prevalence rate of GBS maternal and neonatal colonization were 29.4% and 20.6% respectively while 4% of the colonized neonates had nosocomial GBS colonization. There was no significant association between GBS colonization status and age (p >0.05)), parity (p >0.05), obstetric risk factors (p >0.05) and sex of neonate. There was no incidence of GBS infection observed. Resistance to augmentin (88.8%), ampicillin (60.2%), penicillin (47%), tetracycline (34.7%), ceftriaxone (19.4%), clindamycin (13.3%), vancomycin (10.2%) and erythromycin (7.1%) were observed. one of the 8 representatives of the multidrug resistant isolates harboured tetM gene while other resistant genes examined were negative in all MDR isolates. High prevalence of maternal and neonatal GBS colonization has been established among pregnant women and neonates in the study area. However further research is called for using larger sample size and multiple curve studies for adequate extrapolation into the general population.

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NOTES ON THE WEST INDIES: GEORGE PINCKARD AND LATE 18TH CENTURY TROPICAL MEDICINE

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The British and French fought bitterly for the West Indies in the late 18th century. By the mid-1790s, their forces jockeyed for control of cane-producing islands throughout the region. Both countries committed tens of thousands of troops to this effort, hoping to gain economic control over production and exports of cane-based products as well as strategic military outposts. Infectious disease, not least those that are mosquito-borne, ravaged officers and men alike. As such, military physicians and surgeons busied themselves with cases of yellow fever, malaria, and filariasis. Far more combatants succumbed to tropical diseases than to wounds. This case study will highlight the experiences of Dr. George Pinckard. A member of Sir Ralph Abercromby's "expedition" to the West Indies, he kept a detailed account of his experiences in the region, published in three volumes in 1806 as *Notes on the West Indies: Written During the Expedition Under the Command of the Late General Sir Ralph Abercromby*. Pinckard's *Notes* highlight important aspect of tropical medicine and diseases in the late 18th century.

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NO EVIDENCE OF PARASITIC INFECTION IN A SURVEY OF SCHOOL CHILDREN FROM THE MISSISSIPPI DELTA

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Although recent data from high-risk areas in the American South have suggested a high prevalence of parasitic disease, such data on children living in Mississippi are lacking. We enrolled 166 children (median age 8, range 4-13) from a school-based health clinic in Sharkey County, within the Mississippi Delta. We collected data regarding whether subjects resided in homes with flushable toilets, owned pets (dog or cat), were exposed to soil, or had prior diagnosis or treatment of parasitic disease. Samples for stool microscopy and molecular testing were also collected. Microscopy included Kato-Katz and saturated salt flotation. In parallel, real time PCR with primers specific for *Necator americanus, Ancylostoma duodenale, Ascaris lumbricoides, Trichuris trichiura,* and *Strongyloides stercoralis* (as

described by N. Pilotte, S. Williams and others), was performed. Dried blood spots were obtained for detection of antibodies to *Toxocara* spp., *Strongyloides stercoralis, Fasciola hepatica*, *Cryptosporidium parvum* and *Giardia duodenalis*. Of 166 children surveyed, all reported living in households with flushable toilets, 34% reported having a pet dog or cat and 11% reported soil exposure (not wearing shoes when outside). None of the children had prior diagnosis or treatment of parasitic disease. Of 100 stool samples collected, a subset of 18 processed for microscopy were negative, and all were negative by real time PCR. Antibodies to *Toxocara* spp. were detected in 4/100 (4%), whereas antibodies to *S. stercoralis,* and *F. hepatica* were all negative. Serological evidence of exposure to *Cryptosporidium* and *Giardia* was uncommon (2% and 0%, respectively). Overall prevalence of parasitic infection was low in this population, but larger studies of at-risk populations, including adults, are indicated.

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INFLUENZA-LIKE ILLNESS AND DIARRHEA RATES IN FOUR CAMBODIAN VILLAGES: FIVE-YEAR REVIEW

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Cambodia is an agriculturally productive country in Southeast Asia with large poultry populations, mostly in backyard flocks, often with adjacent pig populations. Highly pathogenic avian influenza has been identified in Cambodia, most recently in January 2018. As of November 2017, a total of 56 human cases (including 37 deaths) and 49 poultry outbreaks of influenza A(H5N1) had been recorded in Cambodia. Establishing baseline rates of diarrheal and influenza-like illness (ILI) for vulnerable communities is key to general public health and pandemic preparedness. From February 2013 through December 2017 a total of 5,531 subjects in 4 villages were enrolled and followed weekly resulting in 835,178 person-weeks of observation with 3,681 diarrhea (≥3 loose stools in 24 hours) and 3,885 ILI (T>38°C and cough or sore throat) cases. Overall, the average monthly incidence of diarrhea was 4.65 cases/1,000 person-weeks (range 0-40, SD=2.68), and ILI was 4.27 cases/1,000 person-weeks (range 0-60, SD 4.47). Defining an outbreak as a monthly incidence rate greater than 2 standard deviations above the mean, villages experienced between 0-36 months of diarrhea outbreak cases and 0-14 months of respiratory outbreak cases. One village had consistently high rates of diarrheal disease, and one village had consistently low rates of both ILI and diarrheal disease. The incidence rate and outbreaks of ILI were larger, but seasonal, while diarrheal disease was endemic throughout the study period. Active surveillance, although resource intensive, is more sensitive, and gives accurate estimates for disease incidence rates, facilitating planning and prioritizing public health interventions and further research into appropriate countermeasures.

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DESCRIBING THE ETIOLOGY AND EPIDEMIOLOGY OF ACUTE FEBRILE ILLNESSES IN TWO PROVINCES IN SOUTHERN CHINA

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Due to the lack of appropriate diagnostic testing, little information is available on the etiology of acute febrile illnesses (AFI) in Guangdong and Yunnan Provinces in Southern China. Both provinces experience recurring outbreaks of dengue due to the presence of *Aedes aegypti* and *albopictus* mosquitos, and are at-risk of Zika virus importations from international travelers. We implemented an AFI surveillance project in Guangdong and Yunnan to determine if Zika and other AFI etiologies are circulating or co-circulating in Southern China. At five sentinel hospitals, we enrolled patients between 2 to 65 years of age presenting with AFI (i.e., fever ≥37.5°C with onset within past 7 days and no clear source of infection). We collected clinical and epidemiologic data and blood specimens for testing using the Trioplex (Zika, dengue, chikungunya) PCR assay and TaqMan® Array Card (TAC, 31 viral, bacterial and parasitic pathogens) diagnostic platforms. Data were entered into a web-based data collection system. From June 2017 to March 2018, we enrolled 243 AFI patients (199 from Yunnan, 44 from Guangdong); 14 outpatients and 229 inpatients. The median age was 39 years (ranging 3-65 years). Among adult case-patients (≥18 years of age), 115 (47%) were farmers, 21 (9%) were officer workers, and 11 (4%) were home makers. Ten patients (4%) reported international travel (Laos [3], Venezuela [2], Thailand [1], Myanmar [1], Indonesia [1], Uganda [1] and United States [1]) prior to AFI onset. Of the 215 case-patients for whom TAC testing results are available, 153 (71%) were positive for dengue, 11 (5%) for *Orientia tsutsugamushi*, and 6 (3%) for *Coxiella burnetii*. Other etiologies included *Rickettsia*, *Plasmodium*, *Salmonella enterica* Typhi, and hepatitis E. One patient was co-infected with *C. burnetii* and hepatitis E and another with *C. burnetii* and *Rickettsia*. All patients tested negative for Zika. Further diagnostic testing and analysis is ongoing. This project addresses a knowledge gap about the cause of AFI in Southern China. We anticipate our findings can help improve AFI case management and assist with prioritization of public health interventions.

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UTILITY OF POINT OF CARE ULTRASOUND BY INTERNAL MEDICINE TRAINEES IN A RESOURCE-LIMITED SETTING

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Point-of-care ultrasound (POCUS) is a quick, inexpensive, and noninvasive tool for bedside diagnosis. Few studies have looked at the utility of POCUS in resource-limited settings. This study aimed to investigate the ability of POCUS to assist with diagnosis and management when used by internal medicine trainees in Malawi. This was a descriptive analysis of POCUS studies conducted at Kamuzu Central Hospital in Lilongwe, Malawi through the University of Pittsburgh Medical Center (UPMC) Internal Medicine program. Residents in the Global Health and Underserved Populations track underwent a week-long ultrasound training course. A SonoSite Nanomaxx with a cardiac probe was used. Residents recorded each POCUS study and information including: type of study, indication for scan, findings, and whether POCUS changed diagnosis or management. Patient identifying information was removed. A total of 48 studies were performed by three residents over two weeks. Abdominal ultrasounds were performed most often (n=21), followed by echocardiograms (n=20). POCUS changed the diagnosis in 11 studies (23%) and the management in 12 studies (25%). Two studies led to new diagnoses of extrapulmonary tuberculosis. Six resulted in medication change, three prompted additional testing, and two revealed an indication for a procedure. Of note, three studies helped distinguish pericardial effusion from dilated cardiomyopathy, and in two studies, unnecessary thoracenteses were avoided. POCUS performed by internal medicine trainees changed diagnosis or management in a significant number of studies, suggesting its utility in resource-limited settings. Limitations of the study include small sample size, performer subjectability, possession of only a cardiac probe, and inability to generalize results to other resource-limited settings. Future studies will include more trainees at additional international sites and validation of trainee interpretations by consultants.

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ENDING MASS TREATMENT FOR LYMPHATIC FILARIASIS IN 87 HEALTH DISTRICTS IN CAMEROON

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According to WHO recommendations, countries may be able to stop mass drug administration (MDA) for lymphatic filariasis (LF) if health districts (HDs) complete ≥5 rounds of MDA with epidemiological coverage >65% and antigenemia prevalence <2%. In 2016, 87 HDs from 9 regions of Cameroon achieved this criteria and in 2017 all conducted a transmission assessment survey (TAS1). A cross-sectional cluster survey was conducted in all 87 HDs which were grouped into 30 evaluation units (EU) according to their epidemiological profile and geographical location. The Survey Sample Builder (SSB) provided the clusters as well as the population sizes required for the sampling. The sampled population consisted of children aged 6-7 years. The survey was conducted in the school settings in regions with school attendance rates >75% and in communities in other regions. The Filariasis Test Strip (FTS, Alere™) was used to diagnose circulating filarial antigen. Data management included electronic data capture through smartphones using ODK technology, storage on an ONA platform and processing through an electronic template with control measures. The teams performed calibrated blood smears (CBS) in *Loa loa* co-endemic areas, when the same child got tested positive with two consecutive FTS. A total of 47,977 children were tested. 16 children had an initial positive FTS. Among these, 9 children had a second positive FTS, but none of them showed the presence of *Loa loa* in the day-performed CBS. The prevalence of antigenemia calculated on the basis of the second FTS is 0.01% (95% CI: 0.01 - 0.04%). In conclusion, this TAS1 conducted in accordance with the WHO criteria showed that the transmission of the disease has been interrupted in the 87 HDs which were previously endemic with LF. Cameroon has achieved the criteria to end MDA in 125 of 137 HDs. The National Program should stop the MDAs in these HDs and start preparing the LF surveillance phase.

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LYMPHANGIOGENIC POTENTIAL OF *WITHANIA SOMNIFERA,* A NOVEL THERAPEUTIC AGENT TARGETS HOST-PARASITE INTERACTION IN FILARIAL INDUCED SECONDARY LYMPHEDEMA

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Lymphatic filariasis is a major parasitic infection caused by nematodes *Brugia malayi* and *Wuchereria bancrofti*. In secondary lymphedema, the natural ability of lymphatic vessels to form new lymphatic channels is compromised. Research studies highlights the therapeutic effects of *Withania somnifera (WS)* extracts; however studies addressing the influence of *WS* extracts are necessary in understanding the inhibitory mode of lymphedemal pathogenesis from reversible to irreversible state. It was assumed that both parasitic products and the host inflammatory responses lead to lymphatic dysfunction and lymphangiogenesis. Hence, our present study aimed at evaluating the anti-bacterial and lymphangiogenic property of *Withania somnifera* (Ashwagandha), a highly revered ayurvedic plant in India. The anti-bacterial activity of the aqueous extract of *WS* was carried out by Disc diffusion and Agar well diffusion methods against lymphedema associated *Staphylococcus epidermidis* (MTCC435). We induced co-infection in the normal HaCaT dermal keratinocyte using *Staphylococcus epidermidis* (1×10^5 CFU) and filarial worm homogenate (50ng/ml) for 2hrs at 37°C in 5% CO_2 incubator. High Content Screening Images showed no changes in morphology. RT-PCR data reveals that expressions of Integrin $\alpha5$, Integrin $\alpha9$ and eNOS were attenuated during co-infection. *WS* (50µg/ml) recovers the expression of integrin $\alpha5$ ($p=0.043$) significantly. *In-vitro* lymphangiogenic activity of *WS* extract was evaluated by 2D matrigel using HDLECs in response to whole *Brugia* worm homogenates. These studies showed that adult worm homogenate attenuated the tubular network formation and branching points ($p=0.0001$). Interestingly *WS* extract restored the endothelial tube formation in a dose dependent manner. Taken together, *Withania somnifera* efficiently kills bacteria, augments lymphangiogenesis and may supports reduction of swelling in lymphedema subjects. Thus, *Withania somnifera* can be a novel therapeutic agent in the treatment of filarial induced secondary lymphedema.

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EXPRESSION CLONING AND ANALYSIS OF A PUTATIVE *BRUGIA MALAYI* POU-HOMEODOMAIN TRANSCRIPTION FACTOR

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Neglected tropical diseases (NTDs) are a series of diseases that afflict individuals living predominantly in developing countries. One of the most impactful NTDs is lymphatic filariasis (LF), a disease caused by the filarial parasitic nematodes *Brugia malayi*, *Brugia timori* and *Wuchereria bancrofti*. Current efforts to combat these diseases have been met with considerable success, but the concern of possible drug resistance underscores a need for the development of novel anthelmintic therapeutics that cause minimal harm to the human host. Expression of the parasite's genome undergoes dramatic changes when the parasite is transmitted from the mosquito vector into the human host. Despite the critical need to understand these gene expression changes, little is known about promoters and transcription factors in filarial parasites. The aim of this study is to identify the transcription factors in *B. malayi* that could serve as potential drug targets to help combat filariasis. Through a comparative analysis between whole genome datasets of *B. malayi* and *C. elegans,* we were able to identify the putative transcription factor Bma-UNC-86 as the best candidate for this initial project. In *C. elegans*, UNC-86 has the critical role of initiating and maintaining the expression of the LIM-homeobox gene *mec-3,* thus enabling mechanosensory function. Bioinformatics analyses revealed high homology between the binding sequences of UNC-86 in the promoter region of its target gene, *mec-3*, in both *C. elegans* and *B. malayi*, as well as in three closely related parasitic nematodes, *Brugia pahangi*, *Loa loa*, and *Onchocerca volvulus*. The putative transcription factor Bma-UNC-86 was then cloned and expressed in *E. coli. E. coli* was later induced to produce large quantities of the UNC-86 protein, which was purified and used to perform binding assays with the *mec-3* promoter isolated from *B. malayi*. Data comparing the UNC-86 binding sequences in the *mec-3* promoter of *C. elegans* and *B. malayi* will be presented along with the results of isolation and purification of the Bma-UNC-86 protein and its binding to the *B. malayi mec-3* promoter.

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BRUGIA MALAYI C-JUN-N-TERMINAL KINASE (BMJNK) AND ITS ROLE IN PARASITIC ANTI-STRESS RESPONSES

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Lymphatic filariasis, also known as elephantiasis, is a parasitic disease caused by thread-like nematodes and affects millions of individuals throughout the tropics. It is globally considered a neglected tropical disease associated with acute and chronic morbidity. Current treatment protocols for the disease are highly lacking. One of the key mechanisms by which parasitic nematodes have evolved to successfully establish infection

in their hosts is via evasion of reactive oxygen species (ROS) generated by the host's innate immune system. It has been previously demonstrated that inhibition of the filarial parasite B. malayi stress-activated protein kinase, BmPMK-1, with small molecule inhibitors renders the parasite unable to respond to oxidative stress, as reported previously. A second stress-activated enzyme, c-Jun-N-terminal kinase (JNK), forms a second component of the anti-ROS pathway. In addition, in C. elegans, JNK plays a role in coordinated locomotion, adult lifespan and response to heat and heavy metals. In this work, a recombinant JNK-1 ortholog (BmJNK) from the filarial parasite B. Malayi was expressed and activated in both mammalian and bacterial systems in effort to evaluate the role of this pathway in anti-ROS responses and its potential as a therapeutic target. Inhibition of BmJNK activity was demonstrated using a panel of known human JNK protein kinase inhibitors, along with their evaluation for their in-vitro potency against B. malayi. The results of this work establish BmJNK as a second key regulator of filarial parasite anti-oxidative stress responses and provide strong incentive for further interrogation of this protein as a promising therapeutic target.

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LECTINS COMPLEXED WITH CIRCULATING FILARIAL ANTIGENS IN HUMAN SERA: A SOURCE OF POTENTIAL DIAGNOSTIC TOOLS

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Lymphatic filariasis surveillance programs rely on rapid diagnostic tests (RDTs) that detect a 250 kDa *Wuchereria bancrofti* circulating filarial antigen via a glycan epitope (the AD12 epitope). This glycan is also present on glycoproteins of other nematodes, and contributes to false positive *W. bancrofti* RDT results in some patients with loiasis. We sought to identify human binding partners for AD12-epitope-containing circulating filarial antigens of *W. bancrofti and L. loa* that might serve as tools to discriminate between AD12 epitope-containing filarial glycoproteins. Using a monoclonal antibody specific for the AD12 epitope, we immunoaffinity purified circulating filarial antigens from sera of *W. bancrofti*- and *L. loa*-infected persons and identified co-precipitating human proteins by mass spectrometry. Among proteins co-precipitating with filarial glycoproteins of both species were multiple human and filarial lectins. Further experiments to confirm an interaction between specific lectins and AD12 glycoproteins are ongoing. Further study of these co-precipitating factors may yield novel tools for diagnostics, as well as insight into the poorly-defined innate immunity against filarial infection.

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DEVELOPMENT OF REPEAT-BASED PCR ASSAYS FOR HIGHER SENSITIVITY DETECTION OF *PLASMODIUM FALCIPARUM* AND *WUCHERERIA BANCROFTI*

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Molecular xenomonitoring (MX) can be utilized as a high-throughput method to monitor mosquito-transmitted diseases such as lymphatic filariasis and malaria. By providing an indirect measure of human infection without relying on human sampling, MX can facilitate the early detection of disease emergence or re-emergence in endemic regions of the world. MX can also be used for intervention monitoring. MX relies on PCR to provide both ultra-sensitive and species-specific pathogen detection. Through successful intervention efforts, the incidences of both malaria and lymphatic filariasis are declining. However, to maintain this progress, it is critical that the assays we have are sensitive enough to detect a decreasing number of targets. When designed and optimized correctly, a PCR assay is capable of detecting very limited numbers of target molecules in a given sample. By designing PCR assays based on repetitive genomic elements, an assay's sensitivity is increased because these targets are more highly represented in eukaryotic genomes. Here we report two novel repeat-based PCR assays for the detection of the causative agent of lymphatic filariasis, *Wuchereria bancrofti*, and the causative agent of malaria, *Plasmodium falciparum*. These new PCR assays show improved performance over currently used detection methods when tested using both control DNA and DNA isolated from field-collected mosquitoes.

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THE STAGE-SPECIFIC TRANSCRIPTOME OF *BRUGIA MALAYI*, *AEDES AEGYPTI*, AND ITS *WOLBACHIA* ENDOSYMBIONT *W*BM THROUGH 16 POINTS IN THE NEMATODE LIFECYCLE

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Brugia malayi is one of three filarial nematodes that cause human lymphatic filariasis. We used RNASeq, edgeR, and WGCNA to identify the transcriptomic alterations in *B. malayi,* its *Wolbachia* endosymbiont *w*Bm, and its invertebrate host, *Aedes aegypti,* across the *Brugia* life cycle. A principal component analysis of the 10,398 differentially expressed *Brugia* genes reveals that the samples group into four clusters: (1) the adult male samples, (2) the adult female, embryo, immature microfilariae, and mature microfilariae samples, (3) the 18 hpi and 4 dpi of the vector samples (L1 to L2) and (4) the samples ranging from 8 dpi in the vector to the 20 dpi male and 24 dpi female samples in the definitive host (vector L3 to mammalian L4). Using WGCNA, differentially expressed genes were grouped into modules based on similarities in expression profile. Kinases, phosphatases, PapD-like proteins with major sperm protein domains, calcineurin-like phosphodiesterase, and BTB/Kelch-associated domains were significantly overrepresented in the 1,481 genes upregulated in adult males. DNA binding proteins, transcriptional regulators, and ATP-binding proteins were significantly over-represented in the 1,615 genes that are upregulated in the adult female, embryo, immature microfilariae, and mature microfilariae life stages while G-protein coupled receptors were significantly under-represented. Ribosomal components and translational processes are significantly overrepresented in the 1,032 upregulated genes 18 hpi and 4 dpi of the vector. The differential gene expression profile shows that these genes are more highly upregulated in the L1 life stage, at 18 hpi, relative to the L2 life stage, at 4 dpi. This suggests that DNA replication was important in the prior life stages, generating proteins becomes the dominant process in L1s and L2s. These results as well as those from the rest of the life cycle, the invertebrate host, and the *Wolbachia* endosymbiont *w*Bm will be presented along with functional validation of differentially expressed genes from adult females.

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ONCHOCERCIAISIS: DISCOVERY OF NOVEL THERAPEUTIC AGENTS FROM SELECTED MEDICINAL PLANTS TO SUPPORT CONTROL AND ELIMINATION EFFORTS

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Onchocerciasis is the world's second leading infectious cause of blindness. Its control is currently hampered by the lack of a macrofilaricidal drug and by severe adverse events observed when the lone recommended microfilaricide, ivermectin is administered to individuals co-infected with *Loa loa.* Therefore, there is the need for a safe and effective macrofilaricidal drug that will be able to cure the infection and break transmission cycles, or at least, an alternative microfilaricide that does not kill *L. loa* microfilariae (mf). Seventeen organic crude extracts from three medicinal plants, *Tragia benthami*, *Piper umbellatum* and *Chromolaena odorata* were screened *in vitro* against the bovine model, *Onchocerca*

ochengi parasite *and L.loa* mf. Activity of extracts was assessed on adult male worms and mf by microscopic evaluation of motility reduction, while percentage inhibition of MTT conversion to formazan was used to assess activity on female worms. Cytotoxicity and acute toxicity of active extracts were tested on monkey kidney cells and Balb/c mice, respectively. Phytochemical screening of active extracts was also done using standard methods. At 500 µg/mL, all extracts showed 100 % activity on *Onchocerca ochengi* males and microfilariae, while 12 of them showed 100 % activity on female worms. The methylene chloride extract of *Piper umbellatum* leaves was the most active on adult males and mf (IC_{50}s: 16.63 µg/mL and 31.25 µg/mL, respectively). On female worms, the methylene chloride extract of *Chromolaena odarata* was the most active with an IC_{50} value of 16.23 µg/mL. Extracts active on *O. ochengi* worms were also highly active on *L. loa* microfilariae, with IC50s of 35.12 - 13.9 µg/mL. Active extracts were generally more toxic to the worms than to cells and showed no acute toxicity to Balb/c mice. Phytochemical screening revealed the presence of saponins, steroids, tannins, tritepenoids and flavanoids in the promising extracts. These results unfold potential sources of novel anti-Onchocerca lead compounds and validate the traditional use of these plants in onchocerciasis treatment.

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A SINGLE-CENTER, FIRST-IN-HUMAN, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL-GROUP STUDY TO INVESTIGATE THE SAFETY, TOLERABILITY AND PHARMACOKINETICS OF ESCALATING SINGLE DOSES OF EMODEPSIDE (BAY44-4400) IN HEALTHY MALE SUBJECTS

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Onchocerciasis (river blindness) is caused by the parasitic nematode *Onchocerca volvulus*. It is the world's second-leading infectious cause of blindness with visual impairment and blindness being the most severe complications of the disease. Programs for the treatment and control of onchocerciasis through mass drug administration (MDA) of ivermectin have been in place for over 20 years. Ivermectin targets the microfilarial stage of the parasite and temporarily sterilizes, but does not kill the adult worms. Therefore, programs require population at risk to be given ivermectin at regular intervals for many years, which is a large economic burden and its implementation being difficult in endemic countries. Thus, there is an urgent need for a macrofilaricide, targeting adult *Onchocerca* worms, as an alternative treatment for case management, to shorten MDA or tackle difficult to treat areas. Emodepside is a cyclooctadepsipeptide anthelmintic drug registered for animal health and marketed by Bayer AG in combination with praziquantel or toltrazuril. Emodepside targets different life stages of *O. volvulus*, including the adults and is being investigated for the oral treatment of onchocerciasis in human. A First-In-Human clinical study investigated the safety, tolerability and pharmacokinetics of single doses of emodepside in healthy male subjects. The trial was conducted in compliance with ICH guidelines and relevant EU Directives. Primary endpoints were safety and tolerability variables (adverse events; physical and neurological findings; vital signs; electrocardiograms; clinical laboratory variables and in the last cohort, also ophthalmology assessments) and pharmacokinetics variables. Emodepside (single dose, 1 to 40 mg) or placebo was administered to 8 subjects (randomized 6:2) in each cohort as an oral solution. Overall, emodepside was found to be safe up to 40 mg and well tolerated up to 20 mg, with rapid absorption, dose-proportional increase in exposure (AUC and Cmax) and a long terminal half-life of approximately 500 h.

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EXPERIMENTS TO IDENTIFY THE INTENDED USE TO DETECT ACTIVE INFECTIONS FOR THE *ONCHOCERCA VOLVULUS* URINARY BIOMARKER *N*-ACETYLTYRAMINE-*O*,β-GLUCURONIDE (NATOG)

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Efforts to eliminate the filarial nematode *Onchocerca volvulus* are based on mass drug administration programs with the microfilaricide ivermectin. Monitoring the efficacy of these programs is largely dependent on existing diagnostic tools that either cannot identify active infections (Ov16 IgG4 serology) or perform poorly in low transmission settings (skin snip microscopy). There is a need for tests to identify infected patients in elimination of transmission settings. Urine *N*-acetyltyramine-*O*,β-glucuronide (NATOG) was recently identified as a specific biomarker for active onchocerciasis. We tested NATOG levels in urine from cases (mf+ and/or qPCR+) and controls (Ov16 ELISA negative, skin snip negative) to evaluate whether it would be an effective biomarker for active infections. The 51 cases consisted of 28 individuals from Kitgum/Lamwo Districts in Uganda, 4 from Jimma Zone in Ethiopia, and 19 from Orientale Province in the Democratic Republic of Congo. The 85 controls lived in areas post-endemic for onchocerciasis; 48 individuals were from Suchitepequez/Chimaltenango Provinces in Guatemala and 37 were from Hoima district in Uganda. Of the 51 cases, 17 (33%) had NATOG levels above the 13 µM threshold for a positive test. Geographical variability was observed as 75% of 4 cases from Ethiopia and 68.4% of 19 cases from DRC were positive, while in Uganda only 3.8% of 28 cases had positive results. The post-endemic population control samples were all negative for NATOG (specificity = 100%). Thus, while NATOG appears to be specific biomarker for onchocerciasis, it has limited sensitivity. Only 1/3 of cases had positive results, though these results appear to have been driven by NATOG levels detected in samples from Ugandan case patients. The explanation for this finding is unclear. Further work is needed to clarify the intended use of NATOG to detect active infections in multiple settings. A highly sensitive test that identifies active infection would assist programs measure progress towards the elimination of transmission.

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UPDATE ON THE BIOLOGY AND ECOLOGY OF *CULICOIDES* SPECIES OF THE SOUTH WEST REGION OF CAMEROON WITH IMPLICATIONS ON THE TRANSMISSION OF *MANSONELLA PERSTANS*

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Culicoides are tiny and stout blood sucking flies that have a worldwide distribution. Where present, they constitute a biting nuisance or are

involved in the transmission of pathogens to humans, domestic and wild animals. Data on the *Culicoides* species of the Southwest region of Cameroon date as far back as the 1950s. Since then there have been ecological transformation in the area due to agriculture and deforestation which may have affected the *Culicoides* fauna. Furthermore, the role of the different species of *Culicoides* of this region on the transmission of *Mansonella sp* is not fully elucidated. We therefore designed this study to fill these gaps in scientific knowledge. Eight species of *Culicoides* (*C. bedfordi, C. inornatipennis, C. fulvithorax, C. grahamii, C. imicola, C. milnei, C. neavei and C. kumbaensis)* were collected using light traps and human baits during this study. *C. grahamii* was the most abundant species, followed closely by *C. milnei*. Three species (*C. milnei, C. grahamii* and *C. inornatipennis)* were common to all breeding sites. Only four species (*C. inornatipennis, C. fulvithorax, C. grahamii,* and *C. milnei)* were collected on humans. Anthropophylic species were more abundant ($p < 0.001$) in the evening (4-7 pm) compared to the morning period (6-9 am). *Culicoides milnei* was the potential host for the development of *Mansonella perstans* microfilariae. The biting cycle of the main vector *C. milnei* shows 3 peaks (9-10pm, 1-2 am, 3-4 am), the highest being from 9-10pm. *Culicoides milnei* has been demonstrated to be the major vector of *M. perstans* in this part of Cameroon and its activity takes place essentially in the night with the most prominent peak between 9 and 10 pm. The findings from this study offer opportunities to carry out transmission surveys for Mansonellosis due to *M. perstans.*

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EVALUATION OF URINARY N-ACETYLTYRAMINE-O,β-GLUCURONIDE (NATOG) AS BIOMARKER FOR *ONCHOCERCA* INFECTION AND/OR *ONCHOCERCA* ASSOCIATED EPILEPSY

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Infection with *Onchocerca volvulus* (OV) is associated with skin and eye disease and possibly also epilepsy (onchocerca associated epilepsy (OAE)), placing a large burden on affected people and their families. Currently there is no biomarker to diagnose OAE. We investigated the potential of the OV metabolite N-acetyltyramine-O,β-glucuronide (NATOG), which is excreted in the urine of infected individuals as a biomarker for active OV infection and/or OAE. OV infection was diagnosed by counting skin microfilariae (mf), nodule count and OV16 IgG4 rapid test (SD bioline). Urine was collected from uninfected individuals without epilepsy (controls, N=20), OV infected individuals without epilepsy (N=20) and infected individuals with mild (N=105) or severe epilepsy (>2 epileptic seizures/month, N=93) in the Democratic Republic of Congo. NATOG concentrations were determined by liquid chromatography tandem mass spectrometry. One-way ANOVA was performed (GraphPad Prism) with α=0.05. We observed a significant trend of increased NATOG levels with increased mf in the skin ($P<0.001$), and there was significantly more NATOG in the urine of individuals with severe epilepsy compared to mild epilepsy ($P=0.002$). No significant difference in urinary NATOG was observed between OV infected and non-infected individuals without epilepsy ($P=0.09$) and there was no increased level of NATOG with an increased number of OV nodules ($P=0.18$). However, there was significantly more urinary NATOG in the urine of OV infected individuals when comparing the entire population (with and without epilepsy ($P=0.015$)). Moreover, there was an increasing trend in NATOG levels from controls to OV infected people, people with mild epilepsy and people with severe epilepsy ($P=0.016$). In conclusion, urinary NATOG only is not an informative biomarker for OAE. Despite an association between urinary NATOG and mf count, there was no significant difference between infected and uninfected individuals without epilepsy. However, there was an increasing trend with more severe epilepsy and urinary NATOG was higher in infected individuals with epilepsy.

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ELEVATED PLASMA INOSINE AND HYPOXANTHINE CONCENTRATIONS, AND URINE *CIS*CINNAMOYL GLYCINE CONCENTRATIONS AS BIOMARKERS FOR ACTIVE *ONCHOCERCA VOLVULUS* INFECTIONS

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The neglected tropical disease onchocerciasis, or river blindness, is caused by infection with the filarial nematode *Onchocerca volvulus*. Current estimates indicate that 17 million people are infected worldwide, the majority of them living in Africa. Today there are no non-invasive tests available that can detect ongoing infection and that can be used for effective monitoring of elimination programs. In addition, to enable pharmacodynamic studies with novel macrofilaricide drug candidates, surrogate endpoints and efficacy biomarkers are needed but are non-existent. We describe the use of a multimodal untargeted mass spectrometry based approach to identify onchocerciasis associated metabolites in urine and plasma and of specific lipid features in plasma of infected individuals (*O. volvulus* infected cases: 68 individuals; lymphatic filariasis cases: 8 individuals; non-endemic controls: 20 individuals). This work resulted in the identification of the plasma metabolites inosine and hypoxanthine as biomarkers for filarial infection, and of the urine metabolite *cis*-cinnamoylglycine as specific biomarker for *O. volvulus*. During the validation study, metabolite-specific cutoffs were determined (inosine: 24.2 ng/ml; hypoxanthine: 1380 ng/ml; CCG: 29.7 ng/ml) and sensitivity (inosine: 74.5%; hypoxanthine: 86.2%; CCG 82.9%) and specificity (inosine: 95.7%; hypoxanthine: 89.2%; CCG 82.2%) profiles were established. With the availability of targeted LC-MS procedures, the full potential of these 3 biomarkers in macrofilaricide clinical trials, MDA efficacy surveys, and epidemiological transmission studies can be investigated. These large-scale validation approaches are necessary prior to the definition of an intended use for any of these markers.

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A LONG-TERM *BRUGIA MALAYI* LYMPHATIC ENDOTHELIAL CO-CULTURE SYSTEM AND ITS VALIDATION AS AND ANTI-*WOLBACHIA* DRUG ASSESSMENT MODEL

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Filarial parasites are the causative agents of lymphatic filariasis (LF) and onchocerciasis. Current elimination programmes rely on up to 12 annual mass drug administrations with standard anti-filarial drugs, targeting only the microfilarial (mf) stage of infection. There is therefore a need to identify new short-course curative drugs (macrofilaricides) in order to accelerate elimination time frames. A promising drug target is the filarial endosymbiotic bacteria, *Wolbachia*, which can be depleted with antibiotics to initiate slow, safe killing of adult parasites. Reproductively active adult female parasites have a limited life-span in culture, therefore filarial drug screens heavily rely on *in vivo* models. Here, we have developed a long term *in vitro* co-culture system to maintain adult female stages of the human lymphatic filariae, *Brugia malayi,* using primary human lymphatic endothelial cells (LEC) To optimise the model, we examined motility and survival, uterine release, metabolic activity and *Wolbachia* titres of female

B. malayi following aseptic isolation from infected immunodeficient mice and cultured parasites over periods up to 28 days. Readouts were compared against freshly isolated *ex vivo B. malayi* or human embryonic kidney (HEK) cell monolayer co-cultures as non-specific feeder cells. Having verified LEC monolayers stably supported these parameters for 14 days+, we then assessed *Wolbachia* reductions and dynamics after treatment with physiologically relevant doxycycline exposures. Doxycycline induced significant reductions in *Wolbachia* after continuous treatment for 7 or 14 days, whilst drug removal after 7 days led to non-significant reduction after 7 days washout. This was in parallel with previous *in vivo* data. Thus, we have robustly validated an *in vitro* co-culture system which may be used to examine anti-*Wolbachia* agents for superiority compared with doxycycline.

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IMPROVING COMMUNITY VOLUNTEER ENGAGEMENT AND IMPLEMENTATION OF MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS THROUGH MICROPLANNING: A CASE STUDY OF PORT-AU-PRINCE, HAITI

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Achieving and maintaining adequate mass drug administration (MDA) coverage for lymphatic filariasis (LF) in urban areas remains challenging. Declining MDA coverage in areas of metropolitan Port-au-Prince (PaP), Haiti is putting the country at risk of not meeting the global goal of eliminating of LF. The Ministry of Public Health and Population (MSPP) and partners proposed a two-phase microplanning protocol in five communes of PaP to improve MDA implementation. In phase one, the 2017 MDA distribution posts were geolocalized to identify access gaps, defined as a maximum walking distance of >500 meters to the nearest distribution post. Supervision area (SA) boundaries were delineated in consultation with community leaders (CLs). Access gaps were identified in 60% of SAs. In phase two, 10 two-day microplanning workshops were held with a total of 73 CLs, 240 community promoters (CPs), and 43 other key MDA stakeholders. The objectives were to review past performance and engage stakeholders in MDA improvement planning; identify and address gaps in SAs; review MDA distribution post analysis and modify strategies to improve access; and review roles and responsibilities of MDA personnel. Retrospective pre-testing was used to evaluate microplanning workshops. Participants used a five-point scale to rate their understanding of past performance, SA boundaries, roles and responsibilities, and their perceived engagement by MSPP. They simultaneously rated their previous year's attitudes and their attitudes following the two-day microplanning workshop. Scores were analyzed using paired t-tests. In comparison to rankings of the previous year, there was an increase in participant understanding of: past performance by 1.34 points (standard deviation [SD]=1.05, p<0.001); the borders of SAs by 1.14 points (SD=1.3; p<0.001); their roles and responsibilities by 0.71 points (SD=0.95, p-<0.001); and sense of engagement by 1.03 points (SD=1.08, p<0.001). Participatory stakeholder workshops during MDA planning increases engagement and may improve personnel performance and contribute to achievement of drug coverage targets.

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COMPARISON OF LYMPHATIC FILARIASIS MASS DRUG ADMINISTRATION COVERAGE IN COASTAL REGION OF KENYA, 2016 - 2017

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Lymphatic Filariasis (LF) is one of the diseases targeted for elimination. It has affected over 120 million in the world and about 3.5 million people in Kenya. We aimed to compare the reporting rates and coverage of LF in 6 counties within the coastal region. This was a programmatic operation where mass drug administration (MDA) of albendazole (ALB) and diethylcarbamazine (DEC) was distributed in 6 counties in both 2016 and 2017 in the endemic coastal region of Kenya. A register of all residents living in the area was developed just before the MDA by community health volunteers (CHV). The registered data was used to estimate the number of tablets needed by each of them. The CHVs distributed the tablets in houses they had registered. The target population were aged two years and above but excluded pregnant women. However, coverage denominator was the total population in the target counties and sub counties. Data was collected and aggregated using registers and tally sheets then analyzed using Microsoft Excel and Epi-Info. A population of 2,209,277 was targeted in 2016 and 3,017,897 in 2017. County reporting rates were above 93% in both years. The targeted population improved across the 6 coastal counties with Kwale County having the greatest increase in target population from 202,358 in 2016 to 588,388 in 2017. County coverage in 2017 was between 71.1% in Kwale and 86.5% in Tana River county while in 2016 was 63.3% in Mombasa and 92.5%. Tana River, Mombasa and Kilifi counties had improved coverage from 65.7%, 63.3% and 77.6% to 86.5%, 73.6% and 83.3% respectively. The other 3 coastal counties had a slight decrease in coverage. Two counties in 2016 compared to 3 in 2017 achieved the 80% recommended coverage. There is an improvement on the numbers targeted and the coverage across the 6 coastal counties.

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ACCEPTABILITY OF A TRIPLE DRUG REGIMEN FOR ELIMINATION OF LYMPHATIC FILARIASIS: RESULTS OF A MULTICENTER COMMUNITY BASED STUDY

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A recent multicenter community-based safety trial compared a standard two-drug treatment (DEC, albendazole, DA) with a three-drug treatment (ivermectin, DEC, albendazole, IDA) for lymphatic filariasis (LF). Acceptability was measured using mixed methods. In each country, acceptability of the treatments was assessed with a survey of 400 randomly selected participants (age ≥14 yrs) within four months of treatment. The mean acceptability score (MAS, range 9 – 36) was

calculated based on 9 indicators. A score of ≥18 was considered acceptable. Focus group discussions were conducted in each country in communities where IDA was administered. 1621 participants were surveyed in 4 countries (n=759 DA arm; n=862 IDA arm). Mixed model analyses revealed no significant difference in acceptability by treatment arm, microfilaremia status, gender or age. MAS was above 18 for all 4 countries, yet scores differed among the countries (India= 26.8+/-2.1; Haiti= 29.1+/-3.7; Indonesia 30.6+/-2.8; PNG= 32.6+/-3.5) (p<0.001). Variables significantly associated with higher MAS included personal knowledge of people with LF, personal concern about getting LF, being happy about quantity of pills, and knowing participation in mass drug administration (MDA) is important for the community. MAS were significantly higher in participants who felt better after taking pills, had improved energy, and perceived reduced itching or skin sores. 12 focus group discussions were analyzed for emergent themes. These results identified the safety study team's prompt management of adverse events (AEs), trust in the drug administration process, and professionalism as key factors affecting acceptability. A history of many rounds of MDA prior to the safety trial may have contributed to lower MAS in India and Haiti. In conclusion, both LF treatments were equally acceptable with high scores in all 4 country studies. Key features of drug distribution during the safety trial were appreciated by communities. Knowledge of LF in the community, being informed about treatment and the importance of personal participation in MDA for community benefit emerged as important messages.

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CONSENSUS DIAGNOSTIC CRITERIA FOR SCABIES ALLOW INTEGRATED MAPPING AND SURVEILLANCE OF NEGLECTED TROPICAL DISEASES

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Scabies was added to the list of WHO neglected tropical diseases (NTDs) in 2017, and the WHO noted the need for further mapping of disease prevalence in many regions. Opportunities exist for integrated mapping of scabies with other NTDs such as filariasis, onchocerciasis, trachoma and STH, but have been limited by a lack of diagnostic standardisation. We aimed to develop consensus criteria for the diagnosis of scabies to facilitate this integration. We conducted an iterative, consensus (Delphi) study involving international experts in the diagnosis of scabies. Panel members were recruited through expression of interest and targeted invitation of known experts from a range of global settings. The Delphi study consisted of four rounds of anonymous surveys, moving from generation and ranking a long list of possible features (rounds 1 - 2), to development and refinement of a series of draft criteria (rounds 3 - 4). Panel participants (n=30) were predominantly highly experienced clinicians, representing a range of clinical settings and all continents. Based on initial rounds, a draft set of criteria were developed, incorporating three levels of diagnostic certainty - Confirmed Scabies, Clinical Scabies and Suspected Scabies. In the final round, there was a very high level of agreement (>90%) for all levels of criteria and subcategories. Adoption of the criteria was supported by 96.4% of panel members. Consensus criteria for the diagnosis of scabies have been established with very high agreement. The 2018 criteria for the diagnosis of scabies can be used as part of integrated NTD mapping to further define the burden of scabies, and for surveillance after control interventions. Formal validation is the next step.

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DEVELOPING THE FIRST NATIONAL DATABASE AND MAP OF LYMPHATIC FILARIASIS CLINICAL CASES IN NEPAL

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Lymphatic filariasis (LF) is widely endemic in Nepal with an estimated 25 million people at risk of infection across 61 endemic districts. The National LF Programme is making good progress towards the elimination targets and in recent years has scaled up managing morbidity and preventing disability (MMDP) activities. This study highlights the LF Programme's approach in determining the number of people affected by lymphoedema and hydrocele, and developing the first national database and morbidity map. This information is essential for validation of LF elimination as a public health problem. The first data on morbidity was collected during national mass drug administration (MDA) campaigns in 2012 when community drug distributers (CDDs) identified and reported 28,855 LF clinical cases (6049 lymphoedema; 19247 hydrocoele cases) across 46 districts. Overall, the highest number of cases were reported in the lowland Terai region of the country and this information has helped to initiate more than 7000 hydrocoele surgeries across 40 district hospitals. To obtain more detail data and to better understand the spatial-epidemiological patterns, extensive patient searching is being conducted across all endemic districts over the next 12 months with specific georeferenced data on the patient's age, sex, condition severity (mild, moderate, severe) and number of acute dermato-lymphangioadenitis (ADLAs; acute attacks). The use of an innovative SMS mHealth tool is helping to created readily accessible databases, and district and regional disease burden and disability maps. Four districts have been mapped to-date with 7541 cases (2049 lymphoedema; 5551 hydrocoele) found, a further eight districts are currently in progress and a further 20 districts planned for November 2018. This information will allow the Nepal LF Programme to appropriately plan and deliver a basic package of care to those suffering from the disabling and debilitating clinical manifestations of LF across the country.

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AN ASSESSMENT OF MOSQUITO COLLECTION TECHNIQUES FOR XENOMONITORING OF ANOPHELINE-TRANSMITTED LYMPHATIC FILARIASIS IN GHANA

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Anopheles mosquitoes are the main vectors of lymphatic filariasis (LF) in Ghana. Monitoring vectors is relevant to ascertain transmission of LF. This may require the best sampling method that can capture high numbers of specific species to give indication of transmission. Gravid anophelines are good indicators for assessing transmission due to close contact with humans through blood meals. It is not clear which sampling method will be appropriate for xenomonitoring of anopheline-transmitted Lymphatic Filariasis. This study compared the efficiency of an Anopheles gravid trap (AGT) with other available mosquito collection methods including the box and CDC gravid, light, exit and BG-sentinel traps, indoor resting collection and pyrethrum spray catches across two endemic regions of Ghana. The AGT showed high trapping efficiency by collecting the highest mean number of anophelines per night in the Western (4.6) and Northern (7.3) regions compared to other outdoor collection methods. Additionally, indoor resting collection was similarly efficient in the Northern region (8.9) where vectors exhibit a high degree of endophily. AGT also,

showed good trapping potential for collecting *An. melas*, which is usually difficult to catch with existing methods. Screening of mosquitoes for infection showed, 3.86%, *W. bancrofti* and 3.57%, *Plasmodium spp.*, in *Mansonia spp.*; 0.80%, *W. bancrofti* and 2.15% *Plasmodium spp.* in *An. gambiae* from the Western region; and a 3.01% *W. bancrofti* and 3.27% *Plasmodium spp.* from the Northern region. The AGT has shown to be appropriate for surveying *Anopheles* populations and can be useful for xenomonitoring both LF and malaria.

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CHALLENGES SURROUNDING THE CONTROL OF SOIL-TRANSMITTED HELMINTH INFECTIONS AND SCHISTOSOMIASIS IN THE PHILIPPINES: PERSPECTIVES OF HEALTH OFFICIALS, HEALTH WORKERS AND COMMUNITY MEMBERS IN TWO RURAL PROVINCES

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In the Philippines, the spread of Soil-Transmitted Helminth (STH) and Schistosomiasis (SCH) infections among school-aged children is controlled through school-based mass drug administration (MDA) of preventive chemotherapy in accordance with World Health Organization guidelines. The Philippine Department of Health (DOH) has also called for improved water, sanitation, and hygiene (WASH) provisions in schools to complement these disease control efforts. However, they have not successfully met their program targets for MDA or WASH in many rural provinces, including Llorente and Oras. Implementation of health policies is further complicated by the newly decentralized health structure in the Philippines, in which local government units (LGUs) enact national guidelines set forth by the DOH. Our objective was to understand the practices and beliefs regarding MDA and WASH in schools from the perspectives of community members, healthcare workers, and government officials within Llorente and Oras. We performed 12 qualitative, semi-structured key informant interviews with members of the DOH and LGUs, and 8 focus groups with community health workers, schoolteachers, and parents. Interviews were either transcribed verbatim in English or translated with the assistance of an interpreter, and were coded in an open, iterative fashion. Codes were then reviewed to identify patterns and themes. Our participants consistently identified the following community-wide challenges as barriers to successful program implementation: 1) lack of health education; 2) limited support from officials in the form of funds and infrastructure; 3) local distrust and misunderstanding of the programs; and 4) persistence of open defecation. Informants attributed the slow progress of STH and SCH control in school-aged children to poor coordination of public health efforts between DOH and community members; and unreliable support of health and hygiene initiatives from LGUs. This study emphasizes the need for leaders of decentralized health systems to evaluate how they partner and engage with both community members and LGUs in implementing MDA and WASH programs.

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IMPACT OF PRE MDA TRAINING ON SCALING UP COVERAGE FOR MASS DRUG ADMINISTRATION- MDA FOR THE FIVE KEY NTDS IN TANZANIA

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NTDs are among the greatest burden to the health and socio-economic well-being of majority poor in Tanzania. Majority of Tanzanians are at risk for co-infection of two or more of the five NTDs. Mass Drug Administration (MDA) for transmission control is a key intervention toward control and elimination of NTDs. The pre-MDA-training using cascade approach is designed to ensure effective mass drug administration, explore areas for improvement and upkeep required coverage for MDA. MDA approach at NTD Control Program is managed through monitoring and Evaluation, Supply Chain Management and MDA's Implementation and Supervision unit. Two coordinators from education and health departments foster the MDA implementation at regional and district level. Front line health workers (FLHW), teachers and community drug distributors (CDDs) are responsible for execution of drugs at community and school level. Our objective was to evaluate the impact of streamlined Pre MDA-cascaded training on raising the effectiveness and required therapeutic coverage for MDA campaigns in Tanzania. To attain high quality training to implementer; training manual for FLHWs and CDDs was reviewed in 2017. The teaching aid for the Regional and District Trainer of Trainee was updated. Standardized test to be administered before and after training for knowledge testing was reviewed. Term of reference for National trainers was refined and bound strict to every trainer. Application of the tools was affirmed at every level of training. Simulation and practical exercises was integrated as key and important sessions during training and supervision during training was strengthened. After institutionalization of revised training approaches, MDA coverage raised from an average 87% in 2016 to 93% in 2017 for all Key NTDs. Increased understanding of the NTDs among implementer and reduced community resistance to the drugs was observes. Mainstreaming pre-MDA training in a way that assures high quality at all levels of implementation remains a promising approach towards effective and increased MDA coverage as we are matching towards total control and elimination goals for NTDs by 2020.

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SUCCESSFUL INTEGRATION OF A TRANSMISSION ASSESSMENT SURVEY FOR ONCHOCERCIASIS AND LYMPHATIC FILARIASIS IN FOUR DISTRICTS IN NIGERIA

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Mass administration of medicine (MAM) with ivermectin (Mectizan) is the recommended strategy for elimination of onchocerciasis (OV) and, combined with albendazole, for lymphatic filariasis (LF). As areas begin reaching sufficient numbers of effective rounds of MAM, decisions on when to stop need to be made. The30-cluster, random-sample transmission assessment survey (TAS) is routinely used by LF programs to guide MAMstopping decisions. In 2016, the World Health Organization (WHO) published criteria for MAMstopping decisions for OV. This can also be used to identify areas of persistent transmission for further intervention. The present study useda modified TAS (iTAS) to informboth LF and OV stopping decisionsin 4 formerly-endemic local government areas (LGA) in Nigeria that had completed 16-17 years of MAM for OV and 5-6 years of MAM for LF. In each LGA, 3,000 children ages 5-9 were targeted for sampling via cluster random sampling of 30 schools. All were tested for LF antigenemia with the filariasis test strip (FTS) and for antibodies against OV and LF via the Ov16/Wb123 Biplex rapid diagnostic test (RDT). Dried blood spots were also taken. All 4 LGAs had FTS prevalence below the critical threshold for passing LF TAS (*i.e.,* stopping MAM).One LGA, Bade, passed the threshold for stopping OV treatment with 0% positive Ov16 results. The other 3 LGAs, Karim Lamido, Gashaka, and Bekwarra, did not pass with 0.03%, 1.7% and 3.6% Ov16 positivity respectively. In Gashaka and Bekwarra, positive results clustered in a few schools. In Gashaka, Ov16 prevalence ranged from 0-23%, with 11 schools having 0 positives. In Bekwarra, Ov16 prevalence ranged from 0-13% with 5 schools having 0 positives. Investigation ofthese 'hotspots' of ongoing OV transmission identified after many years of MAM is needed. This new iTAS appears to be successful in facilitating stopping decisions for both programs and identifying areas in need of further intervention.

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SHADOW PUPPETS AND NEGLECTED DISEASES: EVALUATING A HEALTH PROMOTION PERFORMANCE

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'*Rama and the Worm'* is a shadow puppet production targeting neglected diseases in Central Java. It is an entertainment-based intervention study to promote health by reducing the impact of parasitic diseases such as soil-transmitted helminths (STH). The study uses a traditional Javanese shadow puppetry (*wayang kulit)* as a vehicle in village communities to disseminate health education and promote behaviour change to prevent diseases caused, primarily, by inadequate sanitation and poor hygiene. The health education messages contained in the play, although using traditional characters and themes, required the creation of a completely new narrative script, using characters and plot lines familiar to the *wayang kulit* repertoire, but placing them in new situations that relate specifically to health promotion objectives. The musical accompaniment is a musical-cultural fusion involving both the traditional musical accompaniment of Javanese gamelan and Western instruments, especially rock band instrumentation. The intervention was piloted in a village in Central Java, Indonesia using a pre/post design with both qualitative and quantitative analysis. A total of 96 male and female villagers, aged between 7 and 87 years provided both baseline and follow up data. Participant knowledge and behaviours related to gastrointestinal and helminth-related disease were assessed before and after the intervention through a questionnaire administered by interview. Results revealed statistically significant improvements in both knowledge and behaviour related to gastrointestinal and helminth disease. Findings of the study indicate the *wayang kulit* performance is an effective health education tool. The results provide proof of concept with scaling up the next step forward. The *wayang kulit* production provides a significant additional component for an integrated, comprehensive approach to reduction and elimination of STH infection.

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THE ROLE OF SOCIAL SIGNALING IN COMMUNITY DEWORMING: EVIDENCE FROM A FIELD EXPERIMENT IN KENYA

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A randomized control trial (RCT) was conducted to assess which social incentive intervention (voting ink, calendars, or bracelets) is the most effective at increasing adult community deworming treatment adoption. A community deworming exercise to treat soil-transmitted helminths was implemented in partnership with the Kenyan Ministry of Health in 3 counties in Western Kenya where an existing school-based deworming program operates. The health ministry, through its Community Health Extension Workers, offered free deworming treatment to adults at fixed-point locations, such as churches, and explicitly emphasized the public good aspect of deworming. Two types of social incentives were distributed to dewormed adults in the form of colorful bracelets and ink. The bracelets and ink made the decision to deworm or abstain from treatment observable and allowed adults to signal to others that they contributed to protecting their community from worms. Further, a calendar was introduced as a separate private incentive that was comparable in its consumption value to the bracelet but could not easily be observed by others. Finally, individuals in each study arm were randomly selected to receive text messages as a reminder to get treatment (this intervention included a household visit to recruit subjects). Findings show that offering bracelets incentivizes deworming treatment take-up (8.4 pp), increasing coverage within communities. Offering bracelets was found to be a low-cost intervention and was a more cost-effective incentive to increase treatment adoption when compared to ink and calendars. Text messages led to large increases (12.8-15.3 pp) in deworming take-up across all arms, supporting existing evidence on the power of personal nudges. The combination of bracelets and text message reminders led to treatment take-up as high as 60.9% (a 24.2 pp increase) in the adult community. These findings may help inform other community-based mass treatment and neglected tropical disease (NTD) programs aiming to reach higher treatment coverage.

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TESTING A COMMUNITY BASED VECTOR CONTROL APPROACH FOR HUMAN AFRICAN TRYPANOSOMIASIS IN DRC

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DRC is the most affected country by Human African Trypanosomiasis (HAT). HAT is a parasitic disease caused by the infection with trypanosome through the bites of tsetse flies. WHO's objective is to eliminate HAT by 2020. Mathematic models demonstrate that to reach this objective it is necessary to combine active and passive surveillance, diagnosis and treatment strategies with a vector control (VC) strategy. Tiny targets (pieces of clothes impregnated with insecticide) are a new, cheap and effective tool to control tsetse flies. Currently vector control using tiny targets in the DRC is an expert-directed activity. Communities are little or not involved. However, in the context of vector control, community based approaches are often recommended because the action is implemented near to the daily activities of the affected people. Nevertheless, community based HAT vector control interventions are rare. The aim of this research is to evaluate if a community-led VC activity is feasible and suitable in a HAT elimination context in DRC. A community-led intervention is being implemented since 2017 in three villages in the Kwilu province. This intervention is evaluated through the Action-Research methodology and data is collected with different qualitative and quantitative methods. This research reveals that communities involved are capable of leading an effective VC activity. Further the communities implicated in the community-led VC show a better understanding and acceptance of the presence of the tiny-targets than parallel communities where expert-led tsetse control activities took place. During the study costing data is also collected which allows to present information regarding the costs per km² covered by community-led VC. However, it remains to evaluate if this approach is feasible at a bigger scale, cost effective and appropriate to reach the elimination objective for 2020.

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A COMMUNITY STUDY OF THE IMPACT OF SEMIANNUAL ALBENDAZOLE ON LYMPHATIC FILARIASIS AND SOIL-TRANSMITTED HELMINTH INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Implementation of mass drug administration (MDA) with ivermectin plus albendazole (Alb) for lymphatic filariasis (LF) has been delayed in Central Africa, because ivermectin can induce serious adverse events in people with very high *Loa loa* microfilaremia. In 2012, the WHO recommended use of Alb MDA together with vector control to combat LF in areas with co-endemic loiasis. This strategy has been supported by the results of a 3-year community trial conducted in the Republic of Congo, where baseline circulating filarial antigenaemia (CFA, assessed using the immunochromatographic card test - ICT) and microfilaremia (Mf) rates were 17.3% and 5.3%, respectively. In June 2014, we started a parallel trial in an area with higher baseline infection rates (31.6% for antigenemia

and 11.8% for microfilaremia) in the Democratic Republic of the Congo. Therapeutic coverage for the population > 2 years of age was ~ 70% at all treatment rounds. Evaluation at year 1, 2 and 3 showed that the circulating filarial antigen (assessed using Filarial Test Strip - FTS) rate in the community decreased from 31.6% in 2014 to 28.7% in 2015, 20.6% in 2016 and 15.2% in 2017. Among 438 individuals who were examined both in 2014 and 2017, 149 were positive at baseline; 69 of those 149 (46.3%) cleared their antigenemia in 2017. Mf prevalence in the community decreased to 8.1% in 2015, 3.7% in 2016 and 1.9% in 2017. Mf density (geometric mean of positive counts) decreased from 171 mf/mL in 2014 to 104.4 mf/mL in 2015, 68.1 mf/mL in 2016 and was 197.8 mf/mL in 2017. A total of 63/72 (87.5%) microfilaremic individuals at baseline have cleared their microfilaremia in 2017. Soil-transmitted helminth infections were monitored using Kato-Katz method. Between 2014 and 2017, prevalence of *Ascaris lumbricoides* infection in the community decreased from 14% to 3.6%, prevalence of hookworm infection from 58.6% to 40.9%, and prevalence of *Trichuris trichiura* from 8% to 4.8%. Year 4 and final results from this study will be presented at the meeting. They should provide additional evidence regarding the use of semiannual MDA with Alb for elimination of LF in central Africa.

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TARGETING THREE NEGLECTED TROPICAL DISEASES WITH ONE TRIPLE THERAPY MASS DRUG ADMINISTRATION IN FIJI IS SAFE

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The first case of lymphatic filariasis (LF) in Fiji was reported in 1876 and the first mass drug administration program was implemented in 1961. Local efforts were enhanced by joining the Pacific Elimination of LF program of the World Health Organization with regular annual MDA using diethylcarbamazine and albendazole (DA) from 2002. Despite acceptable coverage of MDA, the Eastern Division of Fiji has been unable to break transmission. The addition of ivermectin to DA (IDA) is hypothesised to be more effective tool in eliminating LF. Fiji was one of 5 sites involved in cohort event monitoring to determine safety of IDA. In 2017, the villages of Rotuma and Gau islands, were randomised to receive IDA or DA in a 2:1 ratio. Over 97% of participants were actively followed up for adverse events (AE) daily for 2 days following treatment and passively followed thereafter to complete 7 days of monitoring. Half of the IDA villages received an additional dose of ivermectin after completion of the safety monitoring period in order to evaluate the efficacy of 1 versus 2 doses of ivermectin on community prevalence of scabies and soil transmitted helminths at 12 months. MDA was given to 3431 of the 3812 enrolled participants, 2272 received IDA and 1159 received DA. Of those treated, 502 (14.6%) were LF antigen positive and 139 (4%) were microfilariae positive. AEs of any severity were experienced by 591 participants (17%), with no difference between treatment groups. AEs were more likely if participants were LF antigen positive (27% vs 16%) or mf positive (43% vs 16%). Women were less likely to be mf positive (2% vs 6% in men) but more likely to report an AE (19% vs 16% respectively). 93% of AEs were mild, not affecting normal daily activities of work or school. Of people experiencing an AE after treatment, the top 5 symptoms were fatigue (32%), headache (10%), nausea (9%), dizziness (7%) and muscle weakness (7%). There were 3 serious advents - 2 occurred in the DA group. None were directly attributable to the MDA. The safety of IDA is comparable to standard DA for LF in Fiji. Adopting this LF elimination strategy is likely to have additional benefits on other NTDs of high burden in Fiji.

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STATISTICAL MODELLING OF THE RELATIONSHIP BETWEEN MICROFILARIAE AND ANTIGENAEMIA PREVALENCE OF LYMPHATIC FILARIASIS INFECTIONS

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Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease targeted for global elimination by 2020. The majority of global cases are caused by three species of nematode worms: *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Historically, surveys for LF have relied upon direct measures of infection (observation of microfilaria in night blood). In recent years, the mapping of LF has been greatly facilitated by the use of simple and rapid detection tests for *W. bancrofti* (antigen-based test) and *Brugia* (antibody-based test), based on the immuno-chromatographic test (ICT card test), which avoids the need to collect blood at night and the time-consuming preparation and examination of blood slides. Whilst highly practical, these diagnostics are less useful for describing ongoing transmission intensity, particularly after treatment. For example, while it is known that estimates of antigenaemia are generally higher than estimates of microfilaraemia, the extent and spatial heterogeneity of this relationship is not clear and changes after the implementation of control activities. Using both geostatistical and machine learning approaches we explore the relationship between microfilaraemia and antigenaemia prevalence across sub-Saharan Africa, and we show how to combine the two approaches to improve risk map predictions. The output of this research can lead to better informed implementantion strategies for the elimination of lymphatic filariasis.

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SUSTAINABILITY ASSESSMENT OF NEGLECTED TOPICAL DISEASE PROGRAM IN ETHIOPIA

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Since the launch of the federal Neglected Tropical Disease (NTD) Master Plan in 2013, significant achievements have been made in putting in place structures at the Federal Ministry of Health (FMOH) to coordinate NTD programing. With these structures in place, NTD control and elimination interventions have resulted in intensified country-wide mapping and, consequently, enhanced evidence-based policies, guidelines and program implementation. While these efforts have contributed to improved health outcomes, one of the main challenges that limits NTD programing from being fully sustainable is limited government ownership of the NTD programs. In partnership with CIFF and the Ethiopian FMOH, Dalberg conducted a sustainability assessment of NTD programing in Ethiopia in October 2017. The objective of this assessment is to provide FMOH and key partners with an understanding of areas where the government needs support to reach sustainability as well as provide recommendations on potential ways to achieve. Ethiopia has shown progress towards sustainability for most approaches. Policy and leadership is sustainable in most aspects, but there have been no detailed conversations about transition at national or regional levels. Budget is largely sustainable, however domestic funding and government ownership of budgets is limited. The government has taken steps to own delivery systems, going forward it needs to strengthen cross-sectoral collaboration. Organizational capacity is sustainable in most aspects, but it lacks sufficient HR capacity to meet programmatic needs. Although there is government demand for data-driven planning, there are parallel reporting systems used and limited data analysis. In order to ensure sustainability is important to develop transition plans which should outline the exact roles and responsibilities played by partners and government stakeholders, and should detail how these responsibilities can slowly transition over time.

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WHO 2016 COVERAGE SURVEY GUIDELINES: A FIRST IMPLEMENTATION AND RESULTS IN TWO HEALTH DISTRICTS IN THE NORTHWEST CAMEROON

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The North West region of Cameroon is endemic to the main PCT NTDS. On the way to achieve elimination goals of onchocerciasis, an MDA campaign was implemented in the region in 2017 targeting at least 80% of the population. The objective was to verify the accuracy of the reported coverage. The study also helped in assessing peoples' perception and compliance on CDI. An ancillary objective of this exercise was to test the feasibility of the WHO 2016 guidelines for MDA coverage surveys and was the first time to be implemented in Cameroon. The survey was cluster-based and took place in two health districts, purposely selected. Data collection was paper-based and quantitative and qualitative interview techniques. Individuals of 5 years old and above were targeted. A total number of 3,454 individuals were surveyed. 57.5% of survey participants were female. The median age of the survey participants was 23 years old. Overall, 86.6% (95%CI: 85.5 – 87.7%) of interviewees received mectizan during the campaign. There were 89.6% in Fundong and 83.6% in Bali. Overall compliance was found at 84.9% (95%CI: 83.6 – 86.0%). The administrative coverage previously reported in Bali was higher than survey coverage, leading to conclusion of an over reporting. In Fundong, these results showed in contrary an under reporting. Reasons for non-compliance were programmatic issues (absenteeism, unawareness) and individual factors (underage, pregnant/breasfeeding, sick). The WHO tool showed to be adapted for a field implementation as tool for onchocerciasis elimination monitoring but implementers will gain in doing daily sample size monitoring through electronic data collection or daily calls to surveyors. Recommendation for program improvement include further investigation of non-compliance with the network analysis method and field supervision. Pictures in the poster used should be enlarged and texts should be made in simple words.

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COMPOSITION OF THE MICROBIOME OF CHAGAS DISEASE VECTORS AND ITS INTERACTION WITH THE PARASITE *TRYPANOSOMA CRUZI* FOR THE DEVELOPMENT OF INNOVATIVE VECTOR CONTROL STRATEGIES

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Chagas disease is caused by the parasite *Trypanosoma cruzi*, which is transmitted to humans and other mammals mainly through the contaminated feces of hematophagous triatomine bugs. This is an anthropozoonosis that constitutes a major public health problem from the southern United States (US) to Argentina. *T. cruzi* infects at least 6 million people in endemic areas and is responsible for 10,000 deaths a year according to the WHO. In the absence of vaccines and effective therapies against the infection, accurate knowledge of the vectors´ ecology constitutes an essential need for the implementation of sustainable control strategies. Among the important vector ecological characteristics, the knowledge of the composition of the intestinal miocrobiome is particularly relevant. Indeed, the bacteria present in the intestine of the insect can positively or negatively affect the infection by *T. cruzi*. Unfortunately, this key aspect involved in the transmission of *T. cruzi* has been very little studied in triatomines and with techniques lacking sensitivity. Here, we use a metabarcoding approach to identify the diversity of intestinal bacteria of *Triatoma dimidiata*, the main vector in Yucatan, México. We analyse the variations of this diversity according to the food sources (blood hosts) of the vector and evaluate the impact of the microbiome on vector infection by *T. cruzi* comparing the microbiome between infected and uninfected insects. Based on these acquired knowledge, we will be able to propose vector control strategies based on modification of the microbiome composition.

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THE TRYPANOCIDAL EFFECT OF BENZNIDAZOLE IS INCREASED BY PROBENECID, A BLOCKER OF CHANNEL BASED IN PANNEXIN AND INNEXIN

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Trypanosoma cruzi is the etiological agent of Chagas Disease, a public health problem in Latin America. Previous studies showed that probenecid (a blocker of channel based on pannexin and innexin proteins) enhances the effect an antimalarial drug, interestingly until now presence of gap junction proteins has not been reported in any unicellular organisms. This study shows that probenecid produces a chemosensitization of *T. cruzi* to benznidazole, enhangins its antitripanocidal effect. This finding also suggest the presence of functional gap junction membrane channel related to innexin. The chemosensitization of probenecid to benznidazole was evaluated by flow cytometry in epimastigote of *T. cruzi* Clone H510 C8C3*hvir*. The proliferation and transformation was evaluated by counting in a hemocytometer, in presence and absence of probenecid. The search of putative sequences of gap junction protein was performed using TritrypBD database, and the membrane topology was analyzed with PROTTER software. Innexon functional assay was performed by dye uptake in epimastigotes, FITC-Dextran (70,000 Da) was used as control for cell membrane damage. Probenecid (400 µM) produce chemosensitization of epimastigotes to benznidazlole (1000 µM) increasing the parasites mortality of 14.7 to 30.5 %, and also reduced the transformation from trypomastigote to amastigote at 4 h (~35%) but did not affect the proliferation. *T. cruzi* presents a putative protein of 257 aa, with 4 TMD, intracellular C and N-terminals, and the highly conserved innexin motif YYQWV. Epimastigotes showed an innexon-like activity promoted by extracellular Ca^{2+}/Mg^{2+} free solution, permeable to YOPRO-1≤DAPI ≤EtBr ≤EvBl. Dye uptake was blocked by probenecid (400 µM), flufenamic acid (50 µM) or heptanol (2 mM). In conclusion, ihe chemosensitization of *T. cruzi* produced by probenecid, suggest the presence of membrane channel that belongs to the gap junction family. This channel cloud be a new molecular target for the development of novel drugs against *T. cruzi.*

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ENDOGENOUS GENE TAGGING OF *PFR2* AND *PFR5* IN *TRYPANOSOMA CRUZI* USING CRISPR/CAS9

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The flagellum of *Trypanosoma cruzi* contains the paraflagellar rod (PFR) an extra-axonemal scaffolding. The PFR consists of a lattice of cytoskeletal filaments that lies alongside the (9 + 2) microtubular axoneme, beginning at the flagellar pocket and extending to the flagellar tip. The PFR has only been observed within the phylums Euglenozoa and Dinoflagellata, although many eukaryotic organisms with long flagella have extra-axonemal structures that accommodate enzymes and regulatory proteins along with serving as scaffolding. The exact function and basic molecular composition of the PFR has yet to be determined although the major structural components, PFR1 and PFR2 and several minor proteins have been identified. The PFR is not only a complex structure that has been shown to be critical for motility, it also constitutes a unique set of proteins that are known to be immunogenic and provide protective immunity in

the *T. cruzi* system. PFR5, a hypothetical minor component of the PFR, contains a PFR internal domain and an SH3 binding domain. Currently it is unknown if the protein product of the *pfr5* gene localizes to the flagellum. We use the Cas9/pTREX-n vector to endogenously tag *pfr5* to investigate the subcellular localization of the protein. PFR2 serves as proof of principle for this system as previous studies have shown that this protein is a major component of the PFR. This gene tagging protocol uses a hemagglutinin (HA) tag system which incorporates a 3xHA tag onto the C-terminal end of the protein. Localization of the 3xHA tagged proteins are determined via immunofluorescence. Study of the organization of the PFR with its restricted evolutionary presence will help improve understanding of this unique structure.

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DEEP SEQUENCING REVEALS MULTICLONALITY AND NEW DISCRETE TYPING UNITS OF *TRYPANOSOMA CRUZI* IN RODENTS FROM SOUTHERN USA

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The parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, a prominent anthropozoonosis in Central and South America that is an emerging concern in the southern United States (US). *T. cruzi* encompasses a diversity of genetic strains that have been grouped into seven discrete typing units (DTUs: TcI to TcVI and Tcbat). Epidemiological assessments of Chagas disease hinge on understanding the diversity and distributions of DTUs, many of which appear to be associated with distinct transmission cycles, geographical distributions, tissue tropism and clinical manifestations. Increasing evidence of multiclonal infection suggests, however, that transmission dynamics are more complex than currently acknowledged. In this study we explored the nature of *T. cruzi* infection in small rodents from New Orleans (LA, USA), an enzootic region of North America. We characterized the full complement of DTUs in rodent hosts through Next Generation metabarcoding, as conventional PCR and Sanger sequencing approaches only detect the dominant genotype in biological samples. We assayed DTU diversity in 5 rodents (3 sylvatic, 2 urban) selected from a cohort of 72 *T. cruzi* positive individuals, confirmed through a conventional PCR assay. The intergenic region of the mini exon gene was then amplified to distinguish TcI from other non TcI DTUs. Phylogenetic trees were inferred by Maximum Likelihood method to identify DTUs in the samples. We detected distinct and varying assemblages of DTUs in the rodent hosts. TcI was the dominant DTU in two sylvatic rodents that also carried TcII, TcV, and TcVI. The third sylvatic rodent was predominantly infected with TcII, TcV, and TcVI. The urban rodents also carried diverse DTU assemblages, but had proportionally more TcII and TcVI than their sylvatic counterparts. These results affirm that mammalian hosts can concurrently harbor a diverse complement of parasites, and indicate that there is greater diversity of DTUs present in North America than previously thought. Further investigation is warranted to understand the role of commensal rodents as a reservoir for *T. cruzi* in sylvatic and peridomestic environments.

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EVIDENCE FOR ASSOCIATION OF THE INTEGRATED ENDOPLASMIC RETICULUM STRESS RESPONSE WITH *LEISHMANIA DONOVANI* -INDUCED CUTANEOUS LEISHMANIASIS IN SRI LANKA

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The integrated endoplasmic reticulum stress response (IERSR) is a pathway that operates to regulate endoplasmic reticulum (ER) homeostasis in various conditions that increase the cellular stress, including infections. Three trans-membrane proteins, namely, IRE-1 that activates XBP-1, the pancreatic ER kinase (PERK) that phosphorylates the eukaryotic translation initiation factor 2 and transcription factor 6 (ATF6) are known to regulate the IERSR pathway. Emerging evidence suggest an important role for IERSR in the pathogenesis of *L. amazonensis* and *L. braziliensis* induced cutaneous leishmaniasis (CL). This study describes the association of IERSR with *L. donovani*-induced CL in Sri Lanka. Skin biopsies from 8 CL lesions and 8 healthy controls were included in the study and proteins were extracted and digested by trypsin. Two –dimensional liquid chromatography separation and subsequent tandem mass spectrometry was performed using a Thermo Orbitrap Fusion HPLC MS/MS system. Proteins were identified by searching against human data bases and the significance threshold for peptide identification was set at $p<0.05$. Patient and control groups were compared by ANOVA method. The p value obtained was adjusted for multiple correction using Benjamini-Hochberg procedure. All the proteins with an adjusted p value <0.01 were considered as significantly expressed between the groups compared. Significantly expressed proteins thus identified were entered into UniProt human database and converted to their corresponding gene names. Pathway analysis was done using the Reactome pathway portal, version 3.2. Out of the three pathways regulating IERSR, two namely ATF6 ($p<0.001$) and IRE-1 ($p<0.05$) were seen to be among the significant pathways. Induction of the IRE1/XBP1 arm of IERSR leads to less oxidative stress and is a mechanism by which these parasites survive within macrophages. Association of IERSR with *L.donovani*-induced CL is evident, which needs to be further validated.

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STUDY OF A NEWLY DISCOVERED ONCOGENIC DOMAIN OF YINP FROM *LEISHMANIA* SP.

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Recently, our group has identified in *Leishmania major* promastigotes a gene, called *LmjYinP* We found that the highest expression of *LmjYinP* occurs during the infective stage of the parasites (metacyclics). Focusing on the relation between *LmjYinP* gene expression and *Leishmania* pathogenesis, we studied the infectivity of *LmjYinP* overexpressing parasites. Our results showed that *LmjYinP* over-expression enhanced parasites infectivity *in vitro*, and, increased metacyclogenesis process. To evaluate LmjYinP suitability as a drug-target, we analyzed *in silico* drugs that may bind to the protein. We built up a model of LmjYinP protein. Our docking results provided us information about the druggability of this protein, supporting that LmjYinP could be consider as a robust therapeutic target. In fact, our data showed that LmjYinP strongly interacts with drugs such as Paromomycin and Amphotericin B, used in clinic. Moreover, the physicochemical properties of the predicted binding sites allow us to propose new drugs potentially suitable against leishmaniasis. We also described that Lmj*YinP* is a highly conserved gene containing two main domains: YinP (N-terminus) and a newly discovered putative oncogenic domain named ONC. Therefore, we aimed to elucidate the function of ONC domain in *Leishmania* proteome as well as in the human host. This domain was overexpressed and downregulated in *L. major*. On the other hand, ONC from LmjYinP was also expressed in different mammalian cells to analyse the impact of the expression of this parasite domain in other

eukaryotic organisms. Additional experiments were also performed to better characterize the aforementioned domain (ONC) as well as Lmj*YinP,* a novel molecule involved in pathogenesis and drug resistance. All our data reinforce the hypothesis that LmjYinP and ONC domain may be robust druggable targets against leishmaniasis.

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COMPARISON OF WHOLE-GENOME SEQUENCING, SANGER SEQUENCING, AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS FOR *LEISHMANIA VIANNIA* MIXED AND HYBRID INFECTION SPECIES IDENTIFICATION

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The prognosis and treatment of leishmaniasis is largely dependent on the infecting species. Varying degrees of disease severity are present, in addition to mixed and hybrid infections, which pose diagnostic challenges. The causative species identification of these types of infections relies on standard techniques such as restriction fragment length polymorphism (RFLP) analysis, and Sanger sequencing (SS). Whole-genome sequencing (WGS) is a robust and increasingly cost-efficient alternative that can improve *Leishmania* species identification. Here, we validated and compared WGS as a potential alternative to RFLP-SS standards in a cohort of 3 ATCC strains (*L. V. braziliensis*, *L. V. guyanensis* and *L. V. panamensis*) and 5 clinical *Leishmania Viannia* isolates of potential hybrids or mixed infections. DNA extraction, followed by *internal transcribed spacer1* (*ITS1)* and heat shock protein 70 (*HSP70*) PCR-RFLP was carried out and SS was performed for the *ITS2*, cysteine proteinase b, and *HSP*70 loci. After *de novo* assembly, sequences were mapped, and homology compared to both ATCC strains and reference genomes at NCBI. All samples went through a diagnostic pipeline to identify species present by all three techniques. Concordant validation was assessed within each isolate, over a 6-week period, to determine if final identification was consistent with the initial. All ATCC isolates were confirmed to be single-species of either *L. V. braziliensis*, *L. V. guyanensis*, or *L. V. panamensis* by WGS; whereas RFLP-SS was unable to definitively speciate two of three isolates. Clinical isolates were identified as a combination of single-species, mixed, and hybrid infections of a variety of *Viannia* species by WGS; while RFLP-SS was largely unable to definitively speciate four of five isolates. We have utilized WGS to differentiate mixed and hybrid infections by species. Ambiguous infection samples, previously speciated by RFLP-SS, were more reliably discerned into single-species, mixed, and hybrid categories by WGS. WGS is a potentially useful alternative to RFLP-SS for the diagnosis and species identification of complex tegumentary *Leishmania* infections.

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THE GP63 GENE CLUSTER IS HIGHLY POLYMORPHIC IN NATURAL *LEISHMANIA (VIANNIA) BRAZILIENSIS* POPULATIONS, BUT FUNCTIONAL SITES ARE CONSERVED

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GP63 or leishmanolysin is the major surface protease of *Leishmania* spp. involved in parasite virulence and host cell interaction. As such, GP63 is a potential target of eventual vaccines against these protozoa. In the current study we evaluate the polymorphism of gp63 in *Leishmania (Viannia) braziliensis* isolated from 41 American tegumentary leishmaniasis (ATL) cases from Corte de Pedra, Brazil. Parasites were obtained from lesions by needle aspiration and cultivation. Genomic DNA was extracted, and 405 bp fragments, including sequences encoding the putative macrophage interacting sites, were amplified from gp63 genes of all isolates. DNA amplicons were cloned into plasmid vectors and ten clones per *L. braziliensis* isolate were sequenced. Alignment of cloned sequences showed extensive polymorphism among gp63 genes within, and between parasite isolates. Overall, 45 different polymorphic alleles were detected. The predicted peptides showed overall conservation below 50%. In marked contrast, the conservation at segments with putative functional domains approached 90% (Fisher's exact test $p<0.0001$). Synthetic peptides based on these short, conserved sequences were capable of significantly inhibiting *in vitro* infection of monocyte derived macrophages by *L. braziliensis* in a dose dependent manner. These findings show that gp63 is very polymorphic even among parasites from a same endemic focus, but the functional domains interacting with the mammalian host environment are conserved.

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EVALUATION OF REAL TIME PCR FOR DIAGNOSIS OF POST-KALA-AZAR DERMAL LEISHMANIASIS (PKDL) IN AN ENDEMIC *FOCI* OF BANGLADESH

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Post-kala-azar dermal leishmaniasis (PKDL) is a sequel of kala-azar or visceral leishmaniasis (VL) that is found in visceral leishmaniasis (VL) endemic countries including Bangladesh. Because of these enigmatic cases, the success of National Kala-azar elimination program (NKEP) is under threat. Unlike other endemic regions, the macular form of PKDL is the most common form in Bangladesh and diagnosis of these cases is more difficult than other forms. Until now, the diagnostic method for PKDL cases in endemic regions is limited to clinical examination and serology using the rK39 rapid test or microscopy. A suitable and accurate alternative method is necessary. In this study, we investigated the application of real time PCR as a potential method for diagnosis of PKDL in comparison with microscopy. For this study, 91 suspected macular PKDL cases from the Mymensingh district, Bangladesh were enrolled following diagnosis through clinical examination and the rk39 RDT. All cases were treated for PKDL and responded well after completion of the treatment. During enrollment, skin biopsy was collected from each patient and both microscopy and real time PCR were performed for detection and quantification of *leishmania donovani* body (LDB) and LD DNA respectively. Real time PCR detected 83 cases among all suspected PKDL patients with an encouraging sensitivity of 91.21% (83.41-96.13) whereas microscopy showed 50.55% (39.86-61.20) sensitivity only. These findings suggest that real time PCR is a promising tool for diagnosis of PKDL in endemic regions. In addition to diagnosis, the quantitative ability of this method could be exploited for after-treatment prognosis and cure assessment of PKDL cases.

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NOVEL TETRACYCLIC IRIDOID COMPOUNDS ISOLATED FROM *MORINDA LUCIDA* BENTH INDUCES CELL CYCLE ARREST, PHENOTYPIC CHANGES AND APOPTOSIS IN *LEISHMANIA DONOVANI*

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Leishmaniasis threatens 350 million people with approximately 12 million people suffering from the disease worldwide. Antimonials remain the first line of treatment for many years. Despite increased progress in drug development, current drugs are challenged by high toxicity, and resistance issues, which necessitates continuous efforts to identify alternative chemotherapy. We previously identified novel tetracyclic iridoid compounds, molucidin, ML-2-3 and ML-F52 from the leaves of *Morinda lucida*, to have anti-trypanosomal activity. In this current study, we hypothesized that these compounds would have anti-*leishnania* properties since *Leishmania* also belongs to the class kinetoplastida and therefore may share some metabolic-pathways to trypanosomes which our compounds could interfere with. We therefore assessed the efficacy of the compounds and their mode of action on *Leishmania donovani* and *L. major*. A 50% inhibitory concentration (IC_{50}) was determined after 48 hours of treatment with or without compounds by Alamar blue assay. Molucidin and ML-F52 showed significant activities against *L. donovani* (molucidin = 2.94 μM, and ML-F52 = 0.91 μM), and against *L. major* (molucidin = 1.85 μM, and ML-F52 = 1.77 μM). No significant difference in IC_{50}s between species and compounds was observed. In further analysis by Nexin assay, ML-F52 induced significantly higher apoptotic effect (64.7 %) relative to molucidin (36.1 %) at twice IC_{50}. Though no significant change in the Kinetoplastid Membrane Protein was observed by immunostaining, Molucidin and ML-F52 induced morphological changes in promastigotes. Also in further analysis, treating of promastigotes at IC_{50}, molucidin induced significantly higher ''nectomonad-like" forms (50%), characterized as slow replicating forms, relative to ML-F52 (7%). ML-F52 induced higher 'cell-rounding' (93%) with either a shortened or complete loss of flagellum observed. Further analysis by Fluorescence-Activated Cell Sorting showed an enhanced cell growth arrest at the G2/M phase induced by Molucidin with significant peaks in sub-G1 populations.

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CAPACITY BUILDING PLATFORM FOR CLINICAL AND OPERATIONAL RESEARCH ON HUMAN AFRICAN TRYPANOSOMIASIS

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For the last 11 years, the HAT platform has been conducting research based on needs in the field and using an approach adapted to realities on the ground. The HAT platform is composed of representatives of national sleeping sickness control programs (NSSCP) and research institutions in its 9-member countries (DRC, Angola, Sudan, Guinea, Congo, Chad, Central African Republic, Uganda, and South Sudan), and foreign research groups such as DNDi, ITM, FIND, Swiss TPH, IRD, MSF, and University of Edinburgh, with the World Health Organization as an observer. The HAT platform is also collaborating with other African research platforms such as the East African Trypanosomiasis Network (EANETT). We will present a summary of the advances made within the framework of the HAT platform in the development of diagnostic and therapeutic tools, with the development and update of target product profile for HAT treatments, discovery of rapid diagnostic tests and development of oral treatments for HAT. Other achievements will be presented in this poster; including an important investment in different trainings, biannual scientific meetings and steering committees, newsletter publication, as well as current advances in clinical and operational trials conducted or in progress. This approach is adapted to the realities of the field and enables local partners, who are the end users of the results, to be important actors involved in clinical research. During these periods, most of NSSCP have adopted the results of this research to adapt their national policies, with or without the support of the HAT platform.

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EVALUATION OF TWO NOVEL POINT OF CARE DIAGNOSTICS FOR CUTANEOUS LEISHMANIASIS

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The diagnosis of CL is mainly based on a variety of clinical signs, but requires laboratory confirmation as these symptoms are not very specific. The laboratory diagnosis of CL is mainly based on microscopic examination of Giemsa's stained skin scrapings or fine needle aspirates, but this approach is reported to have a low sensitivity. Recently, two point of care (POC) diagnostic tests have become available that could aid the diagnosis of CL: 1) a rapid diagnostic test (RDT), the CL Detect™ Rapid Test (InBios International Inc., USA), an immunochromatographic RDT for the detection of the peroxidoxin antigen of *Leishmania* species in CL skin lesions; 2) a molecular diagnostic test based on loop mediated isothermal amplification (LAMP) of a conserved region in the 18 S ribosomal RNA gene of *Leishmania* species and a specific sequence in the kinetoplast DNA of *L. donovani*, which is being marketed as Loopamp™ *Leishmania* Detection kit (Eiken, Japan). In the present study, the diagnostic performance of these two novel diagnostic tests was determined in comparison to microscopy in a specialized dermatology clinic and qPCR performed in a specialised molecular biology laboratory in Suriname, a country endemic for CL mainly caused by *L. guyanensis*. In total 93 suspected CL cases were enrolled in the study with a mean age of 33.6 years, predominantly male (92.5%), and with main location of the lesions on arm or leg. The infecting species was confirmed as *L. guyanensis* (97.5%) or *L. amazonensis* (2.5%). In total 79 (84.9%) of the suspected CL cases were found positive with microscopy. In contrast, only 31 (33.3%) were found positive with RDT testing. Molecular testing in Suriname found 75 (80.7%) cases positive with LAMP testing and 81 (87.1%) with qPCR. The RDT had a very low sensitivity compared to microscopy (36.7%) or qPCR (35.8%), respectively, due to a high number of false negative results. In contrast, the LAMP test had a moderate specificity (42.9%) and good sensitivity (84.8%) compared to microscopy, and a good specificity (91.7%) and sensitivity (91.4%) compared to qPCR. The RDT is not an alternative for the laboratory confirmation of CL by microscopy in Suriname.

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CONGENITAL CHAGAS DISEASE: LONG TERM FOLLOW UP OF TREATED CHILDREN, PRELIMINARY REPORT

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The aim of pharmacological treatment for patients with Chagas is to prevent cardiac involvement. Electrocardiogram (ECG) is the first indicator

of cardiac involvement. A novel echocardiography (ECO) method for quantitative assessment of myocardial contractility deformation has been validated for detection of subclinical left ventricular dysfunction. Children treated for Chagas disease, with benznidazole or nifurtimox (for 60 days), with long term follow-up were enrolled. Clinical (ECG), parasitological (qPCR) and serological (IHA, EIA) data were collected at diagnosis, end of treatment and every 6 months thereafter. ECG was performed at diagnosis and every year after treatment. Also, Holter (24-hour ECG) and strain and speckle-tracking ECO were carried out at end of follow up for this study. As a control group, healthy children underwent the same cardiological evaluations. A total of 50 treated patients and 17 controls were enrolled. Treated patients: median age: 18.6 years (range 7-33). Median age at diagnosis was 2.35 years (range 0.04-20.3). Median follow-up: 12.5 years (range 6-19). Subjects were born in Argentina, and mainly congenitally infected. Persistent negative *T cruzi* qPCR and a decay of *T.cruzi* antibodies titers by IHA, EIA were observed. In 21/43 (48.8%) antibodies became negative. Cardiological evaluation by Holter and strain ECO were normal in 48/50 (96%) and in 41/41 (100%) patients, respectively. Only 2 treated patients showed sinus bradycardia and isolated premature ventricular beats (with normal strain ECO tests) and one beat 1st and 2nd AV blockade (with normal ECO) respectively. In control group (n: 17) median age was 12.91 years (range 6-23.8) One patient shown 2nd degree AV block with Wenckebach pattern and normal ECO and ergometer test. All infected patients showed parasitological and serological signs of treatment response. Only 2/50 patients in treated group and 1/17 in non-infective group showed signs of cardiac pathology, but we cannot rule out a chance finding at this stage. Further patients need to be included to evaluate cardiological involvement related to Chagas disease.

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NEW APTAMER-BASED BIOSENSORS FOR THE DETECTION OF CHAGAS DISEASE

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Chagas is an infectious disease that generates severe morbidity and affects approximately eight million humans in the Americas. Although the clinical symptoms of Chagas disease are well characterized, direct detection of the parasite that causes it remains a challenge. In this work, bioinformatic and *in vitro* evolution techniques were used to identify new biomarkers and to develop aptamers as new biosensors, respectively. Using ribosome profiling databases, ten highly expressed proteins of *Trypanosoma cruzi* were identified as potential biomarkers and the protein encoded by the TcCLB.510323.60 gene was selected as a suitable candidate for aptamer development. This protein was the second most abundant in the metacyclic trypomastigote and was highly conserved among *T. cruzi* strains. Systematic evolution of ligands by exponential enrichment (SELEX) and Next-Generation Sequencing (NGS) allowed to identify a common guanine-rich motif for aptamers. These showed specific binding for a short segment of the protein biomarker (peptide TC1). An ELISA-like assay showed that the aptamers recognized the peptide in a controlled mixture and the complete protein in crude *T. cruzi* lysates. Comparisons between total protein and total membrane protein lysates suggest that the biomarker encoded by the TcCLB.510323.60 gene is membrane associated. The identified aptamers isolated in this work could be used to develop a direct detection assay for *T. cruzi* in biological samples.

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DISCOVERY OF NOVEL SMALL SYNTHETIC MOLECULES WITH ANTI-PROTOZOAN ACTIVITIES

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Chagas disease and Leishmaniasis are neglected tropical diseases caused by protozoans belonging to the family *Trypanosomatida*e. Nearly 20 million people are infected with *Trypanosoma cruzi*, the etiologic agent of Chagas disease. Leishmaniasis, a multi-spectrum disease, is caused by parasites of the genus *Leishmania*. Approximately 12 million people are infected, with an incidence rate ranging from 1 to 2 million new cases annually. No vaccines are currently available for either diseases. The suboptimal effectiveness and significant toxicities of existing systemic therapies for Chagas disease and Leishmaniasis are leading drivers for development of new and more efficacious therapeutic strategies. In previous work, we demonstrated that analogs based on the novel 2-aryl-2-(3-indolyl)-acetohydroxamates (AKS) scaffold displayed significant activities against cancer cells lines that are resistance to standard chemotherapy. While the mechanisms of action of these AKS analogs remain to be fully elucidated, they have been shown to exhibit their anti-proliferative activities through cytostatic non-apoptotic mechanisms. In this work, the anti-protozoan activities of a small library of AKS analogs was investigated. The ability of these analogs to inhibit proliferation of *Leishmania major* promastigotes was interrogated in primary screening. Two analogs, AKS7 and AKS26, effectively inhibited growth of *L. major* at 5 µM. In preliminary experiments, both analogs appear to also effectively clear *L. major* infected monocyte-derived macrophages (MDM) in a dose-dependent manner with minimal host cell cytotoxicity following singular treatment for 48-hrs. Similarly, *T. cruzi*-infected human retinal pigmented epithelium (ARPE) cells, treated with either a single dose of 2 µM or three doses of 1 µM AKS7 daily for 3 days, revealed a clearance of both the extracellular and the intracellular parasites and no detectable toxicity to ARPE host cells. These results provide a promising new lead for the development of therapeutic small molecules that may be effective for the treatment of Chagas disease and Leishmaniasis.

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DIAGNOSTIC PERFORMANCE OF A RAPID DIAGNOSTIC TEST CL-DETECT FOR CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA VIANNIA* SPECIES IN COLOMBIA

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Parasitological confirmation of Cutaneous Leishmaniasis (CL) is mandatory to access treatment in Colombia. Direct smear and culture are the gold standards for diagnosis of CL. However, required technical expertise limits their availability in the remote settings where CL occurs. We assessed the diagnostic performance of the FDA approved rapid diagnostic test (CL-Detect) for CL caused by *L. Viannia* species in Colombia. As secondary aim, we evaluated a non-invasive sampling method for use with this rapid test. Patients ≥ 18 years of age, with cutaneous lesions (>15 days duration) suggestive of CL, were eligible. Mucosal leishmaniasis cases were excluded. Performance of CL-Detect rapid test (donated by *Inbios*) was blindly compared with direct smear and culture. Sensitivity of the test, using sampling procurement from lesions with the supplied dental broach (n=41), one pediatric swab *HydraFlock*® (n=11), one adult swab *Epicentre*® (n=10) and two pediatric swabs (n=29) were tested. Adverse events, Analogous Visual Scale for pain, and patient preferences were measured. Forty-one patients with suspected CL participated. Thirty-one were positive by gold standard (smear and/or culture). *Leishmania* species were identified in 71% of confirmed cases: 68% were *L. panamensis* (15/22), 27% *L. braziliensis* (6/22) and 5% *L. guyanensis* (1/22). Sensitivity of CL-Detect using dental broach was 38.7% (95%CI: 21.8-57.8), compared to gold standard. Sensitivity of CL-Detect using one (pediatric/adult) or two pediatric swabs ranged from 42.9 to 47.8 (95%CI: 26.8-69.4), respectively. The single pediatric swab was preferred (64%) by patients. Diagnostic performance of CL-Detect was similar among the

four tested sampling methods (dental broach versus pediatric and adult swabs). Sensitivity of CL-Detect increased with higher parasite burden in direct smears (p=0.003). CL-Detect had low sensitivity for diagnosing CL caused by *L. Viannia* species in Colombia, suggesting variation in sensitivity by region and species. The findings support the possibility of non-invasive sampling using CL-Detect to extend access to diagnosis to remote communities.

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THE CHAGAS DISEASE STUDY LANDSCAPE: PRELIMINARY ANALYSIS OF A SYSTEMATIC REVIEW OF CLINICAL TRIALS AND OBSERVATIONAL STUDIES TO ASSESS THE FEASIBILITY OF ESTABLISHING AN INDIVIDUAL PARTICIPANT-LEVEL DATA (IPD) PLATFORM

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Significant limitations still exist in our understanding of Chagas disease including its pathologies, factors relating to progression, biomarkers to indicate parasite clearance or cure and optimal treatment regimens for all patient populations. Existing data collected in past trials could be standardised and pooled to address research priorities and knowledge gaps. We conducted a systematic literature review to understand the scope of Chagas clinical studies and assess the technical feasibility of establishing an individual participant-level data (IPD) platform. Following PRISMA guidance, a review of Chagas clinical drug trials and observational investigations conducted between 1998 and 2017 was performed across 4 databases and 2 clinical trial registries. All clinical studies enrolling patients with a confirmed Chagas diagnosis and at least one follow-up measurement was included. Descriptive and thematic analysis of study characteristics has been conducted and R statistical computing software for analysis where possible. A total of 10,319 articles were screened and 43 trypanocidal treatment trials have been identified. The 43 trials cover 12 different countries, 6 interventions, 67 study arms representing 25 different treatment regimens. A total of 9,803 Chagas patients were treated and followed-up, with 37 testing Benznidazole and 5 testing Nifurtimox, accounting for 74% of all patients. 38 out of 43 studies reported parasitaemia-related outcome measures assessed post-baseline. Serology and PCR were the primary methods for outcome measurement in 6,101 and 5,284 patients, respectively. Adverse events were reported in 33 studies representing 9,114 patients. Duration of follow-up ranged from 56 days to 20 years. The clinical need, potential utilisation and quantity of data identified from preliminary analysis of this systematic review suggests development of a Chagas IPD data platform for clinical research would enable optimisation of existing data and more in-depth analyses to strengthen evidence for treatment and diagnosis of Chagas disease and inform prospective data collection.

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EVALUATION OF POINT-OF-CARE TESTS FOR CUTANEOUS LEISHMANIASIS DIAGNOSIS IN AFGHANISTAN

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Afghanistan is one of the major *Foci* of cutaneous leishmaniasis (CL), being this an important public health problem in Kabul, where this is mainly caused by *Leishmania tropica*. Despite its low and variable sensitivity microscopy remains the reference test for CL diagnosis. Sensitive molecular diagnosis (i.e. PCR) cannot be applied routinely due to its technical complexity and logistics requirements. We aimed to evaluate two new point-of-care tests: Loopamp™ *Leishmania* Detection Kit (Eiken Chemical Co., Japan - LAMP) and CL Detect™ Rapid Test (InBios International Inc., USA - CL RDT), for the detection of *Leishmania* DNA and antigen, respectively. We conducted a prospective evaluation of these two new tests at the National Malaria & Leishmaniasis Control Program's (NMLCP) clinic in Kabul. Slit skin samples from lesions from 274 suspected CL cases were subjected to Giemsa's stain microscopy, while samples taken with a dental broach were tested with CL-RDT and LAMP. DNA samples and slides were further transferred to the Academic Medical Center (AMC), Amsterdam for PCR and LAMP analyses. The diagnostic performance of the tests was evaluated against a reference standard combining microscopy and PCR results. In Kabul the CL-RDT returned a sensitivity (Se) of 65.4% and specificity (Sp) of 100%, while this was 89.1% Se and 70.6% Sp for LAMP. LAMP accuracy improved when it was conducted at AMC (92.2% Se, 94.1% Sp). The high specificity CL-RDT enables its use at the peripheral level. The CL-RDT and LAMP could be applied sequentially in an algorithm to diagnose CL patients promptly and minimize the number of patients referred to specialized clinics.

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NEOTROPICAL BATS THAT CO-HABIT WITH HUMANS FUNCTION AS DEAD-END HOSTS FOR DENGUE VIRUS

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Several studies have shown Dengue Virus (DENV) antibodies and /or nucleic acid present in neotropical wildlife including bats, suggesting that some bat species may be susceptible to DENV infection. Here we aim to elucidate the role of house-roosting bats in the DENV transmission cycle. Bats were sampled in households located in high and low dengue incidence regions during rainy and dry seasons in Costa Rica. We captured 318 bats specimens from 12 different species in 29 households. Necropsies were performed in 205 bats to analyze virus presence in heart, lung, spleen, liver, intestine, kidney, and brain tissue. Histopathology, serology and entomological studies were also performed. Histopathology studies from all organs showed no significant findings of disease or infection. Sera were analyzed by PRNT90 for a seroprevalence of 22% (53/241), and by PCR for 8.8% (28/318) positive bats for DENV RNA. From these 28 bats, 11 intestine samples were analyzed by RT-PCR. Two intestines were DENV RNA positive for the same dengue serotype detected in blood. Viral isolation from all positive organs or blood was unsuccessful. Additionally, viral load analysis in positive blood samples by qRT-PCR showed virus concentrations under the minimal dose required for mosquito infection. Simultaneously, 651 mosquitoes were collected using EVS-CO2 traps and analyzed for DENV and feeding preferences (bat cytochrome b). Only three mosquitoes were found DENV positive and none was positive for bat cytochrome b. Our results suggest an accidental presence of DENV in bats probably caused from oral ingestion of infected mosquitoes. Phylogenetic analyses suggest also a spillover event from humans to bats. We conclude

that bats in these urban environments do not sustain DENV amplification, they do not have a role as reservoirs, but function as epidemiological dead end hosts for this virus.

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TRANSLATING PREDICTIONS OF EMERGING ZOONOTIC VIRUSES FOR POLICYMAKERS: PERSPECTIVES FROM CAMEROON

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From the Ebola virus disease epidemic of 2014 to the Lassa fever outbreak this year, zoonotic diseases continue to afflict humans and wildlife. Accurate predictions of where emerging zoonotic viruses occur could improve disease surveillance and response, especially in low and middle income countries. While published maps for emerging viruses such as Ebola virus exist, little is known about how these are perceived by national policymakers. Furthermore, these predictive maps use different models and are often continental in scale, so it is unknown how they compare with one another on a national level. Therefore, we aggregated predictions for 5 zoonotic viruses from the World Health Organization's list of high priority pathogens and analyzed them with national experts in Cameroon, a hotspot for potential zoonotic disease emergence in Central Africa. Together with a One Health team of physicians, veterinarians, scientists, and public health experts, we discussed ways of improving the predictions for national policymakers based on local data. We compiled published predictions for Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, Ebola virus, Marburg virus, and Lassa virus, using a qualitative visual referencing method. We identified 14 published predictions (an average of 3 per virus) and shared these aggregated predictions with national experts who represented 8 Cameroonian ministries and research institutions. We determined that current predictions are mainly based on environmental parameters and species distribution models. We also found that Crimean-Congo hemorrhagic fever virus and Rift Valley fever virus predictions contrasted with national expert opinion likely due to local livestock movements. Lastly, we received feedback on how to improve the presentation of predictive maps so that they may influence policy decisions. Overall, we discovered that including national experts could elicit additional data to improve predictions of emerging zoonotic viruses and help translate them for policymakers. This framework could be used in other tropical countries to improve zoonotic disease prevention.

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SPATIAL DISTRIBUTION OF *YERSINIA PESTIS* FOUND IN A SENTINEL SPECIES ACROSS THE UNITED STATES WHILE ACCOUNTING FOR SAMPLING UNCERTAINTY

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Yersinia pestis is a gram-negative bacterium, primarily transmitted via flea bite, causing plague in numerous mammal species. While human cases are rare in the United States (US), plague can exhibit severe pathology, including death. Surveillance for plague antibodies in wildlife can shed light on disease occurrence and epizootics; however, sampling strategy influences the findings from wildlife surveillance. Therefore, the utility of using wildlife surveillance data to inform our understanding of plague ecology has been limited. Zoonotic diseases are influenced by climate variables because climate determines the ecological niche of and interactions between hosts, vector, and pathogen. *Canis latrans* (coyotes) are historically used as a sentinel species for plague. We applied a kernel density based ecological niche model using ~29,000 locations of coyotes tested for plague antibodies by US government agencies and PRISM Climate 30-Year Average Normals to determine the spatial distribution of *Y. pestis* in the US. A Monte Carlo based statistical comparison of spatial densities was used to detect significantly different habitat types where plague was and was not detected in coyotes. Areas predicted with higher likelihood of plague in coyotes include cooler, semi-arid areas across the Western US including mountain ranges and high-plateaued regions. The *Y. pestis* niche in coyotes was predicted in areas where coyotes were sampled historically and in areas where plague was not directly observed. Also, due to *C. latrans* behavior and limits of sampling resources, coyotes are collected and tested by US government agencies unevenly across the US. When compared to ~14,000 coyote observations from museum collections, coyotes tested for plague are undersampled in high precipitation areas such as the windward side of the Cascade Range and high alpine habitat, historically low plague prevalent areas. Future research will explore modeling-based approaches to incorporate these findings. These results allow us to strategize sampling and testing plans, particularly from historically undersampled areas, to better understand underlying risks.

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POULTRY FARMING PRACTICES IN BANGLADESH: A POTENTIAL CONTRIBUTOR TO THE EMERGENCE AND TRANSMISSION OF ANTIMICROBIAL RESISTANCE IN THE COMMUNITY

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Intensive poultry farming in many developing economies has been associated with unregulated antibiotic use for disease prevention and growth promotion. This may contribute to the emergence of antimicrobial resistant bacteria in poultry with potential for transmission to humans through the food chain and environmental pathways; we explored potential risk behaviors. We conducted a cross-sectional qualitative study using in-depth interviews among purposefully selected farm workers from small-medium commercial poultry farms (n=10); peri-urban backyard poultry raisers (n=3), and urban live poultry market workers (n=5). Among the same groups we also conducted structured observations. In commercial broiler farms antibiotics were given to birds from day one to the end of production. Farm workers were observed and reported to dispose of poultry waste directly in agricultural fields or in water bodies. Farm workers used no biosecurity measures and frequently touched their facial areas and performed various tasks, subsequent to poultry handling without handwashing with soap. Domestic poultry raisers only used antimicrobials when the poultry is ill. They were similarly observed to minimally wash hands with soap after handling poultry, only washing before food preparation or eating. They used no safety precaution during poultry handling and disposed poultry waste into open ditches next to households. In urban live bird markets, poultry were usually

kept for a maximum of 2 days and sellers did not use antimicrobials. Slaughtering and poultry processing was done on site using a knife and bare hands. Hands were rinsed and dipped repeatedly into stored water containers. Poultry feces, feathers and blood were discarded in municipal waste disposal sites on the street adjacent to markets. Poultry offal was commonly sold as feed for farmed fish. To formulate effective intervention strategies, we need further assessment of the prevalence and risks of behaviors along these environmental pathways of antimicrobial resistance transmission.

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SEROLOGIC EVIDENCE OF BAT ORTHOREOVIRUS IN SINGAPORE

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Pteropine orthoreoviruses (PRVs) are a group of double-stranded RNA viruses associated with respiratory and gastrointestinal complications in humans. PRV was first isolated from Australian bats in 1968, but it was not until 2006 when the first human cases of PRV were reported in Malaysia. Since then, multiple countries in Southeast Asia have reported varying degrees of PRV seropositivity; however, no reports have come from Singapore. We sought to address whether members of the Singapore population have been exposed to PRVs through a retrospective study. We developed a Luciferase Immunoprecipitation System (LIPS) assay against PRVs and performed an initial screen on over 800 human samples. Individuals that were deemed positive for at least one PRV strain on LIPS were further tested through serum neutralization assay, leading to a total of seven individuals with neutralizing antibodies against one of the PRV strains, PRV3M. To determine whether other animal populations had prior exposure to PRVs, we next screened wild cynomolgus macaques in Singapore and identified several animals with neutralizing antibodies against not only PRV3M, but up to four other strains of PRV. PRV seropositivity in both macaques and humans suggest that the virus is able to cross over to different animal species, and emphasize the need for further surveillance studies to gain insights on the location and directionality of such events.

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DIVERSITY AND PREVALENCE OF ARENAVIRUSES IN SMALL MAMMAL SPECIES IN SINGAPORE AND CAMBODIA

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Southeast Asia is a hotspot for emerging infectious diseases, several of which are of a zoonotic origin and have the potential to cause disease in human populations. Mammarenaviruses have the potential for widespread zoonotic transmission from a large diversity of small mammal species that are commonly found in and around human settlements. The human disease burden caused by arenaviruses in Africa and America is well known, but relatively little is known about the zoonotic impact in Southeast Asia. We will screen small mammal lung samples from Singapore and Cambodia with a nested RT-PCR protocol targeting the RdRP gene. PCR will be performed to determine the presence of arenavirus in lung samples to understand the diversity and prevalence of arenaviruses in these two countries. We will also determine which species are reservoirs and which are most important in each country. Research is ongoing and we will report the findings.

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BIOSECURITY PRACTICES IN BACKYARD POULTRY IN RURAL BANGLADESH: A MAJOR CONTRIBUTING FACTOR TO THE INCURSION OF NOVEL SUBTYPE OF AVIAN INFLUENZA AND ITS HUMAN SPILL OVER IN THE COMMUNITY

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Bangladesh is at risk of introduction of any emerged subtypes of avian influenza which are circulating in Asia. Being a small country with large human and poultry populations, it has been affected with frequent outbreaks of both high and low pathogenic avian influenza (HPAI and LPAI) since 2007. Very few studies have been carried out to reveal the farm biosecurity at backyard poultry that might have contributed to the spread of avian influenza, specially in rural areas. We aimed to characterize biosecurity practices of rural backyard poultry farms for rapid detection and effective risk management of incursion of HPAI and LPAI viruses. We conducted a cross-sectional study using pre-tested questionnaire among randomly selected backyard Poultry holdings (n=315) in two villages at Kalkini Upazila of Madaripur district. These villages, located within 3-5 km radius of a live bird market, have a relatively low proportion of commercial and high proportion of backyard poultry holdings. We found the water source for backyard poultry is largely based on pond or river (50%) which may facilitate the rapid spread of HPAI in that area. A significant number of farms (39%) used unhygienic ash and 49% farms use no litter materials, the most alarming finding. Few farms (8%) shared night shelter with ducks which is a proven risk factor for HPAI. The poultry was slaughtered within the home yard or adjacent to house at 57% households. The waste disposal system is also favourable for spreading disease. Poultry offal was just thrown away in 44% households. Free roaming of birds in the compound and sick birds remaining with healthy ones were observed in >80% and >60% farms respectively. In addition, daily cleaning of the coop was quite absent in all households. We found 98% farms having another backyard farm within 100 meter which may be a key factor in the spread of airborne infections. All these play an important role in spill over of avian pathogen to human. The findings from this study will support the development of risk-based One Health surveillance and contingency policies to minimize the spread of avian influenza between poultry units and also from poultry to people in Bangladesh.

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NATIONWIDE SEROPREVALENCE AND GEOGRAPHIC DISTRIBUTION OF MURINE TYPHUS IN THAILAND

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Murine typhus is an acute febrile illness caused by Rickettsia typhi, a gram-negative, obligate, intracellular bacterium. Murine typhus is distributed worldwide, is endemic in tropical and sub-tropical coastal areas, and is transmitted by fleas carried by rats and mice. Nonspecific clinical signs (flu-like symptoms) and minimal diagnostic options contribute to murine typhus' status as a neglected disease. This retrospective study was conducted to identify the circulation and geographic distribution of murine typhus in Thailand. Serum from Royal Thai Army recruits (n = 7,760) collected during 2007-2008 were screened for antibodies against murine typhus by ELISA to determine the exposure distribution in Thailand. The nationwide murine typhus IgG seroprevalence was 6.8% (95% confidence interval = 6.3 - 6.4%) and the range by province was 1 - 18%, confirming murine typhus as an endemic disease throughout Thailand. Seroprevalence was slightly higher in rural areas than urban areas, at 7% and 6% respectively, supporting previous studies of increased murine

typhus in urban areas. This study generated a spatial distribution map of murine typhus seroprevalence across Thailand by province, demonstrating the extent of pathogen exposure as well as its dispersion in the natural ecology. The highest seroprevalence was in the South region, which is a peninsula between seas with suitable climate and resources to support murine typhus' lifecycle.Currently there is limited epidemiology data for murine typhus in Thailand. This nationwide distributed seroprevalence data raise concerns regarding implementing public health interventions such as improved reporting and surveillance, better access to diagnostic tools for enhanced disease awareness, and effective public health education and awareness to prevent this disease.

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RETROSPECTIVE CASE HISTORY AND ANALYSIS OF KYASANUR FOREST DISEASE (KFD) IN INDIA

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Kyasanur Forest disease (KFD) was first discovered in Shimoga district in the Indian state of Karnataka. It is caused by a tick-borne pathogen that is deadly to monkeys (*Macaca radiata* and *Semnopithecus entellus*) and can cause hemorrhagic symptoms in humans. Case fatality is 3 - 10%. It is a highly infectious viral disease of the Flaviviridae group and belongs to the Russian Spring Summer Encephalitis complex of viruses. The disease has been expanding recently into new areas; however, there has been inadequacy in case reporting, lack of active surveillance measures and dearth of knowledge on the transmission patterns of this disease. Therefore, we conducted a thorough retrospective analysis to provide a comprehensive perspective of all KFD human cases from 1957 till 2017. Information was collected from peer reviewed journal articles, the ProMED database, historical newspapers, government and technical reports and other grey literature sources. Data was collated into a database that details the annual number and district of cases, and source of information. Upon completion, this meticulous process allowed us to map the cases and review spatio-temporal patterns. KFD has expanded to 12 districts in the states of Karnataka, Kerala, Goa and Maharashtra. The data indicate that there were major outbreaks in the years 1957 - 1958, 1983- 1984, 2002 - 2003 and 2016 - 2017. Factors associated with these outbreaks are major deforestation events, increased contact with infected or dead monkeys, survival of the virus within tick reservoirs, human population migration and climatic trends. The disease continues to expand into new territories and cause new cases and although human to human transmission has been ruled out so far, there is critical information lacking for this emerging infectious disease of concern. The results of this exploratory data will be used to perform further research on epidemiological patterns of Kyasanur Forest disease.

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CHARACTERIZATION OF VIRUSES IN BATS COMMONLY HARVESTED BY HUMANS IN NAGALAND, NORTHEAST INDIA

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Nagaland is largely undeveloped, with numerous remote villages due to its geographical and political isolation from the rest of India. Similar to other sites, protein is often scarce and people maintain their traditional practices and rely heavily on bushmeat, hunting wildlife such as bats as a source of income and sustenance. We collected fresh swabs and tissues from bats (N=103, *Eonycteris spelaea* N=34; *Rosettus leschenaultii* N=69) harvested in 2017 and used family-specific primers and conventional PCR to screen them for viruses that have been associated with recent zoonotic epidemic/pandemic outbreaks (coronaviruses, filoviruses, paramyxoviruses), including astroviruses. We detected paramyxoviruses and astroviruses, but did not detect coronaviruses or filoviruses. Sanger sequencing of the PCR-positive products showed two distinct bat paramyxoviruses (high similarity to African megabat, *Eidolon helvum* paramyxovirus) detected in both bat species. This suggest the transmission of viruses between the species during co-roosting, and a lack of host specificity. Detection levels of the four virus families are low, and could be a result from a number of factors such as low viral load, under sampling (<1% of total bats harvested) or a lack of specificity with viral family screening primers. Further investigation and characterization of these viruses are required prior to making conclusive results. Thus, we will proceed with metagenomics analysis using next generation sequencing methods to obtain additional information about the astrovirus and paramyxoviruses detected, as well as to investigate the pathogen population and diversity between the two bat species that bat harvesters are potentially exposed to.

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GREEN SYNTHESIS OF ISONIAZID-LOADED SILVER-STARCH NANOCOMPOSITE FOR THE TREATMENT OF TUBERCULOSIS

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Tuberculosis (TB) has been declared a public health emergency by the World Health Organization (WHO). Estimates reveal that about 9 million cases occur globally, with Asia and Africa accounting for 85 %. Isoniazid (INH), an effective anti-tuberculosis drug used in many countries is poorly absorbed from the stomach; hence, low bioavailability remains its major challenge. Application of nanotechnology to solve this problem is gaining increasing interest in this regard. Starch isolated and purified from a cheap and renewable source, *Manihot esculenta* was modified to obtain acetylated form. Synthesis was confirmed using NMR, FTIR and Raman spectroscopies while DSC-TGA, SEM, XRD, viscosity profile, water absorption and solubility indices were used to characterize the new polymer. The INH nanoparticles (INH-NPs) were evaluated for mean particle size, polydispersity index, and zeta potential and further characterized by FTIR and SEM. *In vitro* release of INH-NPs as well as the effect of nano-encapsulation of INH on the antibacterial activity of INH against gram-positive and gram-negative bacteria was also evaluated on BACTEC-MGIT960. The results show spherical shapes of INH-NPs with monodispersed size distribution. The nanoparticles had mean particle size (245 nm), polydispersity index (0.320) and zeta potential (-18.16 mV) which were further confirmed by UV, FTIR and SEM. *In vitro* release of encapsulated nanoparticles showed significant rapid release profile in acidic pH of the stomach. INH-NPs exhibited minimum inhibitory concentration (MIC) value of 0.003 ug/mL, while INH-free drug had MIC value of 0.07 ug/mL. This study shows that, INH-NPs prepared from a cheap, non-toxic, renewable and generally compatible natural polymer could considerably improve the antibacterial efficacy of INH while still being economical.

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IMPACT OF THE INTRODUCTION OF PCV7/13 ON ANTIMICROBIAL RESISTANCE IN INVASIVE PNEUMOCOCCAL DISEASE IN THE GAMBIA

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We describe antimicrobial resistance in invasive pneumococcal disease due to all serotypes and non-vaccine types (NVT) pre and post-pneumococcal conjugate vaccine (PCV) implementation in The Gambia in all age groupsWe identified, serotyped, and performed antimicrobial susceptibility testing using disc diffusion methods on pneumococcal isolates obtained from invasive samples collected from standardised population-based pneumococcal disease surveillance in the Basse Health

& Demographic Surveillance System. The study commenced May 2008. PCV7 was introduced in August 2009 and PCV13 in May 2011. Antibiotic susceptibility was interpreted using Clinical Laboratory Standard Institute guidelines. We plotted case counts of invasive pneumococcal disease in the pre-PCV, PCV- introduction, and post-PCV13 implementation period.396 pneumococcal isolates were screened against five antimicrobial agents. There was a moderate decline in antibiotic resistance in all age groups in invasive pneumococcal disease during vaccine implementation. In the 2-23 month age group, annual counts of oxacillin, chloramphenicol, and tetracycline resistant cases fell from 10-15 in 2009 and 2010 to 6-7 in 2014 and 2015. In the 24-59 month age group, there was a large fall in tetracycline resistant cases. In those >5 years, oxacillin, chloramphenicol, and tetracycline resistance fell to zero cases in 2013 and 2014. Resistance fell primarily due to reductions in vaccine-serotypes 1, 5,14 and 23F. The proportion of resistant NVT cases increased over time, particularly in the 2-23 month age group, with tetracycline resistance mainly in serotypes 10A, 12F, 11B, 7C and 25A and tetracycline resistance in serotype 12F in 2016. Isolates were generally sensitive to erythromycin but 95-98% were resistant to cotrimoxazole throughout the study. Although there is an overall reduction in cases of antimicrobial resistant IPD, resistance is emerging in NVT. We hypothesise that increased transmission of NVT after the introduction of PCV and exposure to antimicrobials facilitates the emergence of resistance in NVT. Ongoing surveillance is important.

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DIMINISHED CIRCULATING PLASMA AND ELEVATED LYMPH NODE CULTURE SUPERNATANT LEVELS OF IL-10 FAMILY CYTOKINES IN TUBERCULOUS LYMPHADENITIS

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IL-10 family cytokines are associated with the host immune response to pulmonary tuberculosis (PTB), but their association with host response in tuberculous lymphadenitis (TBL) is not known. Hence, we examined the circulating levels of whole panel of IL-10 family cytokines in TBL (n=44) and compared them with PTB (n=44) and healthy control (HCs, n=44) individuals. We also assessed the pre and post-treatment cytokine levels in TBL individuals following the completion of anti-tuberculosis treatment (ATT). Next, we also compared the levels of IL-10 family cytokines in circulation versus lymph node (LN) culture supernatants in a subset of TBL individuals (n=22). Finally, we also measured the levels of IL-10 family cytokines in tuberculosis antigen (purified protein derivative, PPD) stimulated and unstimulated (UNS) LN culture supernatants. TBL individuals exhibit significantly decreased levels of IL-10, IL-19, IL-20, IL-24, IL-28B and IL-29 cytokines in the circulation when compared to PTB (except IL-10) and HCs (except IL-20 and IL-28B) and significantly increased levels of IL-22 cytokine when compared to PTB individuals. Following ATT, TBL individuals exhibit significantly elevated levels of IL-10, IL-19, IL-20, IL-24, IL-28B and IL-29 cytokines and significantly diminished levels of IL-26 cytokine. Similarly, TBL individuals also exhibited significantly increased levels of IL-10 (P<0.0001), IL-19 (P<0.0001), IL-20 (P<0.0001), IL-24 (P<0.0001), IL-28B (P<0.0001), IL-29 (P<0.0001) and decreased IL-22 (P<0.0001) cytokines in LN culture supernatants compared to plasma. This was associated with enhanced levels of IL-10 (P=0.0002), IL-19 (P=0.0259), IL-20 (P=0.0275), IL-24 (P=0.0246), IL-28B (P=0.0127) and IL-29 (P=0.0351) cytokines upon PPD stimulation of LN cultures. Therefore, we demonstrate that TBL is associated with significantly diminished plasma and elevated LN culture supernatant levels of most of the IL-10 family cytokines; which could perhaps involve in the immune modulation of TBL infection. This to our knowledge is the first comprehensive examination of IL-10 family cytokines in TBL.

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EVALUATION OF THE EPIDEMIOLOGY, AETIOLOGY AND CLINICAL PRESENTATION OF ACUTE LOWER RESPIRATORY INFECTIONS AMONG CHILDREN UNDER FIVE YEARS OF AGE ADMITTED TO THE JIGME DORJI WANGCHUCK NATIONAL REFERRAL HOSPITAL IN THIMPHU, BHUTAN

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Pneumonia remains the major killer of children under five years globally. Most of these deaths could be prevented through the use of vaccines and the early identification and treatment of the disease. Local description of the disease is important to better characterize the major determinants of this clinical syndrome and to support the design and implementation of locally-tailored preventive and therapeutic strategies.We aim to evaluate the epidemiology, aetiology and clinic radiological presentation of WHO-defined pneumonia, and to assess the role of etiologic and prognostic biomarkers, among children under five years of age admitted to the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH) in Bhutan, a lower middle income country locked in the Himalaya. With the exception of a specific influenza surveillance program ongoing in the country, scarce data are available regarding acute respiratory infections, and the Pneumococcal conjugate vaccine is not yet included in their immunization programme. This is a prospective study of children between two and 59 months of age who are admitted to the JDWNRH in Thimphu, Bhutan, with a primary diagnosis of WHO-defined clinical pneumonia, during 12 consecutive months. For each child whose parents consented to participate in the study, we collected demographic and clinical data through questionnaires and physical examination, as well as blood samples, nasal swab and nasopharyngeal washing for etiological study (culture and molecular study), and a chest X-ray. Prognostic and aetiological biomarkers were also assessed. We compared qualitative variables using a χ^2 test or Fisher's exact test, means of normally distributed variables using the Student's t-test or ANOVA and non-normally distributed variables using the Wilcoxon Rank sum or other non-parametric tests. Logistic and linear regression (univariate and multivariate) were used to identify predictors of study endpoints. It is the first time that a study of these characteristics has been conducted in the small kingdom of Bhutan. We will present the preliminary results of the approximately 180 children recruited in the study.

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ISONIAZID AND RIFAMPICIN RESISTANCE AND PATIENT TREATMENT RESPONSE IN A TUBERCULOSIS AND HIV-1 CO-ENDEMIC POPULATION IN WESTERN KENYA

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In 2015, 10.4 million people worldwide had tuberculosis (TB) and 1.4 million deaths occurred, 400 000 of whom were HIV-positive, Sub-Saharan Africa accounted for 81% of these cases. In western Kenya, current data on the distribution of Rifampicin (RIF) and Isoniazid (INH) mutations is not available. In addition, the association of gene mutations with HIV infection and the treatment response of HIV infected and uninfected patients with TB are not known. The current study determined the proportion of drug resistant *Mycobacterium tuberculosis* in sputum isolates and investigated the association of RIF and INH gene mutations with HIV status and monitored the treatment response of TB and HIV co-infected patients. The present study was longitudinal and enrollment was done between 2012 and 2014 after the revision of the TB treatment regimen and patients with confirmed drug resistant TB were followed up for one year to establish the TB treatment response as confirmed by sputum smear microscopy. Patients

with suspected TB symptoms and Ziehl-Neelsen positive patients were targeted for enrollment. A total of 1381 new and 18 previously treated TB patients were enrolled. Sputum samples were cultured on *Mycobacteria* growth indicator tubes, drug susceptibility tests and line probe assay was performed to identify drug resistance and specific mutations on the *rpo* B, *kat* G and *inh* A genes. Discordant samples were sequenced. Conversion rate was calculated by finding the percentage of smear negative and positive patients at follow-up and initial visit, respectively. Regression analysis showed that RIF resistance was associated with HIV status (P = 0.025). Mann-Whitney tests revealed that the conversion time of HIV infected and uninfected patients with TB drug mutations was comparable (P = 0.180). The results of the study showed that INH mono-resistance was common. Detection of INH mono-resistance in TB endemic areas should be scaled-up as well as TB contact investigation studies to increase early detection of resistant strains.

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INOCULUM DOSE DEPENDENCY OF INFLUENZA OUTCOME

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Problems arise when studying influenza infections due to the ephemeral nature of the virus and sudden onset after exposure. Unlike long-term or chronic diseases such as tuberculosis or HIV, influenza patients only experience symptoms for a few days, so the virus must be detected within hours of initial exposure to record data accurately. Also, the amount of pathogen that the patient was exposed to cannot be known when the disease is acquired naturally. Influenza challenge studies have been performed since 1946 to test various prophylaxis and treatment methods as well as to study epidemiological trends. In these studies, volunteers are purposefully inoculated with a known amount and type of influenza virus in a controlled setting. The control groups of these studies, in which the patients are inoculated with the virus and given a placebo in place of prophylaxis or treatment, mimic natural infection but allow researchers to collect information than would be possible in a natural setting. This meta-analysis used data from the control groups of methodically reviewed literature in to determine patterns for how different conditions surrounding inoculations affect the severity of the disease. This information could suggest an efficient way to control the spread of influenza. Information was collected on demographic variables such as age and sample size; medical variables such as antibody titers and the inoculum dose, and outcome variables such as the number of volunteers with fever and the peak mean titer. A low-dose linear model was fitted to the data, and it was effective in showing the difference in infectivity between cold-adapted and wild-type viruses along with other preparations.

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ACUTE RESPIRATORY INFECTIONS IN TRAVELERS RETURNING FROM AVIAN INFLUENZA AFFECTED AREAS

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In the highly inter-connected world today, global travel is common. People who travel to areas with endemic emerging infections are not only at risk of acquiring them, but can also import them to their own countries, potentially contributing to pandemics. In this study, we aimed to explore the array of respiratory pathogens in travelers arriving in Ontario, Canada from avian influenza affected areas. Persons Under Investigation (PUIs) for Avian Influenza were identified as per directives outlined by public health authorities in Canada. Nasopharyngeal samples and/or throat samples were collected and submitted to the Public Health Ontario Laboratory. Influenza viruses were detected by rRT-PCRs targeting the influenza A matrix gene and influenza B nonstructural 1 gene using CDC protocols; influenza A-positive specimens were subtyped. Between April 2013 and June 2017, 230 PUIs were identified. Mean age was 44 years (range <1-92 years); 52.3% were male and 70% arrived from China. For the 62 PUIs for whom data were available, 53 (85.5%) had respiratory samples collected within 14 days (median 4 days, IQR 2-8 days) from symptom onset, which varied from 30 days before return to 7 days after return (median 1 day after return). A total of 284 samples were tested; at least one respiratory pathogen was detected in 86 (37.3%) PUIs. Six PUIs had two different viruses detected. Influenza viruses were most commonly identified [influenza A, 36 (15.6%) PUIs; influenza B, 8 (3.5%) PUIs], followed by rhinovirus (13 PUIs), parainfluenza virus and human metapneumovirus (7 each). No respiratory pathogen was found for 144 (62.6%) PUIs; no PUI tested positive for Avian Influenza. Although the risk for importation of Avian Influenza is low, seasonal respiratory virus infections appear to be commonly acquired abroad or locally after returning to Canada. Further, given the high proportion of infection with influenza virus, vaccination is strongly advised for travelers to endemic areas.

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PREVALENCE AND RISK FACTORS FOR DEPRESSION AMONG PATIENTS WITH DRUG-RESISTANT TUBERCULOSIS IN NEPAL

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The protracted course of illness, reduced efficacy of available treatment, associated stigma and financial implications put the patients with drug-resistant tuberculosis at increased risk of mental illness, particularly depression. The psychiatric side effects of the anti-tuberculosis drugs further aggravate the problem. Studies have reported the prevalence of depression to be as high as 69.5% among these patients. These mental health issues contribute to poor quality of life and influence adherence and cure rates. Addressing these conditions improves treatment outcomes. However, National Tuberculosis Programs insufficiently consider these issues. This study aims to determine the burden of depressive disorders among the patients with drug-resistant tuberculosis and assess the risk factors for the same. It is a cross-sectional study on 129 patients with drug-resistant tuberculosis treatment for at least two months selected from 11 random clusters of treatment centers across the country. Trained healthcare workers conducted face to face interview to collect data on treatment details and administer Patient Health Questionnaire (PHQ) 9 for screening depression. The PHQ9 has been validated in Nepal with a cut-off score of ≥ 10 shown to have a sensitivity of 94% and specificity of 80%. Descriptive statistics were used to summarize the treatment characteristics of the patients. Appropriate inferential statistics were used to test for the association of depression with the risk factors. A p value of less than 0.05 was considered significant. Of the 129 patients, 84.5% had multi-drug resistant tuberculosis and the rest 15.5% had even more extensive forms of drug-resistant tuberculosis. The median duration of illness was 12 months and 78.5% had past history of tuberculosis. Possible depressive disorder was found in 63.1% of the patients which had a statistically significant association with the duration of illness. The study has shown that screening for depression is feasible under program conditions and has revealed a high prevalence of depression among the patients with drug-resistant tuberculosis highlighting the need for actions.

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INADVERTENT INTRAVESICAL BCG ADMINISTRATION IN NEWBORNS AT A TERTIARY CARE HOSPITAL, KARACHI

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Bacillus Calmette-Guérin (BCG) is given to newborns soon after birth in accordance with the immunization program by government of Pakistan. BCG vaccine overdose has been rarely reported. The aim of this study is to determine the frequency of adverse events after accidental administration of high dose intravesical BCG in neonates born at a tertiary

care hospital, Karachi. A crossectional study was conducted in which 25 newborns, who had received BCG overdose accidentally between 14-16th April 2016 in the well-baby nursery of Aga Khan University Hospital, Karachi were included. Participant's demographic; details of adverse reactions and treatment given were recorded from April 2016-Dec 2017 by reviewing medical records. Analysis was carried out by STATA. 24/25 (96%) newborns were followed. 1/25 was (4%) lost to follow up. 13/25 (52%) were males. 23/25(92%) were born on term gestation. 23/25(92%) received isoniazid and rifampicin for 3 months. Rifampicin was discontinued after 2 weeks in 1/23(4.3%) child due to vomiting. 16/25 (64%) developed skin lesions. 9/16 (56.2%) had skin papule, 1/9(11.1%) had ruptured skin papule. Skin erythema observed in 3/16 (18.7%) of children. 2/16(12.5%) each developed skin pustule and skin nodule.1/16(6.3%) had persistent weeping skin lesion and underwent skin biopsy at 3 months of age ,that showed inflammatory changes with eosinophilia. Coagulation was deranged in 2/25(8%) of babies at 1 month of age. Intracranial bleeding was observed in 1/25 (4%) of babies at 1 month of age and managed conservatively. Skin lesion was the most common adverse event observed. Close follow-up is the key for managing such accidental events.

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PREVALENCE, ASSOCIATED FACTORS AND OUTCOMES OF VENTILATOR ASSOCIATED PNEUMONIA AMONG PATIENTS IN INTENSIVE CARE UNITS AT KILIMANJARO CHRISTIAN MEDICAL CENTER

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Ventilator associated pneumonia (VAP) is a nosocomial pneumonia commonly in occurring in patient receiving mechanical ventilation 48-72hrs following endotracheal intubation. Although commonly reported, hospital acquired infection (HAI) in pediatrics than other age groups accounts for about 20% of all HAIs (Galal et al 2016). VAP is associated with an increase in hospital morbidity and mortality, duration of hospitalization (on average 7-9 days) and high health care costs. To determine the prevalence, associated factors and outcomes of ventilator associated pneumonia among the patients in intensive care units at Kilimanjaro Christian Medical Centre, Moshi -Tanzania. A retrospective, cross-sectional hospital based study design was used. A descriptive analysis was done using SPSS v.20 to summarize data using mean, for continuous variables and percentages for categorical variables. Multivariate logistic regression was used to express the magnitude and direction of association with odds ratio showing the strength of association and p-value of <0.05 was considered statistically significant. Of 138 files available, 58(42.0%) were of males and 80(58.0%) were of female patients with the average age of 51.81 years (SD±20). The point prevalence of VAP was 4.1%, in which the sex specific point prevalence was 3.5% and 4.5% for male and female respectively. Tracheostomy, re-intubation frequency, length of staying in Intensive care units (ICUs) were the clinical factors associated with VAP. *Acinobacter baumanii* was microorganism significantly associated with VAP. In this study the proportion of patient dying due to VAP was 38.7%. VAP remains a public health problem given the observed prevalence and proportion of deaths. It is associated with re-intubation frequency and prolonged mechanical ventilation thus effective intervention are necessary in combatting this problem and thus improve patient outcome.

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COMPARISON AND DIAGNOSIS OF *ENTAMOEBA HYSTOLYTICA, E. DISPAR,* AND *E. MOSHKOVSKII* IN STOOL SAMPLE FROM RURAL COMMUNITY OF NEPAL

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Nepal is a developing country which has many health problems. Amebiasis is one of the infectious diseases that is highly seen in rural area of Nepal caused by *Entamoeba* species. Recent reports show that open defecation, contaminated water, unsanitary habits and lack of basic health knowledge cause higher mortality and morbidity in our country. *E. histolytica* is an anaerobic pathogenic parasitic. However, *E. dispar and E. moshkovskii* exists as non-pathogenic. Likewise, *E. histolytica*, *E. dispar* and *E. moshkovskii* are morphologically identical but genetically distinct species. A total of 270 faecal sample were collected from south eastern terai region of Nepal after the informed consent form. The samples were processed by direct wet smear. Eventually, microscopic examination as performed for the detection of *Entamoeba* species along with other intestinal parasites. Furthermore, enzyme immunoassay was executed to detect antigens of *E. histolytica*. Additionally, microscopically positive samples for *Entamoeba* species cysts were further characterized using a Nested- PCR targeting 16S-like ribosomal RNA gene. 8.52% of the total collected samples were microscopically positive for *Entamoeba* cysts either singly or in combination with other intestinal parasites. Among different organisms, *As. Lumbricoids* and *E. histolytica, G. lambia* and *H. nana* were identified in most of the patients accounting for 11.11%, 8.52%, 2.59% and 1.11% respectively. Among several symptoms, diarrhoea seems to be the common symptoms infecting all of the patients followed by fever (55.1 %), vomiting (46.2 %.) and nausea (14.4%). Consequently, 56 cases were PCR positive, 51 cases were ELISA positive whereas 47 were found to be positive by microscopy. In conclusion, This study reports a highly rapid, specific and sensitive molecular technique for detection and differentiation of *E. histolytica*, *E. dispar* and *E. moshkovskii*.

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MOLECULAR CHARACTERIZATION OF *GIARDIA LAMBLIA* IN CHILDREN UNDER FIVE YEARS FROM THE MANHIÇA DISTRICT, SOUTHERN MOZAMBIQUE

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Giardia lamblia, the etiologic agent of giardiasis is responsible for over 2.5 million deaths per-year in children less than 5 years. Currently, 8 morphologically identical assemblages (A to H) are known, with assemblages A and B affecting humans and other mammalians. Data on molecular epidemiology of *Giardia* are scarce in Mozambique. Therefore, we aimed to retrospectively assess the assemblages of *G. lamblia* in stool samples from a case-control study (the Global Enteric Multicenter Study - GEMS1A) conducted in the Manhiça district between November 2011 and November 2012, that investigated the burden and microbiologic etiology of moderate-to-severe (MSD) and less-severe (LSD) diarrhea in children less than 5 years. DNA of 123 stool samples from cases and 259 from controls, positive for *G. lamblia* by immunoassay (TechLab, Inc., Blacksburg, VA, USA) were extracted using Qiagen kit (QIAamp DNA Stool Mini Kit) and typed for 2 assemblages (A and B) by conventional PCR targeting two genes (E1-HP and C1-P21). Overall 50% (191/382) of the samples amplified for at least one targeted gene, with assemblage B being the most frequently found, accounting for 89% of positives (170/191),

followed by A (8.4%; 16/191), and mixed assemblages (A+B) with only 2.6% (5/191). Among cases, 61% of typed samples (75/123) were positive compared to 44.8% (116/259) of controls, with assemblage B being predominantly in cases (59.4%, 73/123) compared to controls (39.4%, 102/259) (p<0.001); while assemblage A was slightly more frequent in controls (12.1%, 14/259) than in cases (2.7%, 2/123) (p=0.19), and mixed assemblages were the least common and found in similar proportions between cases and controls. Our data suggests that *G. lamblia* assemblage B is the most predominant circulating assemblage in children with diarrhea in the rural community of Manhiça, Southern Mozambique. Detailed molecular characterization including the analysis of a higher sample size is underway to better understand the local epidemiology of this parasite.

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MOLECULAR DIAGNOSIS AND GENOTYPE ANALYSIS OF *CRYPTOSPORIDIUM* SP., *GIARDIA LAMBLIA* AND *ENTAMOEBA* SP. IN DIARRHEAL STOOL FROM CHILDREN AGED LESS THAN 12 YEARS IN GHANA

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Diarrhoeal diseases are common among children in developing countries including Ghana. These are caused by several etiological agents including viruses, bacteria and parasites. Parasites that are mostly implicated include *Cryptosporidium spp., Giardia lamblia and Entamoeba spp.* In Ghana, research into childhood diarrhoea mostly focus on children aged less than 5 years and viral isolation. This study aimed at detecting and genotyping *Cryptosporidium* sp., *G. lamblia* and *Entamoeba* sp. from diarrhoeal stool samples from children less than 12 years old. Genomic DNA was extracted from 339 diarrhoeal stool samples and subjected to PCR amplification. PCR-RFLP of the 18S rRNA gene was employed for the sub-typing of *Cryptosporidium* isolates. *Giardia lamblia*-positives were characterized by multilocus genotyping of SSU rDNA, gdh and tpi genes of the parasite. Detection and differential diagnosis of *Entamoeba* species was done by nested PCR. The overall prevalence of intestinal protozoan parasites among study participants was 48.9%. Overall prevalence of *Cryptosporidium* sp., *G. lamblia* and *Entamoeba* sp. were 23.9%, 12.7% and 20.4% respectively. Genotyping of *Cryptosporidium* sp. revealed 35/81 as *C. hominis*, 2/81 as *C. parvum*, *C. andersoni* (2/81), *C. meleagridis* (1/81) and *C. baileyi* (1/81). For *G. lamblia*, 12 out of 14 were identified as assemblage BIII whilst one each was identified as assemblages AII and BIV. *Entamoeba* isolates were 37/339 *E. histolytica* and 24/339 *E. moshkovskii* whilst 6 were *E. histolytica*/*E. moshkovskii* mixed infections. This is the first report of *C. andersoni*, *C. meleagridis*, *C. baileyi*, *E. moshkovskii*, *G. lamblia* assemblage AII, BIII and BIV in humans in Ghana. Our results suggest that cryptosporidiosis, giardiasis and amoebiasis represent a significant burden in Ghana and of public concern that need urgent attention.

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THE "BRAIN- EATING" AMOEBA: A QUANTITATIVE ASSESSMENT OF SEASONAL VARIATIONS IN *NAEGLERIA FOWLERI* AND FECAL INDICATOR BACTERIA IN LAKE PONTCHARTRAIN OF LOUISIANA

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Naegleria fowleri is a free-living, pathogenic amoeba causing primary amoebic meningoencephalitis (PAM), a degenerative brain disorder with a 98% case fatality rate and median death within five days. PAM occurs when *N. fowleri* enters the nose and reaches the olfactory nerve, usually during swimming or sinus irrigation with neti pots. Given Louisiana's two fatal PAM cases in 2011, the goal of our study was to assess the presence of *N. fowleri* in brackish Lake Pontchartrain, which is used extensively by Southeast Louisiana residents for recreational purposes. Furthermore, we aimed to determine seasonal differences and its dependence on specific environmental conditions. We collected 160 surface water samples over a 10- month time period from 10 recreational sites in Lake Pontchartrain. Physical and water quality parameters were measured in situ. Using quantitative polymerase chain reaction (qPCR) methods, we quantified *N. fowleri*, *E. coli*, and *Enterococci*. *N. fowleri* target sequence was detected in 35.4% of the water samples ranging from 11.6 to 457.8 gene copies per 100 ml. *N. fowleri* had statistically significant positive correlations with water temperature (r = 0.62, p-value < 0.01) and *E. coli* concentration (r = 0.64, p-value < 0.01). *E. coli* and *Enterococci* were present in 90.6% and 95.8% of the samples, respectively. Multiple linear regression models confirmed seasonality of *N. fowleri*, *E. coli*, and *Enterococci*. Average concentrations of *N. fowleri* and *E. coli* were significantly higher during the summer than winter, and *Enterococci* concentrations were higher during the winter than summer. *N. fowleri* was widespread at the 10 recreational sites during our sampling period. Our study highlights the persistence of *N. fowleri* in a brackish water environment and its strong relationship with water temperature and *E.coli*. Future research examining subsurface water and whether sediment is a source of *N. fowleri* in the water column will lead to a better understanding of favorable conditions for *N. fowleri*, thus further describing its potential risk to human health.

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GIARDIA/CRYPTOSPORIDIUM QUIK CHEK ASSAY FOR THE DETECTION OF *CRYPTOSPORIDIUM* DIARRHEA IN CHILDREN IN BANGLADESH

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Cryptosporidium is a major cause of childhood diarrhea. Current modes of cryptosporidiosis diagnosis involve procedures which are costly and require both a well-equipped laboratory and technical expertise. Therefore, a cost effective, user friendly and rapid method for point-of-care detection of *Cryptosporidium* species in fecal samples is desirable. A total of 832 diarrheal stool specimens collected from 200 children aged below two years were tested by *Giardia/Cryptosporidium* QUIK CHEK®, ELISA and qPCR to compare the performance of the individual techniques. We also tested the presence of other diarrheal pathogens in qPCR positive samples with a TaqMan Array Card (TAC) to assess whether *Cryptosporidium* was the sole causative agent for the diarrheal episodes. Of 832 samples, 4.4% were found positive for *Cryptosporidium* by QUIK CHEK®, 3.6% by ELISA and 8.8% by qPCR. Using "TAC attributed *Cryptosporidium* diarrhea" as the gold standard, the sensitivities of QUIK CHEK®, ELISA and qPCR were 92.3%, 71.8% and 100% respectively; while the specificities were 97.1%, 94.3% and 0% respectively. Analysis of the qPCR positive and QUIK CHEK® negative samples by TAC identified other enteropathogens as more likely than *Cryptosporidium* to be the causative agents of diarrhea. QUIK CHEK® was more sensitive and specific than ELISA and allowed for point of care use for the diagnosis of *Cryptosporidium* diarrhea. While qPCR detected *Cryptosporidium* in more samples than QUIK CHEK®, most of these were instances of qPCR detecting small quantitites of *Cryptosporidium* DNA in a diarrheal episode caused by another enteropathogens/ We concluded that QUIK CHEK® was comparable in sensitivity and superior in specificity to qPCR for the diagnosis of *Cryptosporidium* diarrhea.

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IS *ENTAMOEBA DISPAR* PATHOGENIC?

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Entamoeba dispar is generally considered non-pathogenic while *E. histolytica* is known to cause human infections. Because the two species cannot be distinguished based on morphological characteristics, the Wadsworth Center Parasitology Laboratory within the New York State Department of Health, developed a real-time PCR assay to distinguish between the two species. Over 250 specimens were tested using stool samples received in 2017 and 2018. Interestingly, a majority of submitted specimens contained *E. dispar* and not *E. histolytica.* Only 5.3% of the cases submitted were positive for *E. histolytica* whereas a much greater proportion, 83.3%, were positive for *E. dispar.* Samples that were positive for *Entamoeba* based on microscopy but negative for both *E. dispar* and *E. histolytica* by molecular analysis account for 11.4% of specimens tested. Because the specimens were collected from individuals seeking medical attention for intestinal illness, our results suggest that the pathogenicity status of *E. dispar* should be revisited.

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IMPACT OF *GIARDIA* ON INTESTINAL MICROBIOTA AND VITAMIN B12 BIOSYNTHESIS IN PRESCHOOL CHILDREN

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Depending on the species, parasites can disrupt intestinal bacterial flora affecting nutritional status. Using multi-parallel quantitative real-time PCR (qPCR) and whole genome sequencing analysis for bacterial microbiota and *Giardia lamblia* (assemblage A or B). Stool samples were collected longitudinally from 100 children sampled at 3 and again at 5 years old from Ecuador. Uninfected versus *Giardia* only groups were analyzed for microbiome and metagenomic differences and compared to growth delays. For *Giardia* only infected children, longitudinal sequencing data showed an increase in bacterial biodiversity compared to those uninfected that correlated with increasing *Giardia* burden (Shannon alpha diversity (*Giardia* only 2.7; uninfected 2.1, $p = 0.0317$; Spearman $r = -0.5491$, $p = 0.0244$)) within each age group but showed a significant increase in diversity from paired 3 to 5-year-old children ($p = 0.01838$). In *Giardia* only infections, microbiome taxonomy shifted to *Prevotella copri*, with the degree of shift related to the intensity of infection compared to uninfected (43.2 % versus 12%, $p = 0.012$). Metagenomic analysis of the bacterial microbiota showed the proportion of vitamin B12 producing bacteria (*Bifidobacteriaceae*) were diminished in the *Giardia* assemblage A group infected group compared to the non-infected group and also assemblage B group ($p = 0.038$). Specific genes in the cobalamin synthesis pathway (cobinamide kinase, ATP corrinoid adenosyltransferase) were proportionally decreased with the burden of *Giardia* infection ($p < 0.05$). Z-scores for both height and head circumference was decreased in the *Giardia* infected children at both time points ($p < 0.05$). The rate of decreased growth was larger in children infected with *Giardia* at both 3 and 5 years old ($p < 0.05$). Our data provide evidence for an effect of parasitic infections allowing permissive growth of anaerobic bacteria such as *Prevotella* and *Bifidobacteriaceae*, altering capacity of vitamin B12 biosynthesis and impacting growth in children.

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VALIDATION OF A MULTIPLEX REAL-TIME PCR GASTROINTESTINAL PARASITE PANEL

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Microscopy is the conventional method for identification of gastrointestinal parasitic pathogens in fecal samples, however, it presents numerous challenges including high technical expertise and prolonged turnaround time. Molecular methods provide higher throughput and potentially higher sensitivity and specificity. We sought to validate a commercial multiplex parasitic real time PCR panel detecting 6 protozoal pathogens: *Blastocystis hominis (Bh)*, *Cryptosporidium*, *Cyclospora*, *Dientamoeba fragilis (Df)*, *Entamoeba histolytica (Eh)* and *Giardia lamblia (Gl)* in unpreserved fecal specimens submitted for diagnostic parasitology. We analyzed 192 specimens, including 84 banked, frozen known positive specimens containing all of the targeted pathogens (8 *Bh*, 13 *Crytosporidium*, 13 *Cyclospora*, 10 *Df*, 15 *Eh*, 13 *Gl* and 12 mixed protozoal infections) and 108 fresh specimens randomly selected from our prospective parasitology submissions, including 4 *Bh*, 3 *Df*, 2 mixed infections, and 99 microscopy negatives. DNA extraction and PCR were setup with the Hamilton Starlet automated platform and Seegene's extraction and PCR kits. Microscopy was the reference standard for all organisms with stool ELISA as an additional reference assay for *Eh*. Sensitivity, specificity, positive predictive and negative predictive values of the enteric multiplex were: 96%, 90%, 60%, and 99% for *Bh*; 100% for *Cryptosporidium*; 79%, 100%, 100%, and 98% for *Cyclospora*; 86%, 86%, 86%, and 98% for Df; 81%, 100%, 100%, and 98% for Eh; and, finally, 94%, 85%, 85% and 99% for *Gl*, respectively. The platform had high sensitivity for *Bh*, *Cryptosporidium* and *Gl*, but suboptimal sensitivity for detection of *Cyclospora, Df*, and *Eh*. Low positive predictive value for *Bh* may reflect challenges to accurate microscopic identification of this organism. Negative predictive value was excellent for all targets, supporting that the platform accurately determines true negatives. This particular enteric multiplex platform provides a useful diagnostic tool for *Bh*, *Cryptosporidium,* and *Gl*. Further optimization of the assay is required for *Cyclospora*, *Df,* and *Eh* prior to clinical use.

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GENETIC DIVERSITY OF *TRICHOMONAS VAGINALIS* ISOLATES IN WESTERN AUSTRALIA, THE NORTHERN TERRITORY OF AUSTRALIA AND SOUTHERN GHANA

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Genetic diversity of *Trichomonas vaginalis* among regional populations has become more evident in studies over the last decade, with increasing cases of treatment failures and variable clinical presentations. We applied next generation-multilocus sequence typing (NG-MLST), comprising seven single-copy housekeeping genes to genetically characterize isolates of *T. vaginalis.* We examined one hundred and seventy-six archival and recently sampled *T. vaginalis* isolates from Western Australia, the Northern Territory and female patients visiting selected health care facilities in Southern Ghana, to assess the level of intra- and inter-population genetic diversity of *T. vaginalis* in these regions. Twenty-two zero-radius operational taxonomic units (ZOTUs) and 106 sequence types (ST) were distinguished among 176 isolates, suggesting diverse *T. vaginalis* populations within the three geographical regions. Each characterized locus comprised more than one allele and nucleotide diversity for the loci based on pairwise difference averaged 0.0175 differences/site. The number of different alleles for each locus ranged from 2 to 8. Eleven multiple infections with different genotypes were found among 6% of the samples, mostly those from Ghana. ZOTU diversity was greater among isolates from Ghana and one

novel *T. vaginalis* genotype was found in 1% of isolates from Ghana. We discuss how this genetic variation may affect clinical presentation and treatment of *T. vaginalis* infections.

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PEDIATRIC CRYPTOSPORIDIOSIS IN SUB-SAHARAN AFRICA: GAPS IN ACTIONABLE GUIDANCE FOR CLINICAL MANAGEMENT AT THE BEDSIDE

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Cryptosporidium has emerged as a major contributor to pediatric diarrhea-associated harm in sub-Saharan Africa (SSA), accounting for an estimated 3 million cases and 125,000 deaths annually in children aged 0-24 months. Despite the high disease burden, it is unclear if frontline health workers in endemic settings are adequately equipped with knowledge and skills to detect and treat cryptosporidiosis at the bedside. We sought to understand what guidance is available for clinical management of this parasite in young, non-HIV infected children in SSA through analyses of four commonly used resources: published literature, World Health Organization (WHO) guidelines, national Standard Treatment Guidelines (STGs), and Essential Medicine Lists (EMLs). Inclusion criteria were materials that were developed or updated in the past 10 years. When relevant, we investigated for specific discussion of nitazoxanide, the only approved therapy for *Cryptosporidium* (acknowledging its clinical efficacy is variable, especially in malnourished children). Systematic review of Embase, Biosis, and Medline databases identified 528 unique articles that examined cryptosporidiosis in Africa, but only 20 discussed clinical diagnostics (15 articles) or therapeutic approaches (5 articles). WHO pediatric and malnutrition guidelines did not address *Cryptosporidium* as a cause of acute diarrhea in young children without HIV. We searched for STGs from 54 countries in SSA and reviewed guidelines from 15 countries that were retrievable online; none described specific guidance for cryptosporidiosis diagnosis or treatment. Finally, we reviewed EMLs from WHO and 24 SSA countries; only one (Zambia) listed nitazoxanide. Our study suggests that the epidemiologic discoveries revealing cryptosporidiosis to be an important diarrheal pathogen have yet to be translated to actionable guidance that could help practitioners advance population health. To improve survival, global stakeholders will need to empower health workers with effective tools to execute a paradigm shift in the way diarrheal illness is diagnosed and treated in young children in SSA.

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GENETIC DIVERSITY OF *CRYPTOSPORIDIUM* IN A BANGLADESHI COMMUNITY

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Cryptosporidiosis is one of the top 5 causes of diarrhea in children < 2 years in age from low-income countries. Between June 2014 to 2017 disadvantaged children were enrolled at birth at one of two study locations in Bangladesh (urban Mirpur: 231; rural Mirzapur: 254). During the study period *Cryptosporidium* infections were identified at the urban (240) and rural (138) sites. *C. hominis* was prevalent at the urban location whereas *C. meleagridis* was most common at the rural site. *C. hominis* and *C. meleagridis* are both transmitted by the fecal-oral route however the *C. hominis* parasite source is human whereas that of *C. meleagridis* is usually avian. Both these pathogens can be transmitted via contaminated water but infections increased only at the urban location during the monsoon season. We both gp60 genotyped and sequenced high burden parasites. In 32 samples >80% of the genome was covered with a depth of > 50X, sufficient depth to detect the SNPs present in an intra-host parasite population arising from a single infecting oocyst. 36,780 SNPs varied between the Bangladesh *C. hominis* isolates however only 1,582 occurred with a frequency > 20% (common SNPs). No linkage was observed between common SNPs if they were separated by more than 300 bp in the genome indicating that in Bangladesh parasites recombination was frequent. It was not therefore surprising that the genome clusters did not necessarily reflect the *gp60* genotype. Several hypervariable regions encoding membrane or secreted proteins were identified and the Tajima's D score was computed to confirm that variability was due to unusual selective pressure. As expected the hypervariable gp60 gene was picked out - other regions spanned the Cops-1, cgd8_690 and secreted SKSR family protein and two members of the insulinase family. Both non-synonymous changes in the encoded amino acids, internal deletions and the premature termination of some ORF occurred. Yet to be determined is if the regions of genomic diversity identified here are responsible in part for the high rate of reinfection, seasonality and varied clinical presentations of cryptosporidiosis in this population.

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THE *THEILERIA PARVA* GENE CATALOG

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Theileria parva is a protozoan parasite that causes East Coast Fever (ECF) in cattle, killing over a million animals a year, and resulting in significant economic loss in the 12 countries in central, southern and eastern Africa where ECF is endemic. Currently, the only effective vaccine against *T. parva* is a live, multi-strain vaccine administered through an "infection and treatment method" (ITM) approach, which has several drawbacks, perhaps the most critical of which are standardization of vaccine production and the fact that cattle immunized through ITM become asymptomatic carriers. The importance of securing food resources in Africa has created an incentive to develop next generation of vaccines against ECF. To facilitate this goal, we generated a publicly available online resource named *Theileria parva* Gene Catalog (TpGC) that allows the user to prioritize *T. parva* proteins as vaccine candidates based on a variety of properties inferred from the amino acid composition of each protein. These properties are based on the recently updated *T. parva* genome annotation, and include physical attributes, antigenic potential and expression data. Also included are estimates, for each protein, of non-synonymous polymorphism in *T. parva* isolates from cattle and African cape buffalo, an asymptomatic reservoir of the parasite. Polymorphism estimates are based on whole genome sequence data we generated for 25 *T. parva* isolates from cattle and 28 others derived from buffalo and buffalo-associated *R. appendiculatus* ticks. Here, we evaluate the potential of polymorphism data for the identification of novel antigens. We hypothesize that firstly known antigens are frequently highly enriched for among proteins with the highest amount of non-synonymous polymorphism and, secondly, that the set of polymorphic proteins is highly enriched for genes encoding secreted and GPI-anchored proteins, presumably as a result of selection imposed by the host or tick vector.

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DETECTION OF *URBANORUM SPP.* EMERGING MICROORGANISM BY AUTOFLUORESCENCE IN WET MOUNT COMPARED TO EXAMINATION WITH SALINE SOLUTION AND LUGOL IN HUMAN FECAL SAMPLES. NATIONAL INSTITUTE OF CHILD HEALTH, LIMA- PERU; APRIL TO OCTOBER 2017

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In the world literature and especially in Latin America, autofluorescence of *Urbanorum spp*., an emerging microorganism that causes diarrhoea in humans, has not yet been reported. Detection routinely is performed in wet assembly using saline solution and lugol with microscope light. Our objective was to use the autofluorescence of *Urbanorum spp.* in human fecal samples for laboratory diagnosis. 200 human fecal samples from the National Institute of Child Health were studied. They were received for routine parasitological diagnosis. Before discarding, wet mount and observation were performed using a fluorescence microscope with a blue excitation filter (length of wave = 450-490 nm) with 20X and 40X objectives to observe the autofluorescence of *Urbanorum spp.* At the same time, parasitological techniques with saline solution and lugol were used wet mount with 10X and 40X objectives of the light microscope. Autofluorescence of *Urbanorum spp.* - yellowish green color and size of 80-100um- was observed in 21/200 cases (10.5%), and 21/200 using saline solution and lugol, giving a 100% concordance; microorganism images are presented in microphotographs. Autofluorescence of *Urbanorum spp* facilitates laboratory diagnosis compared to saline solution and lugol techniques in wet mount. Autofluorescence, which is reported for the first time in this study, would be very useful to detect the presence of the emerging microorganism.

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URBANORUM SPP. MICROORGANISM EMERGING IN HUMAN FECAL SAMPLES FROM THREE PERUVIAN HEALTH INSTITUTIONS, FROM OCTOBER 2017 TO MARCH 2018

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Urbanorum spp. has been reported as an emerging microorganism in Colombia, Ecuador, Venezuela and Mexico and recently in Peru, with 4 reports from 2016 to 2017; described as a protozoon similar to amoebas, presenting rounded or oval shapes of 80-100um that emit "pseudopods", found in coproparasitic exams of patients, adults and children, with prevalences of 0-16%. Research on *Urbanorum spp.* in human fecal samples in three health institutions of Lima, Peru: National Institute of Child Health (INSN), Maternal and Child Institute of San Bartolomé (IMISB) and Daniel A. Carrión National Hospital (HNDAC) of Callao. Descriptive, transversal study. Before discarding, the fecal samples for routine parasitological diagnosis in the Microbiology Services of said institutions were collected by adding physiological saline solution v / v and transferred to the Institute of Tropical Medicine Daniel A. Carrión, Universidad San Marcos University (UNMSM), Lima, Peru, for the research of *Urbanorum spp.*, between October 2017 and March 2018. 2,800 samples were processed: 1,800 from the INSN, 500 from the IMISB and another 500 from the HNDAC, Callao; Samples were processed with saline solution and lugol. *Urbanorum spp.* was found in 556 of 2800 samples (20%); with 306 / 1,800 (17 %) at INSN, 105/500 (21%) at IMISB and 145/500 (29%) at HNDAC. *Urbanorum spp.* was found in a greater percentage (29%) in fecal samples of the HNDAC of Callao, followed with 21% in the IMISB and 17% in the INSN. Given the high percentage found, it is necessary to perform microbiological studies including electron microscopy, immunology, molecular biology, clinical epidemiology, pathogenesis, treatment and control of this emerging microorganism, find the reasons for the percentage difference between Lima and Callao.

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INVESTIGATION OF NEURORETINAL INFILTRATION BY *TOXOPLASMA GONDII* IN A MOUSE MODEL OF OCULAR TOXOPLASMOSIS

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The conversion of tachyzoites into bradyzoites is a way for *Toxoplasma gondii* to establish a chronic and asymptomatic infection and achieve lifelong persistence in the host. The bradyzoites form tissue cysts in the retina, but not much is known about the horizontal distribution of the cysts or their interactions with glial cells in the retina. A chronic ocular toxoplasmosis model was induced by per oral administration of *T. gondii* Me49 strain cysts to BALB/c mice. Two months after the infection, retinas were flat-mounted and immunostained to detect cysts, ganglion cells, Müller cells, astrocytes and microglial cells, followed by observation under fluorescence and confocal microscope. The horizontal distribution showed a rather clustered pattern but the clusters were not restricted to certain location of the retina. Axial distribution was confined to the inner retina, mostly in ganglion cell layer or the inner plexiform layer. Both ganglion cells, a type of retinal neurons, and Müller cells, predominant retinal glial cells, could harbor cysts. The cysts were spatially separated from astrocytes, the most abundant glial cells in the ganglion cell layer, while close spatial distribution of microglial cells were observed in two thirds of retinal cysts. In this study, we demonstrated the retinal cysts were not evenly distributed horizontally and were confined to the inner retina axially. Both neurons and one type of glial cells could harbor cysts and topographic analysis of other glial cells suggests role of microglial cells in chronic ocular toxoplasmosis.

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SCHISTOSOMIASIS CONTROL IN EGGUA: COMBINING HEALTH EDUCATION, DRUG TREATMENT AND ALTERNATIVE WATER SOURCE

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This was a pilot intervention study for health communication in schistosomiasis control. A critical aspect of schistosomiasis disease control is the role of health communication for behavioural changes.In 2016, we used Focus Group Discussions, (FGD) to find out what the people living in Eggua Ward know about schistosomiasis after close to 10 years of research in Yewa North Local Government Area, and what sort of health initiatives they expect from the stakeholders in schistosomiasis control (the local and state governments, researchers, NGOs, etc). We then conducted a health education campaign using pamphlets, radio, town-crier announcements and seminars for the community. Trainings were also conducted for teachers, community health workers and officials of the State Schisto Control Programme. At the same time, anyone who was tested and positive for the disease was treated with praziquantel. A bore hole was provided for the community. The knowledge of the correct natural history of the disease was acquired by the people, and there was an increased awareness of how their behavior can impact the transmission

of the disease in their village. Prevalence was slightly lower a year later, but there were cases of reinfection. Open defeaction continued mostly unabated, since most people had no latrines. Indeed, we are aware that any behavioural change achieved may be temporary, and some even unfeasible, as long as there are limitations on infrastructural changes.

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ADAPTIVE STRATEGIES FOR SCHISTOSOMIASIS CONTROL AND ELIMINATION IN HETEROGENEOUS ENVIRONMENTS: A MODEL-BASED ANALYSIS OF PUBLIC HEALTH GUIDELINES

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Schistosoma infection is endemic in many parts of the world, and WHO has made its control and elimination a high priority. Recently, a large-scale trial and multiple country control-surveillance programs have been conducted, including SCORE (Schistosomiasis Consortium for Operational Research and Evaluation, U. Georgia) and SCI (Schistosomiasis Control Initiative, Imperial College, London). The 5-year SCORE trial explored several control strategies with different target age groups and drug regimens. However, these studies have yielded mixed results in seemingly similar settings; whereas some communities achieved substantial gains, others deemed "hotspots" were highly resilient to the effects of mass drug treatment (MDA). We developed a simulation-based study with dynamic transmission models to investigate how complex environmental and life cycle aspects (e.g. host demographics, in-host biology, transmission environment, and host-vector interactions) can drive the differential response to MDA seen in SCORE and country programs, and compared new adaptive decision making strategies (updating strategy based on initial treatment response) to current WHO guidelines and targets. The models were fit to datasets from SCORE villages and related studies. Our model-based analysis revealed many key predictors of response to MDA, including those of intermediate snail host biology and infection dynamics. The new set of adaptive strategies achieved more rapid control of schistosomiasis with fewer treatment rounds compared to WHO guidelines. Finally, an adaptive strategy that included an integrated approach (MDA + snail control with molluscicide) was found to be optimal in control. This study found that across a broad range of host communities and environmental settings that adaptive decision making with integrated strategies (MDA + molluscicide) could achieve control of schistosomiasis in shorter time periods compared to current WHO guidelines and targets. Future policy decisions should consider adopting adaptive decision making to address "hot spot" settings.

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SCHISTOSOMA MANSONI INFECTION IS DYNAMIC AND INCOMPLETELY TREATED WITH SINGLE-DOSE PRAZIQUANTEL IN ADULT WOMEN

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Current WHO guidelines recommend annual mass praziquantel treatment in areas with high schistosome endemicity. Yet little is known about incidence and reinfection rates after treatment in women with daily exposure to schistosomes. We sought to quantify incident *Schistosoma mansoni* infections in this population and characterize their response to praziquantel treatment. We followed a cohort of rural women in northwest Tanzania starting in July 2017. Every 3 months, women provided urine, stool, and serum for schistosome testing and completed a survey. Microscopic examinations for ova were performed on filtered urine and Kato Katz slides, and serum Circulating Anodic Antigen (CAA) was quantified. We defined schistosome positivity as either positive microscopy or CAA >30 pg/mL. We enrolled women who were positive for *S. mansoni* ova and uninfected women. Those with schistosome infections were given praziquantel. We calculated cumulative incidence and incidence rates for schistosome infection. At initial screening, 150/349 (42.3%) women were schistosome positive. We enrolled 43 egg positive and 54 uninfected women. 31/43 (72%) and 43/54 (80%) respectively were followed up at 3 and/or 6 months. Of the *S. mansoni* infected group, 9 were CAA positive at 3 months and 7 were CAA positive at 6 months, including one who had been negative at 3 months. In total, 10/31 women with *S. mansoni* infection at baseline had infection at 3 or 6 months (32.3%). In addition, 3/43 women (7.0%) who were originally uninfected were newly CAA positive at 3 or 6 months. Only 2 women were egg positive at follow-up. Overall, the incidence rate was 3.5 per 100 person-months. No significant demographic differences were found between women who were successfully treated and those with recurrent or persistent infection. Our data suggests that annual praziquantel treatment is insufficient for nearly a third of women in endemic communities. Furthermore, microscopy lacks adequate sensitivity to evaluate efficacy of treatment in this population. The cohort is ongoing, and our work suggests that further investigation into treatment efficacy and reinfection rates in adults is warranted.

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PROTEASE-BASED BIOREPORTERS FOR THE DETECTION OF *SCHISTOSOMA CERCARIAE*

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The parasitic infection Schistosomiasis affects over 200 million people worldwide. The causative agents are fluke worms of the *Schistosoma* genus, and infection only occurs when the cercarial larvae penetrate the hosts' skin barrier. To facilitate this, the cercariae secrete a serine protease called elastase, which degrades elastin in the skin barrier, enabling the parasite to burrow through the skin. Using a synthetic biology approach, we designed and characterised several modular whole-cell bioreporters that detect *Schistosoma* cercarial elastase activity. Our bioreporters incorporate a cercarial elastase detection system that is based on the specific recognition of its proteolytic activity, and upon detection to produce a bioreporter output that is easy to measure. Here, we report new data on our elastase bioreporter designs. We show how sensitive the bioreporters are in terms of number of cercariae required for detection. We also have additional data and exciting progress to report.

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EPIDEMIOLOGY OF *FASCIOLA HEPATICA* INFECTION AMONG CHILDREN IN THE ANTA PROVINCE OF CUSCO, PERU

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Fasciola hepatica is the most widely distributed trematode infection, but half of human infections occur in countries of South America. Most *Fasciola* infections in the community remain undiagnosed. Children are disproportionately affected and develop anemia and under nutrition, which may cause devastating long-term consequences. We conducted a cross-sectional study on the epidemiology of fascioliasis among all children attending pre-school and school in 26 communities of the Anta province (elevation 3,350 meters) in the Cusco region of Peru. Interviews at school and at home collected information on demographics, socioeconomics, medical history, and nutrition. Blood from subjects was tested for

complete cell count, FAS2 ELISA antibodies, and transaminases. Three stool samples per subject were tested with Kato-Katz and Lumbreras rapid sedimentation for parasites. Overall, 2515 children were included, with mean age of 9.6 years and female/male ratio of 0.99. Few children had a history of treatment for anemia (6.4%, 154/2394), under nutrition (4.4%, 105/2,394), and parasites (14.2%, 338/2,388). The median HAZ was -1.45 (IQR: -2.07 – -0.81) and the mean BMI was 17.7 (±2.75). Ten percent (253/2,513) of the participants had one or more tests indicating *Fasciola hepatica* exposure with a range from 0% to 20.4% depending on community. Microscopy was positive in 6.1% (154/2,515) and Fas2 ELISA in 8.4% (211/2,513). The prevalence of chronic infection was 6.1% (154/2,515) and acute infection was 0.8% (19/2,512). *Fasciola* exposure was associated with older age (10.7 years (±3.4) vs. 9.5 years (±3.6), p < 0.01), higher altitude (3,486 meters vs. 3,453, p = 0.01)), and fewer years of education among parents (7.2 vs 7.8 in fathers p=0.02, 5.4 vs 6.3 in mothers, p<0.001). Those living in adobe houses and with higher probability of living in poverty were more likely to have *Fasciola* exposure. Fasciola exposure was not associated with anemia or stunting. The logistic regression analysis showed that age, district, and probability of living in poverty were associated with *Fasciola* exposure.

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THE BURDEN OF *SCHISTOSOMA MANSONI* AND SAFETY OF PRAZIQUANTEL TREATMENT IN PRESCHOOL AGE CHILDREN FROM WESTERN KENYA

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Despite evidence of significant schistosome infection in preschool-aged children (PSAC), these young children remain excluded in many national mass drug administration (MDA) control programs partly due to paucity of data on the safety of praziquantel in treating these young children. This study therefore determined the burden of schistosomiasis, safety and side effects associated with praziquantel treatment in preschool children aged 1-5 years in western Kenya. The cross-sectional study was conducted between January-August 2017 among 877 children. Stool samples collected on three consecutive days were examined based on duplicate slides for eggs of *Schistosoma mansoni*. All children diagnosed with *S. mansoni* infection were treated with praziquantel (crushed, 40 mg/kg), and any observed or reported drug events documented within 3 days of drug administration. Overall, 181 (20.6%) were infected with *S. mansoni*. Of those surveyed, 115(63.5%), 51(28.2%) and 15(8.3) had low, moderate and heavy intensities of infection, respectively. Of the 181 treated children, 30(16.6%) experienced side effects which included loss of appetite 2 (6.7%), diarrhea 5 (16.7%), fatigue 3 (10%), fever 2 (6.7%), nausea 2 (6.7%), skin rash 4 (13.3), stomachache 8 (26.6%) and vomiting 4 (13.3%) which cleared within 3 days of drug administration. No serious adverse event was reported. Higher *S. mansoni* egg burdens were significantly associated with increased odds of adverse events; moderate infection intensity (OR=2.86 (95%C.I= (1.194, 6.865), P=0.018) and heavy infection intensity (OR=5.945 (95%C.I= (1.81, 19.48), P=0.003). There was no significant association between gender and adverse events. In conclusion, Side effects of praziquantel treatment in PSAC were mild, transient and non-life threatening. Our findings add to the body of evidence indicating the need for a policy change to include PSAC in routine mass deworming control programs.

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IDENTIFYING INDIVIDUAL RISK BEHAVIORS AND COMMUNITY-LEVEL CONTRIBUTIONS TO REINFECTION WITH *SCHISTOSOMA MANSONI* IN SCHOOL-AGED CHILDREN IN RURAL UGANDA

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Globally, over 200 million people are infected with *Schistosoma* parasitic helminths, and 400 million are at risk of infection, with school-aged children (SAC) disproportionately affected. The World Health Organization identified Uganda as one of the ten priority countries, highly endemic for schistosomiasis, with an estimated 4 million people infected. The national control programme focusses on mass drug administration (MDA) with praziquantel. However, MDA coverage is only ~37% of SAC and even lower in community members, and hotspots with high prevalence remain. SAC are also found to become rapidly reinfected after treatment and mean infection intensities and associated morbidity continue to be high. My interdisciplinary study uses ethnographic and population genetics in conjunction with standard epidemiological methods to better understand how, why and where certain children in Mayuge District, Uganda become rapidly reinfected with *S. mansoni* after treatment. Results from observational ethnographic appraisals of rapidly reinfected and non-infected children and focus group discussions with parents on water contact attitudes and practices will be presented. These methods help elucidate group and/or individual behaviours that affect children's risk of reinfection and how such risks might be reduced. Concurrently, intermediate snail hosts were collected from key water contact sites identified through the ethnographic appraisals. DNA extracted from *S. mansoni* cercariae shed from these snails will be compared to DNA from *S. mansoni* miracidia found in previously collected samples from SAC and community members to understand better who is driving these reinfections. Analyses of collected data and interpretation of results help provide recommendations for improvements to the national control programme with the aim to reduce *Schistosoma* reinfection in Uganda. I will present preliminary findings from Bugoto from Nov 2017 and March 2018.

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THE ROLE OF HIGH RISK GROUPS IN MAINTAINING *SCHISTOSOMA MANSONI* TRANSMISSION DESPITE OVER A DECADE OF PRAZIQUANTEL TREATMENT

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Hotspots of schistosomiasis persist in Uganda despite over 14 years of mass drug administration (MDA). Although praziquantel is donated for free and recommended for community-wide treatment in high endemic areas, coverage remains low and global supplies are limited. A key question is how to optimize drug distribution to maximise success, reduce transmission and, in particular, reduce rapid reinfection of treated school-aged children (SAC). We describe a cross-sectional survey of a *Schistosoma mansoni* endemic community alongside a longitudinal survey of SAC to identify groups driving reinfection. We recruited 700 SAC (6 – 14 yrs) from

three schools by Lake Victoria, Mayuge District, Uganda, and collected samples 7 times over the 13 month study from March 2017. In Nov 2017, we recruited 103 pre-SAC (9 mths – 5 yrs) and 250 adults (≥15 yrs) from communities to compare infection rates and determine their contribution to transmission. At each timepoint we collected three days of stool for Kato-Katz to estimate infection intensity as eggs per gram (epg) and stored miracidia for genetic analyses with 18 microsatellites. Prevalence, ranging from 61.8% to 88.5% pre-treatment in SAC, and 39.8% in pre-SAC and 50% in adults, was partially explained by treatment history, age, household factors, and geographic location. Following national MDA, infection intensities were significantly higher in pre- SAC (μ = 118.0 epg) and young adults (15-29 yrs; μ = 162.34 epg) compared to SAC (μ = 72.6 epg). High infection intensities were driven by high exposure rates (water contact frequency and duration), ineligibility for MDA, and young adults systematically not offered treatment. We present differences in parasite genetic diversity between risk groups: pre-SAC, SAC, fisherfolk and other community members and results from statistical models exploring demographic, behavioural, and household indicators of parasite infection intensity and genetic diversity of community members. While SAC are often the focus of control programs, we provide genetic evidence that these target groups can be rapidly re-infected by an untreated reservoir of high-risk pre-SAC and young adults.

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COMPARISON OF STOOL KATO-KATZ AND URINE POINT-OF-CARE ANTIGEN TEST TO DIAGNOSE SCHISTOSOMIASIS INFECTION IN A LOW PREVALENCE ENDEMIC AREA OF KENYA

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The Kato-Katz (KK) method to detect *Schistosoma mansoni* infection has limitations for program use. A commercial point-of-care (POC) urine test that detects *S. mansoni* circulating cathodic antigen (CCA) is an attractive potential replacement. In highly endemic areas, KK and POC-CCA estimate a similar prevalence. However, the limited data collected in lower prevalence settings suggest a much higher prevalence estimate when POC-CCA is used compared to KK. We aimed to collect additional data in areas with lower KK prevalence to better define the relationship between POC-CCA and KK measures. We sampled 30 schools at different distances from Lake Victoria for presence of *S. mansoni* using KK and POC-CCA. The number of children tested per school ranged from 69 to 107 with an average of 98 students per school. POC-CCA was measured as negative or positive with visual band intensity results labeled as negative = 0, trace = 0.5, 1+ = 1, 2+ =2 and 3+ = 3. KK prevalence and intensity were based on three stool samples with two slides per sample. Mean eggs per gram and POC-CCA visual band intensity were used as measures of intensity of infection. Nine schools with < 10% prevalence by KK had a median POC-CCA prevalence (95% CI) of 54.2 (36.8, 64.8) with a median visual band intensity of 0.4 (0.3, 0.5). For eight schools with KK prevalence of 10-19%, median POC-CCA prevalence (95% CI) was 60.8 (34.9, 67.6) and median intensity was 0.5 (0.2, 0.7). For six schools with KK prevalence of 20-29%, median POC-CCA prevalence (95% CI) was 59.0 (43.4, 74.7) and median intensity was 0.4 (0.3, 0.5). The remaining seven schools had KK prevalence > 30%; their median POC-CCA prevalence was 64.5 (41.4, 78.2) and median intensity was 0.5 (0.3, 0.7). Using Spearman's correlation coefficient, prevalence as measured by KK and POC-CCA ($r = 0.33$, $p = 0.08$) and intensity ($r = 0.13$, $p = 0.50$) were not correlated. We saw less variation in POC-CCA prevalence measures and visual band intensity scores by KK prevalence than we had anticipated. Additional investigation, including in the context of annual mass drug administration, will be necessary to determine whether POC-CCA can be used for monitoring control programs.

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NOVEL SCHISTOSOMIASIS EDUCATION PROGRAM DEVELOPED FOR CHILDREN IN THE MAROLAMBO DISTRICT, MADAGASCAR

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Improving health education and understanding of schistosomiasis is necessary to reduce transmission rates of this disease. A pilot education programme focussed on disease transmission and prevention in children in an area of Madagascar (Marolambo district) where 45% of children have daily contact with infested water and there is a Schistosoma mansoni infection prevalence of over 90%. This complements a series of prevalence and morbidity studies with treatment provision to this rural region in the East of Madagascar. A novel education programme was developed using the character of 'Scolly', a schistosome, to target understanding of schistosomiasis transmission, symptom awareness and prevention. Interactive teaching methods included a mix of stories, songs, games and theatre. This was delivered to children and adults from six villages. Knowledge, Attitude and Practices (KAP) questionnaires were used pre and post education in 298 children (5-14 years old) to assess understanding of the disease. Significant gaps in knowledge pre education were identified, with only 18% of children correctly identifying praziquantel as treatment. Post-education questionnaires showed consistent improvements in knowledge. Increases were seen in percentages of children able to identify water contact as a transmission mode (from 45% to 79%), showing knowledge of key symptoms (24% to 54%), understanding avoiding infested water as a preventative measure (11% to 37%) and awareness of praziquantel as treatment (18% to 80%). These early results show promising effects of a bespoke education programme on this high-risk population. Further studies with a longitudinal approach via annual research and education based expeditions will continue in 2018. This will assess efficacy and longevity of these novel education methods in improving disease understanding and attendance rates at treatment programmes, and in reducing disease transmission and morbidity.

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THE PREVALENCE OF *SCHISTOSOMA MANSONI* INFECTION IN SNAILS IN SALVADOR, BAHIA, BRAZIL

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Human schistosomiasis is a neglected tropical disease transmitted by contact with fresh water contaminated with larvae of the trematode parasite of the genus *Schistosoma* In Brazil, schistosomiasis is still considered a serious public health problem primarily of rural areas, and affects around 7 million people. Bahia state has the second highest prevalence, but constitutes the largest endemic area. Salvador, the state capital, is Brazil's 4th largest city. The occurrence of schistosomiasis is dependent on the distribution of the intermediate snail host of the genus *Biomphalaria*. We evaluated the populations of *Biomphalaria sp* in urban water collections that were previously positive for *S. mansoni* to determine the prevalence of infection, to characterize the parasite populations, and to describe their distribution. We collected 828 *Biomphalaria glabrata*, which were distributed in 12 out of 17 water collections. Morphological identification of snail species was performed observing characteristics of the shell and mantle. Snails were evaluated for *S. mansoni* infection by

repeated light exposure for 30 days and then qPCR with an *S. mansoni*-specific primer. Five snails were positive by classical cercarial shedding, with infection rates of 1.9% and 5.5% in Horta de Saramandaia and Lagoa do IAT, respectively. Non-*Schistosoma* larvae were observed in 2.4% of the snails. They belonged to the genera *Xiphidiocercaria*, *Strigeidae*, *Spirorchiidae* and *Clinostomidae*. qPCR, however, detected *S. mansoni* in 3.4% to 60% of snails. These results demonstrate that *B. glabrata* species are widely distributed in the city of Salvador. The *S. mansoni* positivity rates found in the snails show that there are potential areas with active schistosomiasis transmission and additional surveys should be performed. qPCR technique can be used to complement the light exposition method. Estimates of snail *S. mansoni* prevalence only by light exposure may underestimate the problem.

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QUANTITATIVE RISK ASSESSMENT OF NOROVIRUS AND ADENOVIRUS FOR THE USE OF RECLAIMED WATER TO IRRIGATE LETTUCE IN CATALONIA

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Wastewater is an important resource in water-scarce regions of the world, and its use in agriculture requires the guarantee of acceptable risk levels in public health. The presence of fecal bacteria, indicators of contamination, does not correlate with the presence of viruses, which are the main potential health risks transmitted through water. Using viral pathogens as indicators could complement the use of fecal indicator bacteria in the evaluation of water quality. In this study, we characterized the concentration of human adenovirus (HAdV) and norovirus genogroup II (NoV GII), the most important human viruses found in wastewater, in two wastewater treatment plants (WWTPs) that use different tertiary treatments (natural wetland vs conventional UV, Cl and Actiflo treatments) for a year in Catalonia. The main objective of this study was to develop a quantitative microbial risk assessment to estimate the health risk associated with the ingestion of lettuce irrigated with tertiary effluents from these WWTPs. The results show that the disease burden of NoV GII and HAdV for the consumption of lettuce irrigated with tertiary effluent from either WWTP was higher than the WHO recommendation of 10^{-6} DALYs for both viruses. The WWTP with natural wetland showed a higher viral reduction on average (3.9 and 2.8 logs for NoV GII and HAdV, respectively) than conventional treatment (1.9 and 2.5 logs) but a higher variability than the conventional WWTP. Sensitivity analysis demonstrated that the input parameters used to estimate the viral reduction by treatment and viral concentrations accounted for much of the model output variability. The estimated reductions required to reach the WHO recommended levels in tertiary effluent depended mainly on the treatments developed in the WWTPs, and additional average reductions are necessary. The results suggest that the analyzed reclaimed water would require an extra disinfection treatment to achieve acceptable risk in the irrigation of vegetables with reclaimed water.

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IDENTIFICATION OF EXPOSURES TO PATHOGENS CAUSING GASTROINTESTINAL DISEASE USING A ONE HEALTH APPROACH IN THE GALAPAGOS ISLANDS

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The rich ecology of the Galapagos Islands draws many tourists and researchers to the locale, but approximately 25,000 Ecuadorians inhabit four of the islands in the archipelago. Hospital data obtained directly from doctors on San Cristobal, Santa Cruz, and Floreana Islands from 2014 indicate that the yearly prevalence of gastrointestinal infections is approximately 30% on each island, but records provided fail to identify specific pathogens causing disease. Previous research has revealed that fresh water sources in the Galapagos Islands are often contaminated by saltwater and human waste. Researchers have also isolated many pathogens in animals on the islands that can be transmitted to humans. We hope to provide more conclusive data on the pathogens causing diarrheal disease on these islands, and the probable sources of contamination. Data from hospital laboratory results of fecal sample evaluations and approximate location of residence of patients consulting the hospital physicians for the last year (May 2017 – May 2018) will be collected. This information will be entered in to ArcGIS in order to identify hotspots of gastrointestinal disease. Once the cases have been entered into the GIS software, water, soil, and animal fecal samples will be collected from areas with high prevalence of disease to attempt to identify sources of contamination. These samples will be analyzed with Next Generation Sequencing techniques to determine if the soil and water samples are contaminated, and with what organism they are contaminated. Animal fecal samples will be analyzed with standard fecal float techniques to determine if parasitic organisms colonize the animals, and with which organism animals are colonized. We are in the beginning stages of this research project, and expect our results to align with previous research, but we expect to provide a more defined model of the transmission cycle between animals, the environment, and the native population of humans on the Galapagos Islands. We expect to have preliminary results by the time of the annual meeting in late October.

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IN VITRO EVALUATION OF ANTI-SNAKE VENOM PROPERTIES OF THE ETHANOLIC EXTRACTS FROM *ANNONA SENEGALENSIS* (PERS) AND *CINNAMOMUM ZEYLANICUM* (BLUME)

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Snakebites cause serious health problems globally and mostly in poor rural communities in sub-Saharan Africa and South Asia resulting in about 20,000 deaths out of about 1,000,000 cases per year. Many more of its victims are left permanently disabled or disfigured. The clinical management of snake envenomation is mainly by the use of anti-venoms (anti-venin) which are in most cases specific for particular snakebites (monovalent) or in some cases for a number snake bites (polyvalent). It is well known that anti-venoms are not for treatment of local tissue damage which contributes to the high morbidity and mortality rates associated with snakebites. Anti-venom sera are also expensive and not readily available in remote farming communities where there is much demand. It is important that an alternative readily available and cheaper source of anti-snake venom agent be developed. In this work, the anti-snake venom properties of ethanolic leaves extracts of *Annona senegalensis* and *Cinnamomum zeylanicum* were evaluated. The anti-coagulation and anti-haemotoxic effects of the extracts were assessed against *E. ocellatus* and *B. gabonica* venom using *in vitro* assays. The results revealed that 50 mg/ml of *A. senegalensis* extract completely inhibited the coagulation activity of *E. ocellatus* venom on citrated human plasma whiles 25 mg/ml of *C. zeylanicum* extract was necessary. Indicating that *C. zeylanicum* extract had much stronger anti-coagulation activity than *A. senegalensis* with respect to *E. ocellatus* venom. On the anti-haemotoxic studies of the extracts, *A. senegalensis* exhibited stronger inhibition concentration (IC_{50}) 1.96 mg/ml against *B. gabonica* venom and 4.48 mg/ml against *E. ocellauts* venom. The *C. zeylanicum* extract had IC_{50} of 13.701 mg/ml against *B. gabonica* and 16.06 mg/ml against *E. ocellauts* venom. Thus, *A. senegalensis* and *C. zeylanicum* have anti-snake venom activities. This

study provides important data on anti-snake venom coagulation and haemolysis properties of ethanolic extracts from *A. senegalensis* and *Cinnamomum zeylanicum,* which is essential for drug discovery in anti-snake drugs development.

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OUTBREAK INVESTIGATION OF FOOD POISONING AMONG PARTICIPANTS IN A WORKSHOP AT A HOTEL IN KOUDOUGOU, BURKINA FASO, NOVEMBER 2017

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On November 7th, 2017, 4 participants of a workshop were admitted in Koudougou's Regional Hospital Center (CHR) for acute diarrhea and vomiting in one hour time. The Hospital staff informed Health authorities and an investigation was conducted to describe the outbreak, identify the source, risk factors and to prevent future outbreaks. We conducted a retrospective cohort study from November 7th to 24th, 2017. We reviewed medical records and interviewed participants and cooks. We defined a case as any person from the workshop in the Hotel with abdominal pain or vomiting and/or diarrhea (≥3 soft stools in 24 hours) between November 7- 8th , 2017. We conducted active search to identify other cases. We collected food and stools specimens for laboratory analysis. We observed food preparation and kitchen sanitation. We analyzed data using Epi Info 7.2. We calculated proportions, relative risks and 95 % confidence interval. Among the 65 attendees, we identified 18 cases (attack rate 27.69%), 67% (12/18) had nausea/vomiting, 61% (11/18) had abdominal pain and 50% (9/18) had acute diarrhea. Median age was 46 years old (range 40-63 years). Median incubation period was 1 hour 30 minutes (range 30 minutes-11 hours 20 minutes). Consumption of cucumbers (RR =1.6 [1.9-2.14]), lettuce leaves (RR = 1.45 [1.11-1.88]), tomato (RR=1.44 [1.06-1.86]) and onions (RR=1.34 [1.01-1.80]) were items significantly associated with the disease. Direct examination of untreated lettuce's leaves isolated lot of vegetative forms of *Balantidium coli* and larvae of *Strongyloides stercoralis*. Cultures of food samples and stools were negative for *Salmonella, Shigella, Vibrion* and *staphylococcus aureus* species. The laboratory was not able to measure toxins. In conclusion, this outbreak was associated with consumption of raw vegetables, possibly by poor hygiene or contaminated by enterotoxin. We recommend hotel to reinforce the kitchen hygiene measures, to sensitize and to train cooks and waiters on food safety.

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FEASIBILITY AND ACCEPTABILITY OF AN INTERVENTION WITH HAND SANITIZER USE AND RESPIRATORY HYGIENE EDUCATION IN BANGLADESHI PRIMARY SCHOOLS

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Transmission of respiratory pathogen among schoolchildren is common. Handwashing has been shown to decrease infections; however, it is often a challenge in Bangladeshi schools due to limited supply of soap and water. We evaluated the feasibility and acceptability of hand sanitizer in schools to promote hand hygiene. During Jun-Sep 2015, we implemented a hand sanitizer intervention with respiratory hygiene education at 12 schools in Dhaka (5,077 students). Both students and teachers of the study schools received hand sanitizer with hygiene education and were asked to use hand sanitizer at specific times each day while at school. We provided teachers with training about behavior change for students, and both teachers and students received training about hand sanitizer use and proper cough etiquette. We conducted interviews with 12 purposively selected teachers from six of the 12 schools to ask about experiences with the intervention. We also conducted six group discussions with students across all grades to understand their experiences with the intervention (48 students). At the end of the 10-week intervention period, most children had knowledge of the importance and benefits of hand and respiratory hygiene and recalled intervention messages. During the group discussion, children reported that if they did not wash their hands, germs could transmit to their friends. Students also reported that covering their mouth and nose while coughing and sneezing was very easy to practice and might protect classmates from germs. Teachers reported that it was easy to provide intervention messages since these were delivered in conjunction with regular hygiene classes. The majority of teachers thought the intervention was feasible but anticipated problems continuously procuring hand sanitizer for the primary schools. Two schools agreed in principle to buy hand sanitizer while the other ten requested hand sanitizer from the government. This school-based intervention was found to be acceptable and feasible for students in the short term; however, additional studies are needed to understand the sustainability, uptake and impact of such interventions.

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DETECTION OF *SALMONELLA TYPHI* AND *S. PARATYPHI A* IN DRINKING WATER AT HYDERABAD, PAKISTAN: AN OUTBREAK AREA OF CEFTRIAXONE RESISTANT TYPHOID FEVER

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The availability of safe drinking water remains a challenge in developing countries, approximately one third of the population in developing countries combating serious morbidities and fatalities due to unsafe drinking water. Water sources in developing countries are often polluted with sewage run off and serve as a reservoir for diseases likes of typhoid and cholera. Typhoid fever continues to be a substantial threat in developing countries, with rates as high as 500-1000/100,000 in countries like Pakistan. An outbreak of ceftriaxone resistant typhoid due to *Salmonella.typhi* reported from Hyderabad, Pakistan last year. For the investigation of the source of the outbreak, water cultures had been performed. However, samples tested by routine water cultures showed high coliform counts but no *S. typhi* or *paratyphi* could be isolated. Later, PCR had performed on the same drinking water samples, n= *115* samples were tested from different drinking water sources in Hyderabad area. DNA extraction from water samples were performed, using Qiagen/ Mobio PowerWater® Sterivex™ DNA Isolation Kit. Briefly, water samples were spiked with 05 µl of PhHV, followed by sterivex filtration, lysis, bead beating, washing and elution. Multiplex real-time PCR for the detection of *S. typhi* and *S. paratyphi A* was performed, as described by Nga etal in 2010. Preliminary results showed an overall positivity of 11.3 % with 6.08%, 1.73% and 3.47 respectively for *S. typhi, S. paratyphi A* and co-infection of *S,typhi* and *S.paratyphi A.* our data suggests that drinking water sources in Hyderabad are the possible sources for transmission of *S. typhi* and *paratyphi.* Further, work on genotype of the organisms from the environment and human infection would close the circle on the role of the water delivery systems as a possible cause of this outbreak.

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WHO IS SAFE; RICH OR POOR? A SANIPATH MICROBIAL ENVIRONMENTAL EXPOSURE PATHWAY ASSESSMENT IN DHAKA CITY

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Rapid urbanization has led to a growing sanitation crisis in urban areas of Bangladesh. Limited data available on bacterial contamination in different environmental pathways in Dhaka slums but no data available from the non-slum settings. We conducted a cross-sectional study to explore the magnitude of fecal contamination in the environment of slum and non-slum areas in Dhaka city. We collected 10 different types of environmental samples [shared latrine walls and door, soil, produce, street food, drain, bathing water, municipal, and non-municipal drinking water (private wells or 20L jar water), surface water and flood] from 10 areas (4 poor, 4 non-poor and 2 floating (bus and train station)) in Dhaka city. From April-June 2017, ten samples of each category (N=[10*10*10])=1000) were collected from each area and analyzed with IDEXX-Colilert-24®and Quanti-Tray/2000 to determine most-probable-number (MPN) of *E. coli*. Among the 10 samples, drain (log_{10} mean=9.0[sd 0.91]), surface water (log_{10} mean=7.3 [sd 1.29]), flood (log_{10} mean=6.7 [sd 0.84]), soil (log_{10} mean=6.1 [sd 1.06]), municipal drinking water (log_{10} mean=2.6 [sd 1.19]), bathing water (log_{10} mean=2.65 [sd 1.09]), street food (log_{10} mean=2.7 [sd 1.12]), and produce (log_{10} mean=2.4 [sd 1.29]) had high *E. coli* contamination. We found similar level of *E. coli* contamination between poor and non-poor areas in latrine swabs, drain, municipal water, produce and street food samples. *E. coli* concentration between slum and non-slum varied for soil (0.70 log_{10} MPN/gram, P = 0.002), bathing water (0.63 log_{10} MPN/100 mL, P = 0.007), non-municipal water (0.61 log_{10} MPN/100 mL, P = 0.002), surface water (1.73 log_{10} MPN/100 mL, P < 0.001), and flood water (0.39 log_{10} MPN/100 mL, P = 0.038) samples. Almost all environmental samples in Dhaka city were highly contaminated with *E. coli*. Level of *E. coli* contamination was similar in both slum and non-slum neighbourhoods for samples which people directly exposed to. Future studies should assess health impacts of environmental contamination among the non-slum and slum communities in Dhaka city.

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MEASURING IMPACT OF MENSTRUAL HYGIENE MANAGEMENT INTERVENTIONS ON SCHOOLS ATTENDANCE AND WOMEN'S EDUCATION IN BANGLADESH

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Girls from low-income countries, including Bangladesh, miss school days from lack of menstrual hygiene management (MHM) facilities. However, weak absenteeism measurement approaches limit the ability to detect impact of school MHM interventions and women's education. In this study, we piloted 4 absenteeism measurement systems: fingerprint device, barcode scanner, records from class captains, and mobile messaging in 2 urban and 2 rural schools from April-May 2017. From each enrolled student we took details for ID cards for the barcode and fingerprint scans. Researchers distributed attendance sheets to class captains, and a local software company sent mobile messaging and recorded absenteeism. Additionally, school girls recorded absenteeism causes on menstrual calendars. Researchers conducted 16 interviews with teachers and students, compared data with schools' absenteeism register book, and conducted biometric test efficiency to evaluate the 2 digital systems and compare with registers. Class captain and mobile messaging were feasible to implement. During interviews, class captains sometimes forgot to collect data, and students mentioned they sometimes forgot their ID cards for barcode scanning. The software company depended on schools' absenteeism register book to message staff for absenteeism data. Both fingerprint (US$ 400) and barcode (US$ 138) devices were expensive. Compared to school register and other 3 systems, the fingerprint device was accurate recognizing11% of 439 absences not recorded in the register, and students could not fake presence. However, 5% (12/244) of fingerprints could not be read due in part to dirty fingers, and school computer operators were initially reluctant to operate the system. Out of 171girl calendar users, 11% mentioned that they missed school due to menstruation. The fingerprint device provided most accurate absenteeism measure in terms of authentication which has the potential to measure the impact of MHM on school attendance and women's education. To prevent mismatch problems, we recommend using higher quality fingerprint scanners and train students to scan their own fingerprints.

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SIMPLE SOLUTIONS TO LARGE PROBLEMS: CLEAN WATER AND HANDWASHING LOWER DISEASE BURDEN

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Mpopongi Primary and Destiny Shaper Primary schools are located in a rural area of Kenya and did not have access to clean water or handwashing. We partnered with these schools to provide clean water, handwashing stations, and menstrual pads. Both schools are in areas that are difficult to access and do not have proper sanitary facilities. Mpopongi Primary has over 570 students and Destiny has over 118 students under their care. Each school spends over 10% of their yearly budget on hospitalization visits for their students, which are normally for easily preventable water-borne diseases. A water filtration system was implemented along with water storage at both schools. The water filter used was the LifeStraw Community Filter, which can remove 99% of Bacteria, 99% of protozoa, 99% of viruses, and can filter 70,000-100,000 liters. The storage containers hold at least 10,000 L each and provide water in times of drought. Additionally, rudimentary handwashing stations, including anti-bacterial soap were implemented at each school, where one handwashing station will service about 50 students per day. Reusable female menstruation pads, which are built to last for 3 years, were given to the students. Educational classes on menstrual hygiene, handwashing, and clean water were taught to the students and staff. Data was collected daily to measure the impact of each program. Within 1 year >90% of the students were using the handwashing stations and water filters daily, there were no indicences of typhoid and dysentry and only 2 cases of malaria, and an increase in student attendance was observed. We also showed that schools were able to save 40% on hospitalization visits after the implementation of these programs. These data suggest that simple general hygienic methods and brief educational classes reduce the rates of disease among primary age students, while increasing their school attendance.

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OCCURRENCE OF *CRYPTOSPORIDIUM* IN DOMESTIC WATER IN THE SOUTHERN CARIBBEAN

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Parasitic protozoans are often considered less important than bacterial agents in causing water borne disease outbreaks. However, a lack of routine monitoring and sensitive detection methods may have contributed to the underreporting of water-borne morbidity and mortality from these agents. *Cryptosporidium* is the most important protozoan responsible for water borne diseases because minimal exposure can result in infection. As such this neglected tropical disease is important in domestic water quality in the Caribbean. There is limited data available on the occurrence of *Cryptosporidium* in water in the Caribbean region. This study investigated the prevalence of *Cryptosporidium* in domestic water in rural communities in the Southern Caribbean region. Two hundred and thirty-four domestic water samples were collected in the dry and wet season over a period of one year in the predominately rain-water harvesting communities in Carriacou island, off Grenada; Nariva, Trinidad and Speightstown, Barbados. Water samples were filtered and DNA extracted before polymerase chain reaction (PCR) based detection of *Cryptosporidium* was done based on amplification of the small subunit (SSU) of ribosomal RNA gene. The overall prevalence rate was 73% for the three islands, with more samples from the wet (75%) than dry (69%) season being positive for the parasite. The prevalence rate of *Cryptosporidium* in water samples was higher in Cariacou (wet season = 91%; dry season = 60%) and Nariva (wet season = 73%; dry season = 82%) as compared to Speightown (wet season = 25%; dry season = 40%). Generally, water from natural sources (stored rainwater, wells, springs, boreholes) had a higher prevalence rate (93%) than public water supplies (36%). Future work will focus on genotyping to determine the contributing species to further characterize Cryptosporidium in the region. The study shows potential risk of *Cryptosporidium* infections from domestic water in rural communities in the region. There is urgent need for implement appropriate water disinfection strategies for these communities.

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SILICATE PNEUMOCONIOSIS IN DOMESTIC AND WILD ANIMALS FROM THE CARIBBEAN ISLAND OF ST. KITTS

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Silicate pneumoconiosis is a well-known environmentally acquired lung disease in humans characterized by silicate crystal depositions within the pulmonary interstitium. A high incidence of silicate pneumoconiosis has been observed in domestic and wild species on the Caribbean island of St. Kitts. To estimate the prevalence, 258 lung samples, from selected species (chickens, mongoose, cattle, swine, small ruminants, felines, canines, equines, monkeys), were collected at the abattoir or from previous studies and examined by histopathology, scanning electron microscopy with energy dispersive x-ray analysis (SEM/EDXA), and x-ray diffraction (XRD). Microscopic findings, seen in 201/258 (77%) samples, included perivascular and interstitial accumulations of heterogeneous crystalloid particulate material, either free or intracytoplasmic within macrophages. The material was birefringent, acid-fast positive, and largely composed of SiO_4^{4-} based on SEM/EDXA. Forty-eight passive eight hour air samples were obtained from random geographical areas and animal collection sites throughout the island and analyzed by XRD for presence and density of silicates. Local topsoil samples and lung tissue were submitted for EDXA and XRD to determine the elemental composition and crystalline structure of particulate material. The lung lesions were graded based on severity (normal, grade I, grade II, grade III) based on affectation of larger airways. By assessing the prevalence of pneumoconiosis in the animal population and the composition/structure of the particulate material within samples (lung, air, topsoil), we intend to elucidate the silicate source of origin and to correlate environmental exposure with the presence and severity of silicate deposition within the lungs. Results from this investigation my warrant future studies on the prevalence of environmentally acquired pulmonary disease within the human population of St. Kitts.

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LESSONS FROM A COMMUNITY-LEVEL WATER INTERVENTION STUDY IN KARNATAKA, INDIA

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Community-based studies assessing the health effects of interventions aimed at improving water quality and/or quantity in resource poor areas have provided mixed results. We performed a study in rural Karnataka, India, to evaluate the health impacts of an intervention delivering riverbank filtration technology (RBF)-treated water to community tanks. Our ultimate aim was to generalise conclusions of the utility of RBF for other rural villages situated in river basins in Southern India that are similarly affected by poor river water quality, monsoonal flooding and/or dry season water shortages. We employed a stepped wedge RCT design with villages as the cluster unit. Our primary outcome was self- or carer-reported diarrhoea, and we incorporated questions about symptoms unrelated to water consumption/exposure to check the validity of responses. During the study, inundation of RBF wells due to monsoonal rains and difficulties securing 24-hour electricity connections resulted in temporary RBF supply interruptions. Coincident with intervention delivery, the state government rolled out a rural water delivery scheme providing rural villages with reverse osmosis water stations, which resulted in the availability of an alternative improved water source for villagers. Reported rates of diarrhoeal illness were lower than expected, and qualitative data indicated a decline in willingness of villagers to answer questions about health as the study progressed. We failed to observe a measurable health benefit due to the RBF intervention. We expect that future studies may experience similar problems associated with unexpected introduction of new water treatment alternatives, infrastructure limitations, and waning community engagement. To counter these we recommend greater protection of the water supply and treatment system, contingency planning, close engagement with government authorities, careful measurement of intervention uptake and the incorporation of qualitative study components to understand the drivers of intervention uptake by villagers.

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OCCUPATIONAL EXPOSURE OF HEALTH CARE WORKERS IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO

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Globally, health care workers (HCWs) are on the front line of illness management, treating sick patients who may carry and transmit any

number of infectious diseases. Their occupational health and safety is critical to disease control, especially during disease outbreak events. In November 2016, we conducted a survey on occupational risks in 3 distinct health zones of Kinshasa province, Democratic Republic of the Congo (DRC) as part of a larger serosurvey determining prevalence of Ebola antibodies in health care workers. Interviews and blood specimens were collected from all consenting individuals enrolled in Lingwala, Kinshasa and Maluku health zones. Questionnaires collected sociodemographic data, occupational and general exposure histories, and information on any previous known exposures to Ebola. In total, 263 HCWs (206 nurses, 15 physicians, and 42 room attendants) were included in this analysis. 54 HCWs reported needle-stick injuries (23.3% of nurses and 40% of physicians), 32 reported coming in contact with other sharps containing biological materials (13.6% of nurses and 26.7% of physicians), 126 reported contact with droplets of biological specimens (53.9% of nurses, 66.7% of physicians, and 11.9% of room attendants), and 38 reported contact with blood near an open wound (16.0% of nurses, and 33.3% of physicians). Respondents were also asked about their accessibility to personal protective equipment (PPE), including gloves, gowns and facemasks. Physicians reported the highest rate of regular PPE access (80%), followed by nurses (58.7%), and room attendants (50%). In aggregate, only 58.6% of participants reported regular access to PPE, with room attendants left the most vulnerable to unmet PPE needs. HCWs in Kinshasa appear to be highly exposed to patients' biological specimens, either through direct patient contact or via other occupational fomite exposures. This information may be used to make recommendations for improving HCW safety and increasing workplace access to PPE throughout DRC.

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NEONATAL INFECTIONS IN LOW INCOME COUNTRIES: INCIDENCE, ETIOLOGY, RISK FACTORS AND OUTCOMES - EVIDENCE FROM A MULTICENTRIC COMMUNITY-BASED COHORT STUDY IN MADAGASCAR, SENEGAL AND CAMBODIA

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Almost 3 million neonatal deaths occurred yearly, mostly in low income countries, and around a quarter of which are due to infection. In these settings, data on the burden of neonatal infection is scarce and there is a particular lack of data regarding infections occurring in the community, which may differ from cases admitted to the hospital. Also, the role of the different factors involved in the transmission of bacteria remains unclear, particularly mother-to-child. Data are needed for these countries to prioritize interventions to decrease neonatal infections. We conducted a prospective cohort of 2500 newborns in Madagascar, Cambodia and Senegal between September 2012 and April 2018, both in rural and urban areas. Newborns were enrolled at birth at the community level and were actively followed-up. Data on clinical symptoms developed by the children and all results of biological and bacteriological samples taken were collected. The global incidence of community-acquired neonatal infections was 35.8 cases per 1,000 live births [95% CI, 25.4-50.8], with a great majority during the first week of life (75%). The most common bacteria isolated were gram-negative (70%). Almost two-thirds of the pathogens isolated were resistant to current WHO-recommended treatment for neonatal sepsis. Prevalence of extended-spectrum beta-lactamase producing Enterobacteriaceae and *agalactiae streptococcus* carriage among pregnant women were heterogeneous between the different countries and ranged from 19% to 78% and from 5% to14%, respectively. 48, 17 and 13 deaths occurred during the follow-up in Madagascar, Cambodia and Senegal, respectively. Preliminary analysis showed a high incidence of bacterial neonatal infections in the community and highlighted that current recommended treatment for neonatal sepsis is no longer adapted. Risk factors for neonatal infection, in particular the role of the mother to child transmission, and mortality outcomes will be presented. These results should help the implementation of interventions to improve the prevention, early diagnosis, and case management of neonatal infections to decrease neonatal mortality.

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GUT BARRIER AND SYSTEMIC INFLAMMATION IN A MODEL OF MODERATE ACUTE MALNUTRITION

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Moderate acute malnutrition (MAM) affects 35.1 million children worldwide. Children with MAM exhibit some signs of systemic inflammation, but responsible mechanisms are not known. We used a protein-energy and micronutrient deficient mouse model to investigate mechanisms of malnutrition-driven inflammation. We hypothesize that malnutrition-driven changes in intestinal microbiota and intestinal barrier dysfunction allows translocation of bacteria and bacterial products that lead to an exaggerated inflammatory response. After 4 weeks on either a well-nourished (WN) control or malnourished (MN) diet, MN mice exhibited increased translocation of bacteria to the spleen, liver, and mesenteric lymph nodes. MN mice also had decreased expression of tight junction proteins and cytokines related to barrier function in the ileum and colon. Peritoneal macrophages from MN mice exposed to lipopolysaccharide (LPS) or lipotechoic acid produced more TNF-α than cells from WN mice suggesting that the macrophages in MN mice were endotoxin and LTA primed. The cecal microbiota composition showed increased proportions of gram - Proteobacteria in MN mice, similar to what has been found in malnourished children. To determine if expansion of pathobiont groups coupled with increased gut leakiness was driving endotoxin priming, we gave MN mice oral colistin or vancomycin to selectively deplete intestinal gram - or gram + species. We found that vancomycin-treated, but not colistin-treated, mice exhibited significantly increased serum TNF-α and IL-6 upon intradermal challenge with LPS. This increased endotoxin priming is likely due to expansion of gram - groups upon depletion of gram + species. Similar trends of increased LPS-stimulated expression of neutrophil-related cytokines were observed in the skin of vancomycin-treated mice. Collectively, these data suggest that the altered intestinal microbiota, impaired intestinal barrier function and translocation of gram - bacteria or bacterial components drives dysregulated innate immune function and inflammation in malnutrition.

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EVALUATING THE GUT MICROBIOME IN CHILDREN WITH STUNTING: FINDINGS FROM A SOUTH AFRICAN BIRTH COHORT

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Stunting remains a significant burden to child health globally. A large proportion of children in low and middle income countries develop stunting despite preventative nutritional and environmental interventions. Environmental Enteric Dysfunction (EED), a subclinical condition characterized by small intestinal villous blunting and increased permeability, is an important cause of stunting. Recent evidence suggests that the intestinal microbiome contributes to the development and

persistence of EED and stunting, however, there is a paucity of data on the microbiome in stunted African children. This study aims to describe the microbiome of African children with stunting compared to those with normal linear growth. Children enrolled into the Drakenstein Child Health Study (DCHS), a birth cohort, had stool collected at regular study visits during the first year of life. The DCHS was situated in a low income, peri-urban setting in South Africa. Anthropometry was measure during routine study visits by trained study staff according to standard procedures. Stool samples underwent standard 16S rRNA sequencing and of sequencing data was conducted using QIIME2 and the R statistical package, Phyloseq. Alpha (Shannon index) and beta diversity (Unifrac) were compared between the stunted and control groups and significant differences in microbial relative abundance between the 2 groups were assessed using DESEQ2 and MaAsLiN. We analyzed a subset of 90 children with complete clinical and microbial data. The mean age of participants was 8.7 months (SD =6.77) with 44% being female. The mean weight was 8.0kg (SD=2.49) and mean height 66.7cm (SD=9.30). A total of 34 children were stunted of which 17 (50%) had severe stunting (height-for-age z score < -3). The results of our microbiome analysis are currently pending. This is the first descriptive analysis of the intestinal microbiome in a cohort of African children in a peri-urban setting with stunting. Characterization of the microbial signatures in this condition may help inform the development of novel defined microbial therapeutics for the treatment of EED and stunting.

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SPATIOTEMPORAL CLUSTERS OF YAWS ON LIHIR ISLAND, PAPUA NEW GUINEA ENCOMPASS MULTIPLE VILLAGES

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Yaws is a neglected tropical disease caused by the bacterium *Treponema pallidum* subspecies *pertenue*. It causes skin lesions, particularly in children. The World Health Organization has targeted yaws for eradication, and a better understanding of its spatial epidemiology is necessary to inform eradication strategies. We sought to understand the spatial and temporal clustering of yaws on Lihir Island (area 200 km^2, population about 16 000), Papua New Guinea. We analyzed 11 years (April 2005 through May 2016) of passive surveillance data on incident yaws (n = 1448) using SaTScan, a cluster detection algorithm commonly used in spatial epidemiology. After adjusting for age, sex, and spatial and temporal trends in health-seeking, we detected three statistically significant ($p < 1 \times 10^{-17}$, $p = 1.8 \times 10^{-14}$, $p = 1.6 \times 10^{-8}$) non-overlapping spatiotemporal clusters. These varied in duration from 28 to 47 months and each encompassed between four and six villages. In the geographically smallest cluster, the component villages were up to 5.1 km apart by road and in the geographically largest cluster, they were up to 10.7 km apart. In a second analysis, we assessed spatial clustering of prevalent serologically confirmed yaws cases (n = 532) that were detected in 7 biannual rounds of active case finding beginning in 2013. We identified 1 statistically significant ($\alpha = 0.05$) cluster in each round. The number of villages per cluster varied from 1 to 16 and most Lihir villages were part of a cluster at least once. We considered the possibility that schools that serve multiple villages might be loci of transmission. Using a permutation test, we found no evidence that incident cases of yaws among 8- to 14-year-olds clustered within primary school attendance areas (p = 0.6846). To our knowledge, our analysis is the first to analyze yaws spatial epidemiology at the scale of multiple neighboring villages. These clusters likely reflect transmission of yaws across village boundaries; villages may be epidemiologically linked to a degree such that mass drug administration may be more effectively implemented at a spatial scale larger than the individual village.

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IDENTIFICATION AND CHARACTERIZATION OF A NOVEL ADHERING RECEPTOR FOR SPOTTED FEVER GROUP *RICKETTSIAE*

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Spotted fever group (SFG) rickettsioses are tick-borne zoonotic diseases of global importance caused by obligatory intracellular bacteria of the genus *Rickettsia.* Although rickettsial infection is controlled by appropriate broad-spectrum antibiotic therapy, untreated or misdiagnosed SFG rickettsioses are frequently associated with severe morbidity and mortality. Endothelial cells (ECs) are the main mammalian host target cells of SFG rickettsiae. The most prominent pathophysiological effect is increased microvascular permeability, causing vasogenic cerebral edema and non-cardiogenic pulmonary edema with potentially fatal outcomes. The underlying mechanism(s) of rickettsial attachment to and anchoring on the endothelial cell luminal surface remains incompletely determined, including how the bacteria overcome shear stress from blood flow prior to host cell invasion. Here we examined the role of endothelial surface annexin A2 (ANXA2) during rickettsial adherence to human endothelial cells. We demonstrated that endothelial surface ANXA2 contributed to rickettsial attachment to endothelial cells as an adhering receptor. In vivo data from an anatomy-based *in vivo* quantitative bacterial adhesion analysis system revealed that global depletion of ANXA2 diminished rickettsial adherence to the blood vessel luminal surface. Investigation of *a protein-protein interaction* at a single-molecule level by *atomic force microscopy* (AFM) biomechanically characterized endothelial apical surface ANXA2 as a receptor for richettsial adhesin out membrane protein-B to bind. Coupled with site-directed mutagenesis investigation of protein-living cell interaction with AFM probed phosphorylation of the N-terminus of ANXA as a switch to control rickettsial adhering. Our study, targeting the pivotal initial step in successfully establishing bacterial infection, delineates both biomechanical and biochemical mechanisms underlying rickettsiae hijack endothelial surface ANXA2 for their adherence to non-phagocytic vascular endothelial cells.

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HIGH-THROUGHPUT MULTI-PARALLEL NL-QPCR CHIP FOR THE DETECTION OF 17 ENTERIC PATHOGENS

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Molecular diagnostics deliver important insights on enteric pathogen load; however cost is still a barrier in assessing a broad suite of pathogens in large epidemiological studies (n > 1000). Recent developments in microfluidic technology offer the opportunity to increase throughput for quantitative analyses while decreasing the per-sample cost. Here, we introduce a nano-liter qPCR (nL-qPCR) chip configured for duplicate quantification of 17 enteric pathogens and total bacteria, archaea, and fungi in 96 samples, at a cost of approximately $10 per sample. Pathogen-specific targets include *Campylobacter jejuni/coli*, *Clostridium difficile* and *C. perfringens*, pathogenic *E. coli* (EAEC, EPEC, ETEC, STEC, EIEC/*Shigella* spp.), *Helicobacter pylori*, *Vibrio cholerae*, *Salmonella* spp., *Yersinia enterocolitica*, *Cryptosproidium* spp., *Entamoeba histolytica*, *Giardia* spp., *Ascaris lumbricoides, and Trichuris trichura*. The chip achieves a detection limit of 100 copies per 100nL reaction for most assays. Assay efficiencies, measured over a dynamic range from 10-10^6 copies, are between 90 and 112% with a mean standard error of 2.3. Reproducibility of standard curves across 10 chips run at two facilities over a minimum timespan of two years, yielded a standard deviation of C_T values (measured across all points of the standard curve) ranging from 0.41 to 1.45 (mean 0.68), with all pathogen-specific assays except *Cryptosporidium* spp. maintaining a mean standard deviation less than 1.0 C_T. We used the nL-qPCR chip to analyze 399 fecal samples. The mean coefficient of variation for the

calculated target copy number of four replicates analyzed over two separate chips was 0.26 [range 0.004 -1.33]. Specificity was verified by sequencing PCR amplicons. We compare results for the 399 fecal samples analyzed on both our nL-qPCR and an enteropathogen TaqMan array card (TAC) that has been widely used for large epidemiological studies.

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IMPACT OF ENTEROPATHOGEN INFECTION ON LINEAR GROWTH USING QUANTITATIVE MOLECULAR DIAGNOSTICS: RESULTS FROM THE MAL-ED STUDY

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Exposure to enteropathogens early in life not only causes diarrhea, but may also contribute to poor linear growth. In the multi-site longitudinal birth cohort, MAL-ED, we measured enteropathogen exposure in monthly non-diarrheal stools and during diarrhea with qPCR assays for 29 pathogens. We estimated the effects of etiology-specific diarrhea and subclinical enteropathogen infection on linear growth in 3 month intervals, at 2 years of age, and using a longitudinal model that accounted for temporality and time-varying confounding. We then used the parametric g-formula to estimate the impact of hypothetical interventions that would reduce enteropathogen infection. Among 1,469 children who completed follow-up to two years of age, 35,622 stool samples were tested and yielded valid results. Diarrhea episodes attributed to bacteria and protozoa, but not viruses, were associated with small decrements in length-for-age z-score (LAZ) after 3 months, but these associations were not maintained at 2 years of age. Conversely, subclinical infection with *Shigella* (LAZ difference: -0.15, 95% CI: -0.28, -0.02), enteroaggregative *E. coli* (-0.21, 95% CI: -0.37, -0.05), *Campylobacter* (-0.18, 95% CI: -0.34, -0.03), and *Giardia* (-0.18, 95% CI: -0.31, -0.06) were associated with significant decrements in LAZ at 2 years. A perfect quadrivalent vaccine against these four pathogens would be expected to raise the average LAZ at age 2 by 0.24 LAZ (95% CI: 0.08, 0.41). Conversely, individual-level provision of improved water and sanitation would not be expected to substantially reduce pathogen infection, such that no improvement in average LAZ would be expected after this intervention. Subclinical infection and quantity of select enteropathogens particularly *Shigella*, enteroaggregative *E. coli*, *Campylobacter,* and *Giardia*, had sustained negative associations with linear growth. The potential impact of a perfect vaccine was similar to effects of nutritional interventions, and though the impact of a realistic vaccine would be smaller, prevention of enteropathogen infection in early childhood may contribute to reductions in global stunting.

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CLINICAL AND LABORATORY FEATURES ASSOCIATED WITH PROGRESSION TO SEVERE DENGUE AMONG POTENTIALLY HIGH-RISK DENGUE PATIENTS HOSPITALIZED IN SOUTHERN SRI LANKA

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Dengue is an important cause of acute febrile illness globally. The Sri Lankan government recommends hospital admission when the platelet count is <100x10^9/L. We determined predictors of severe dengue to identify patients for intensive vs ambulatory management. A prospective, cohort study was conducted from Jun-Oct 2017 at one tertiary and one secondary care hospital in southern Sri Lanka. Consecutive patients ≥1 year with fever were enrolled within 7 days of admission if they developed a platelet count <100x10^9/L and two clinical symptoms consistent with dengue with or without warning signs, as per the 2009 WHO criteria. Sociodemographic and clinical data were collected. Acute dengue was confirmed if NS1 rapid antigen test was positive (fever <6 days) or if dengue IgM test was positive (fever ≥6 days or NS1 test negative). Severe dengue was defined per the 2009 WHO criteria; features associated with severe dengue were determined using the Fisher exact and Kruskall-Wallis tests. Of 414 enrolled patients, most patients (323, 78.2%) had laboratory-confirmed dengue. Median age was 33 years (IQR 25- 46) and 200 (61.9%) of dengue patients were male. Overall, 59 patients (18.3%) developed severe dengue: 39 (12.1%) plasma leakage with respiratory distress, 12 (3.7%) severe organ failure, 10 (3.1%) shock, and 9 (2.8%) severe bleeding. Patients with severe vs non-severe dengue were more likely to be females (49.2% vs 35.6%, p=0.05). At admission, patients with severe dengue had a higher white cell count (4.3 x10^9 vs 3.9 x10^9 cells/L, p=0.05) and a lower platelet count (38.0 x 10^9/L vs 80.0 x 10^9/L, p=0.02) and were more likely to have a transaminitis (AST/ALT ≥120 U/L, 21.1% vs 12.6%, p=0.003). Patients with severe dengue were more likely to have ultrasound evidence of pleural effusions (14.0% vs 3.0%, p<0.001), fluid in the Morrison's pouch (17.5% s 8.7%, p=0.04), and pelvic fluid (8.8% vs 0.4%, p<0.001) at admission. Approximately one-fifth of hospitalized dengue patients developed severe dengue. Several laboratory and ultrasound findings at admission were associated with progression to severe disease and may be used for triaging patients with dengue.

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EVALUATION OF THE CARESTART™ GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) RAPID DIAGNOSTIC TEST AT COMMUNITY AND HEALTH CENTER LEVEL IN CAMBODIA

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Primaquine (PQ) is the only FDA-approved drug for radical cure of *Plasmodium vivax* (*P.v*) malaria, but treatment can result in life-threatening hemolysis if given to a glucose-6-phosphate dehydrogenase deficient (G6PD*d*) patient. Therefore, the G6PD status of the patient with *P.v* must be known prior to prescribing PQ. To increase PQ access in Cambodia in the malaria endemic community, performance of CareStart™ G6PD rapid diagnostic tests (RDTs) needs to be evaluated in healthcare workers (HCWs) and village malaria workers (VMWs). Training materials on G6PD and PQ were developed for HCWs and VMWs, and each performed G6PD test on 8 -12 adult male volunteers, with pre- and post-training questionnaires completed by trainees and volunteers. The field performance of CareStart™ RDT for G6PD*d* screening was assessed against a quantitative G6PD test (Pointe Scientific, Inc., Canton MI). Descriptive and inferential statistics were used to analyze the data. 96 trainees and 960 G6PD volunteers were recruited in Anlong Veng, Cambodia from December 2017 to February 2018. Of the 960 volunteers, 156 (16%) were G6PD deficient based on a quantitative test activity threshold of 30%. The sensitivity, specificity, PPV and NPV of CareStart™ RDT was 96.77%, 95.54%, 80.21%, 99.37% for HCW/VMW trainees vs. 96.15%, 97.24%, 86.71%, and 99.26% for trained study staff in the field and 94.23%, 98.80%, 93.63% and 98.92% for experienced laboratory staff, with no statistical difference among the groups. The mean knowledge score pre-training was 33.86% (VMWs) and 56.4% (HCWs), with improvement to 89% and 90% post training ($p < 0.001$). The improvement in knowledge scores was independent of the years of experience, profession and education level. HCW/VMWs' perception of PQ risk based on RDT results was assessed pre and post counseling and will be presented. With minimal training, CareStart™ RDT is highly specific, feasible and a practical option for the identification of G6PD*d* male patients and its use may enable safer prescribing of PQ to decrease the burden of *P.v* relapse.

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MULTIBEAD BASED ASSAY PROVIDES ONE-STEP SPECIES-SPECIFICITY FOR THE DIAGNOSIS OF FILARIAL INFECTIONS AND *STRONGYLOIDES STERCORALIS* IN TRAVELERS AND IMMIGRANTS

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Previously, antifilarial antibody testing in the evaluation of returned travelers and immigrants to North America has relied on IgG- and IgG4-specific responses to crude filarial extracts (BmA), tests that suffer from significant cross-reactivity with Strongyloides and cannot distinguish among the infecting filarial species. Nevertheless, between 1992 and 2016, testing of >11000 samples have been tested; close to 20% were able to be definitively diagnosed with a filarial infection (based on IgG4 anti-BmA reactivity) and ~50% had the diagnosis excluded (based on IgG anti-BmA seronegativity). Thus, to create an all-in-one assay for screening travelers and immigrants where infections with filariae or *Strongyloides stercoralis* (Ss) is being considered, we configured a multibead-based assay to measure simultaneously the IgG and IgG4 responses to BmA, and the species-specific recombinants Ll-SXP1, Ov-16, Wb-123, Ss-NIE and Ss-IR in a CLIA-certified fashion. By tuning the assays to >99% specificity for the species of interest (using ROC-based cutoffs) we have utilized this new assay to prospectively test samples from 712 unique patients. Of these 712 samples we have found that 166 (23%) were seropositive by IgG anti-BmA and 71(10%) were seropositive by IgG4 anti BmA. From these 237 (either IgG or IgG4 seropositive) we have been able to provide species-specific diagnosis for 137 samples (72 *Loa loa*, 3 *Wuchereria bancrofti*, 6 *Onchocerca volvulus*, and 56 *Strongyloides stercoralis*). Using this multiplex assay we have been able to deconvolute the anti-BMA reactivity and identify the species of infecting parasite responsible for the antibody positivity for better accuracy in the diagnosis of individual filarial and Ss infections and to help direct specific therapeutic options.

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COMPARISON OF LUMINEX METHODS FOR ANALYSIS OF ANTIBODY RESPONSES AGAINST MARKERS OF VACCINE PROTECTION AND PARASITIC, WATER-BORNE AND NEGLECTED TROPICAL DISEASES

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Serosurveillance is a useful tool for monitoring biomarkers in a population to estimate exposure to pathogens or vaccination protection. We use a multiplex bead assay (MBA) based on Luminex technology to simultaneously measure antibodies against antigens derived from malaria, neglected tropical diseases (NTDs), vaccine preventable diseases (VPDs), waterborne diseases, and arboviruses. These antigens can be combined into panels customized for conducting integrated serosurveillance in different epidemiological settings. Because international deployment of this technique may require use of different MBA instrumentation and beads, we compared assay performance of two commonly used Luminex instruments and bead types. 430 dried blood spot samples representing broad geographic areas were tested for antibody responses against 40 antigens from 28 different pathogens. Antigens were coupled to both magnetic and non-magnetic beads and read on Luminex 200 and MAPGIX instruments; assays were read immediately after completion and after overnight storage at 4°C. Linear regression was used to do pairwise comparisons of antibody responses for each antigen under different conditions. Percent seropositivity was calculated for each condition for the four VPDs (rubella, tetanus, measles and diphtheria) using international serum standards. The majority of antigens (37/40) had an $R^2 > 0.85$ across 5 pairwise comparisons. The comparisons with $R^2 < 0.85$ were based on comparisons that used different lots of beads on the two machines. Among tested samples, we measured seropositivity rates of 86-87% for tetanus, 91-94% for measles, 80-81% for rubella and 47-51% for diphtheria; 95% confidence intervals were overlapping for all estimates. Overall, there was excellent correlation between instruments and among samples tested at different times, indicating that with robust quality assurance and control, comparable data can be obtained from a variety of assay conditions run on different instrumentation. This gives programs flexibility in choosing platforms for integrated surveillance.

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IS MELIOIDOSIS A SIGNIFICANT CAUSE OF ACUTE FEBRILE ILLNESS IN BANGLADESH?

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The etiology of febrile illness remains poorly characterized in many countries including Bangladesh. This carries the twin risks of incorrect treatment and inappropriate use of antibiotics fuelling antimicrobial

resistance (AMR). Melioidosis is a neglected tropical disease with a high case fatality rate caused by the Gram negative bacterium *Burkholderia pseudomallei.* It is rarely diagnosed in countries with limited facilities for accurate microbiology diagnosis, but may be a significant undiagnosed cause of infection and death in Bangladesh. We conducted a prospective clinical study of the etiology of fever in consecutive adult patients admitted to two hospitals in Dhaka: Dhaka Medical College Hospital and BIRDEM from June to October 2017. The capacity of the microbiological laboratory to identify *B. pseudomallei* and other bacteria from culture was strengthened by training prior to study commencement. Blood culture was performed for every patient, with further cultures, serology and imaging as clinically indicated. 210 patients were recruited: 57% male, 26% with diabetes and with a median (IQR) age of 35 (23-55) years. A definitive diagnosis was made for 74 (35%) cases. Overall, 28 (13%) had positive bacterial cultures including 18 (9%) with positive blood culture. Six patients (3%) had a positive culture for *B. pseudomallei,* with other positive cultures including *E. coli, Salmonella* Typhi*, Pseudomonas* species, *Klebsiella pneumoniae, Acinetobacter* species and *Staphylococcus aureus.* Antimicrobial resistance included extended-spectrum beta-lactamase production in Gram negatives and multi-drug resistance in 75% of *Salmonella* Typhi. Non-bacterial causes of fever included dengue (4%) and Chikungunya (2%). Overall mortality was 16% but 67% in melioidosis. We found melioidosis to be the commonest cause of bacteremia in Dhaka, Bangladesh with high rates of AMR in other Gram negative infections meaning that empirical cephalosporin treatment would likely have been ineffective. Strengthening the capacity of microbiology laboratories in Bangladesh is vital for accurate diagnosis of melioidosis and for surveillance of AMR.

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NOVEL 3-DIMENSIONAL OPTICAL SCANNING IN THE ASSESSMENT OF PATIENTS WITH MYCETOMA

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Magnetic resonance imaging (MRI), CT and ultrasound imaging modalities are currently used in the diagnosis of mycetoma lesions. The availability, cost or expertise needed for such modalities often preclude wide application under field conditions. Therefore, in practice, response to treatment is often assessed clinically by examining the affected area, which is subjective and may be inaccurate. Currently, 3-dimensional (3D) optical scanning technologies are used for an increasing range of medical applications. The resulting 3D surface models allow objective, quantitative 3D analysis of any body part over time with superior point accuracy up to 0.1 mm, and 3D resolution up to 0.5 mm. In this study we examined the usability of 3D optical scanning in patients with lower limb eumycetoma with a limited lesion size (2-10 cm) who were recruited in a randomized controlled phase 2 clinical trial in Khartoum, Sudan. Patients were monitored using MRI and optical 3D scans acquired at regular intervals. The resulting MRI and 3D scan images were reconstructed and the volume of the mycetoma lesion was measured. The skin surface volumes of the lesions acquired were compared during follow-up to monitor changes in volume and skin texture. The first evaluation obtained in ten patients studied the volume of the lesion varied between 3 and 30 ml. A reduction in size between months 1 and 2 could be detected in 3 of them. For the first time, we have shown that using optical 3D scanning, the volume of mycetoma lesions can be accurately quantified and monitored during treatment, allowing statistical assessment of treatment response. Optical 3D scanning was well accepted and the alignment and superimposition of 3D scans with the corresponding MRI scans was feasible in this setting. Novel, portable 3D scanning technologies offer an alternative to existing medical imaging technologies such as MRI and to clinical assessment when monitoring mycetoma lesions. This innovative and cutting-edge digital tool is suitable for use under field conditions and may be explored in other (tropical) skin lesions.

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BRUCELLOSIS AMONG PATIENTS WITH PERSISTENT FEVER IN A RURAL HOSPITAL IN EASTERN SUDAN

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Brucellosis is a classic cause of prolonged fever but for most African countries, few data are available. We aimed to assess prevalence and clinical presentation of brucellosis among patients presenting with persistent fever in rural Sudan. This study was part of the Neglected Infectious Diseases Diagnosis (NIDIAG) project. Consecutive patients (more than 5 years old) with persistent 7 days or more) fever were recruited at Tabarak Allah Hospital, Gedaref State, eastern Sudan. Blood cultures and serum antibody testing (Rose Bengal test (RBT), Serum Agglutination Test (SAT) and cELISA) were performed in all patients to diagnose brucellosis, one of the study target diseases. Isolates were characterized by multiplex PCR (Bruce-ladder, using IS711 and bcsp31 primers). From February 2013 to June 2014, 667 patients were recruited; 28 of them (4.2%) had blood culture-confirmed (n=15; 2.2%) or probable (positive RBT, SAT or cELISA; n=13; 2.0%) brucellosis. All *Brucella* isolates were identified as *Brucella melitensis* biovar1. Patients with confirmed brucellosis had a median age (interquartile range (IQR)) of 23 (16-36) years and 9 were men; 5 were farmers, 2 herdsmen and 1 butcher. At time of presentation, median duration of fever was 14 days (IQR 8-26; range 7-56); median axillary temperature was 37.2°C (IQR 36.6°-38.2°C; range 35.9°-40.7C°). Other complaints were headache (12/15), joint pain (10/15), cough (10/15), abdominal pain (7/15) and sore throat (3/15). Of 41 working diagnoses at presentation (1-7/patient), the most frequent were visceral leishmaniasis (n=11), enteric fever (n=10) and brucellosis (n=6). In 2 patients, co-infections were diagnosed: enteric fever (blood culture-confirmed *Salmonella* Paratyphi) and visceral leishmaniasis (based on serology and response to treatment). In conclusion, brucellosis was a significant cause of persistent fever in rural Sudan. Local care-givers should have this diagnosis in mind and laboratories should install appropriate biosafety measures. Lack of accurate and field-friendly diagnostic tools remains an obstacle to proper diagnosis of clinically suspect patients in rural tropics.

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PHASE 2 CLINICAL RESULTS - CHIKUNGUNYA VACCINE BASED ON MEASLES VECTOR (MV-CHIK) INDUCES HUMORAL AND CELLULAR RESPONSES IN THE PRESENCE OF PRE-EXISTING ANTI MEASLES IMMUNITY

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Chikungunya is a rapidly spreading viral disease affecting significant parts of the Americas, India and South East Asia. Regional outbreaks are of increasing intensity, long lasting and difficult to control. In the majority of patients, the febrile acute disease turns into a chronic disease causing severe, debilitating arthritis. The chronic symptoms can last from months to years post-infection. So far, no vaccine or treatment has been licensed. MV-CHIK, a live recombinant measles vector-based vaccine induced a functional humoral immune response against CHIKV in healthy adult human subjects as tested in a Phase 1 clinial trial (EudraCT:2013-001084-23). Previously, interim safety and immunogenicity data from the ongoing Phase 2 study (NCT02861586) were presented. The vaccine presents with a safety profile in humans that is comparable to the approved control vaccine in the trial. The vaccine induced functional, neutralizing antibodies in 96% of the subjects after two vaccinations. Here, we present the final data collected during the Phase 2 clinical trial. We will show the immune profile, including cross-neutralizing antibodies and antibody persistency induced by the vaccine in a population of up to 300 subjects. In addition, we analyzed the cellular responses induced by the vectored vaccine in human subjects. Importantly, the vaccine induced a functional immune response even in the presence of pre-existing anti-measles immunity as demonstrated in a subset of subjects who received a measles prime immunization prior to the Chikungunya vaccine. The data show that the live recombinant MV-CHIK vaccine is a safe and tolerably vaccine that induces a robust immune response. The data clearly path the way for the preparation of a late stage clinical development program.

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AUTOCIDAL GRAVID OVITRAPS LIMIT INTRA-COMMUNITY TRANSMISSION OF CHIKUNGUNYA VIRUS

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Global public health responses to dengue, chikungunya (CHIKV), and Zika virus outbreaks are hampered by the inability to control *Aedes aegypti* mosquitos. Autocidal Gravid Ovitraps (AGO traps) have been shown to sustainably reduce the density of *Ae. aegypti* mosquito populations by >80%. To evaluate the effectiveness of AGO traps in limiting CHIKV transmission among humans, soon after the introduction of CHIKV to Puerto Rico in 2014 we conducted representative household surveys in two intervention communities (IC) where traps had been present for 3 years and two communities where no traps were present (NIC). Serum specimens were tested by anti-CHIKV IgM/IgG ELISA. We evaluated differences in CHIKV seroprevalence in IC and NIC using generalized linear models with a log-link adjusting for clustering and including an interaction between community type and human mobility. Of 1,218 and 1,284 IC and NIC residents, respectively, 175 (14%) and 152 (12%) participated. There were no differences between participants from IC and NIC by gender or age. IC participants less often reported daily or weekly mosquito bites (41% vs. 73%, $p < 0.001$) and daily use of repellent (6% vs. 24%, $p< 0.001$) or a bed net (1% vs. 9%, $p < 0.001$). Among IC participants, 47% spent more than half of weekly daylight hours in their community, while in NIC residents it was 73% ($p < 0.001$). After adjusting for differences in human mobility, seroprevalence of CHIKV antibodies in residents of IC and NIC was 26% and 44%, respectively (adjusted prevalence ratio [aPR] = 0.5, 95% confidence interval [CI]: 0.4-0.7). Seroprevalence in IC and NIC residents who spent less than half of daylight hours in their community was 34% and 59%, respectively (aPR = 0.6, CI: 0.4-0.9), whereas it was 17% and 38% in residents who spent more than half of daylight hours in their community (aPR = 0.4, CI: 0.3-0.7) (interaction $p = 0.48$). These findings demonstrate the effectiveness of AGO traps in protection of humans from CHIKV infection, which did not vary by time spent in their community. Evaluation of the effectiveness of AGO traps in a larger, urban setting is merited, potentially in combination with other prevention strategies.

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TRANSMISSION OF CHIKUNGUNYA VIRUS USING TERRAFORMA ARTIFICIAL ECOSYSTEMS

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Vector-borne and zoonotic diseases comprise the majority of emerging infectious diseases that impact human and animal health. These pathogens are part of a complex system of multifaceted and deeply integrated components that, when certain criteria are met, can lead to epidemics that are often poorly understood and difficult to contain. Our approach to elucidating some of the factors related to disease emergence is to integrate ecological pressures into controlled laboratory settings to tease out the drivers of an emergent event. The system we have designed, referred to as TerraForma, uses models in a BSL3 laboratory that mimic barnyards, wet markets, or terrariums where different animal species intermingle and abiotic factors can be altered to investigate causation of emergent events. Here we report using Chikungunya virus, a known human pathogen with an undetermined enzootic cycle, to answer the question of whether reptiles and amphibians can serve as reservoir hosts for viral maintenance. Previous studies have demonstrated that Chikungunya viremia develops in garter snakes, leopard frogs, and common toads following needle inoculation; in this experiment, we allowed these animals to comingle, and then introduced infected *Aedes albopictus* mosquitoes into the environment. Twenty-eight days following the mosquito introduction, one toad seroconverted; viremia was not detected. In a follow up experiment, naïve mosquitoes were released into the ecosystem with infected snakes, toads, and frogs. While all of the animals were viremic at the time of exposure, none of the mosquitoes that were harvested from the system developed disseminated Chikungunya infections following extrinsic incubation. While this model did not provide substantial evidence regarding the potential of these ectothermic vertebrate hosts to source an infection, it does provide the basis for any number of similar experiments to be performed utilizing a variety of hosts, including birds, rodents and bats, with varying vectors and pathogens.

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IMPAIRED EXPRESSIVE LANGUAGE IN 24-MONTH-OLD INFANTS EXPOSED TO THE CHIKUNGUNYA VIRUS IN EARLY CHILDHOOD

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The chikungunya virus (CHIKV) was first reported in Grenada in July 2014, infecting about 65% of the population in six months. Approximately 1,750 women were pregnant, became pregnant, and/or gave birth during the outbreak. Little is known about the rate of CHIKV

transmission between mother and fetus during pregnancy or the impact of transmission on the developing fetus; however, a cohort study of 33 mother-to-child CHIKV infected 2-year old children in La Réunion showed significantly lower development quotient scores and greater rates of global neurodevelopmental delay compared to uninfected children. We used the Intergrowth-21st Neurodevelopment Assessment (Inter-NDA) to assess 2-year old infants born to mothers infected (n=332) and not infected (n=80) with CHIKV during the outbreak in Grenada and did not detect any differences in cognitive, fine motor, gross motor, receptive language, expressive language or overall neurodevelopment score between the groups. We also assessed neurodevelopment at 2 years of age in CHIKV infected infants (n=18) versus uninfected infants (n=413). CHIKV infected infants showed a trend for lower total composite score on the Inter-NDA than non-CHIKV infected infants [M=3.218 (95% CI: 2.904 - 3.533) v. M=3.488 (95% CI: 3.425 - 3.551), p=.086] but significantly lower scores on the overall language subscale [M=2.760 (95% CI: 2.385 - 3.135) v. M=3.090 (95% CI: 3.024 - 3.156), p<.05], which was driven by lower scores on the expressive language subscale [M=2.607 (95% CI: 2.246 - 2.969) v. M=2.931 (95% CI: 2.870 - 2.992), p<.05]. These results are driven partly by post-partum (mosquito-borne) infection as infants who were CHIKV IgG positive born to mothers who were IgG negative (n=7) had lower expressive language subscale scores than all the other infants (n=425) [M=2.191 (95% CI: 1.703 - 2.678)] v. [M=2.932 (95% CI: 2.871 - 2.992)], p<.01. These results confirm findings from La Réunion: while rates of maternal-fetal transmission are low, infant infection with CHIKV can have a significant impact on early neurodevelopment. Negative impact of post-partum infection on language function is a novel finding in this study.

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A BRIEF HISTORY OF TIME AND ORIGIN OF CHIKUNGUNYA VIRUS SPREAD IN THAILAND AND PHILIPPINES

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Chikungunya virus (CHIKV) was first reported in Thailand in 1958, and cases of infections have been continuously described until the outbreak of 1995-96. This was followed by a period of low activity until 2008, when the disease emerged again. In order to investigate the spread of CHIKV in Thailand in greater detail, we sequenced and analyzed additional CHIKV samples from this country, collected between 1976 and 2012. Additional samples from the Philippines (2013-2014) were also available and analyzed to increase resolution of our results. Maximum likelihood trees showed that our new genomes fell within both Asian and ECSA lineages, which were subsequently analyzed separately by Bayesian tree inference. Thai genomes from 1976-1996 and the genomes from the Philippines were found within the Asian lineage. Our estimates indicate CHIKV entered Thailand in 1956 and moved into Indonesia around 1981. Indonesia seeded transmission to many other countries, including two separate introductions into the Philippines, in late 1984 and late 2006. This second introduction established a successful spread of the virus and the most recent Philippines genomes from 2013-14 still fall within this group of viruses. All the genomes from the Asian lineage, including Thailand and Philippines, had the E1-A98T mutation that imposes an epistatic constraint on the E1-A226V change, preventing the emergence of enhanced infection within *Aedes albopictus.* Thai samples collected 2008-2013 were found exclusively in the ECSA lineage, strongly suggesting complete replacement of the Asian lineage in Thailand. Our results indicate several introductions of the ECSA virus into Thailand, all seeded from Malaysia around 2007, with a single dominant cluster establishing successful spread until 2013. All Thai genomes from the ECSA lineage contained several mutations that enhance infection and fitness of CHIKV in both *Ae. albopictus* and *Ae. aegypti.* Although detection of the ECSA lineage in the Americas has been limited and sporadic, these adaptive mutations may increase the risk of virus spread to these regions and potential lineage replacement, as already observed in Thailand.

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SOCIAL AND ENTOMOLOGICAL DRIVERS INTERCONNECT IN DRIVING SPATIAL PROPAGATION OF CHIKUNGUNYA VIRUS IN BANGLADESH

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An individual's risk of arboviral infection depends on behaviour and local environment. Understanding how these factors interconnect to drive transmission across spatial scales is pertinent for better preparedness and control efforts. Recent advances have been made at neighbourhood and country levels, but the drivers of pathogen dispersal between communities, the scale at which control programs often act, are poorly understood. We performed a household-based seroprevalence study in individuals of all ages in 40 randomly selected villages in Chapainawabganj, Bangladesh, following a chikungunya outbreak in the district. We collected information on demographics and travel behaviours, and trapped mosquitoes. Participants provided blood samples that we tested for evidence of chikungunya infection (IgG ELISA). We used final size distribution methods in a Bayesian framework to estimate local transmission parameters across villages and characterized proportion of transmission within the household and main determinants of spatial propagation. Among 1502 participants, 130 were seropositive (8.7%). We found significant spatial variability in seroprevalence across villages, ranging from 0% to 74% (median: 3%). The probability of vector-mediated transmission between household members was stable across the district yet varied by household size (2-person household:12%, 95% credible interval [95%CI]: 4-20%; 5-person household:5%, 95%CI 2-8%). Within-household transmission was responsible for 5% of infections. The abundance of *Aedes albopictus* (proportion of trap nights positive) explained 46% of total variance in community transmission as well as the observed spatial dependence between communities. Mosquito abundance was elevated in villages with high connectivity, as measured by the average time inhabitants spent in villages other than their own. This study shows that infection risk is driven by factors outside the household, including distance from affected villages, mosquito abundance, and connectivity. Surveys that identify *Aedes* habitat could help target interventions to the most at-risk communities.

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IDENTIFICATION OF POSSIBLE MAYARO VIRUS VECTORS

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Mayaro virus (MAYV) is an *Alphavirus* (family Togaviridae) initially described in 1954 following isolation from the blood of five febrile workers in Mayaro County (Trinidad). Since then, minor MAYV outbreaks have been reported in several countries of South America and the Caribbean. More recently, international travel from endemic areas has increased the number of imported MAYV cases. MAYV thus poses a concern regarding

its invasion potential, similar to spread of Chikungunya and Zika viruses. With the exclusion of *Haemagogus* sp. and *Aedes aegypti*, little is known about the range of mosquito species that are competent to transmit MAYV. We tested vector competence of 2 different MAYV genotypes (D and L) for six different mosquito species across 3 genera (*Aedes aegypti*, *Anopheles gambiae*, *An. stephensi*, *An. quadrimaculatus*, *An. freeborni*, *Culex quinquefasciatus*) using the focus forming assay (FFA). Despite its status as the putative major vector of MAYV in South America, *Ae. aegypti* was almost completely refractory to infection by MAYV genotype L, and while genotype D was able to infect and disseminate, only one mosquito was able to transmit. *Cx. quinquefasciatus* was completely refractory to infection by genotype L, and very poorly infected by genotype D, with no mosquitoes transmitting. In contrast, all species of *Anopheles* were able to be infected with both MAYV genotypes. *An. stephensi, gambiae* and *quadrimaculatus* were able to transmit both genotypes, while *An. freeborni* was able to transmit the L genotype. This is the first study to indicate that *Anopheles* mosquitoes are competent vectors of MAYV. The 4 *Anopheles* species included in this study are highly divergent and native to widely separated geographic regions (Africa, Southeast Asia, and North America), suggesting that *Anopheles* mosquitoes may be important in the invasion and spread of MAYV across diverse regions of the world, including North America.

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REACTIVE CASE DETECTION FOR THE ELIMINATION OF MALARIA IN CAMBODIAN FOREST GOERS: RESULTS FROM THE PACES TRIAL

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Cambodia is one the Greater Mekong Sub-region aiming for malaria elimination. Challenges include multi-drug resistant *Plasmodium falciparum* malaria which have necessitated a recent switch back to artesunate- mefloquine from dihydroartemisinin-piperaquine and a hard to reach high risk population of mobile forest-goers. In order to inform decision on how to operationalise key strategies for malaria elimination in this region we conducted mixed-methods operational research study in 130 villages on Cambodia-Thai border. At the core of the study was a cluster randomised control trial of reactive and proactive case detection using rapid diagnostic tests, PCR, LAMP and ultra-sensitive RDTs. Between May 2016 and Dec 2017 we received 344 notifications of symptomatic *P. falciparum* index cases we carried out 192 reactive case investigations and screened over 1000 contacts. Overall malaria positivity by PCR was 13%, predominantly *P. vivax*. Co-travellers who had visited the forest with the index case in the last month, and high-risk neighbours had the highest positivity rates for *P. falciparum*. Adherence to the 3-day course of treatment and general acceptance of the intervention was high. We will present final results from this study and discuss implications for scaling up and sustainability.

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THE COST OF ACTIVE CASE DETECTION FOR MALARIA ELIMINATION: FINDINGS FROM THE PACES CLUSTER-RANDOMIZED TRIAL IN CAMBODIA

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Cambodia aims to eliminate all malaria by 2025. Key strategies include reactive case detection and a focus on the forest going population. In 2016-2017, we conducted a cluster-randomisedtrial aiming at reducing malaria parasite reservoirs, with an active case detection approach targeting the forest goers. Case investigations were triggered upon notification of symptomatic falciparum malaria cases; high risk contact were screened using rapiddiagnostic tests and high sensitivity diagnostics. An economic evaluation was conducted alongside the trial. We assessed the financial and economic costs of implementing the intervention, using cost data from project accounts. We analysed separately the setup activities, investigation team, laboratory analyses, and other intervention costs. We adjusted costs to anticipate implementing in operational circumstances rather than under trial conditions. Research-related costs (project management, social sciences research, baseline and endline surveys) were excluded. For resources that were used for both research and intervention activities, we estimated the proportion allocated to one or the other from observations of the activities and interviews conducted with the study team. Over two malaria seasons, a total of 1,657 individuals were tested for malaria. After excluding research costs, we found that the financial cost to deliver the intervention was US$74 per person tested. The main cost drivers were the employment and other costs of the intervention team (63% of total costs), laboratory analyses (15%), and setup costs (13%). Our assumptions are tested by a series of sensitivity analyses. We also estimate the cost impact of delivering the intervention to the high risk population only.

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RESULTS FROM THE CORE TRIAL IN SOUTHERN PROVINCE, ZAMBIA, COMPARING TWO REACTIVE RESPONSES IN A QUEST TO ACCELERATE ELIMINATION OF MALARIA

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Zambia has seen impressive reductions in malaria transmission, such that the government has set ambitious targets of eliminating the disease by 2021. One key intervention in this success has been training community health workers to test and treat suspected cases, using rapid diagnostic tests and artemether-lumefantrine, as well as perform reactive focal test and treat (RFTAT) in the index case and neighbouring households. To accelerate toward zero, the use of a more aggressive approach; reactive focal drug administration (RFDA) using the longer lasting dihydroartemisinin-piperaquine, was evaluated against RFTAT in the Community-led Responses for Elimination (CoRE) randomized

controlled trial. Data from this two-year trial include: 1) a cross-sectional endline survey to measure seropositivity and *P. falciparum* prevalence in children under 15 years of age, to be performed in May 2018; 2) routine, aggregate health facility catchment area (HFCA) data, including community health worker health posts; and 3) longitudinal cohorts enrolled in reactive responses to measure clearance and re-infection rates for each arm. During the study, malaria incidence has dropped dramatically, with no significant difference in incidence between the two arms after the first year. However, in 2018, the control arm has reported 68 confirmed cases of malaria compared to only 27 in the intervention arm. PCR analysis of reactive responses strongly demonstrates the focal nature of transmission with >95% of all positives found in the index household. Further analyses of this study will follow final data collection. Optimizing the approach to malaria elimination through the use of community health worker responses to malaria cases is critical for rapidly achieving national strategic goals.

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REACTIVE CASE DETECTION FOR MALARIA ELIMINATION IN ZANZIBAR - SYSTEM EFFECTIVENESS AND COST

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Reactive case detection (RCD) for malaria is a surveillance strategy in which the detection of a malaria case by passive surveillance triggers a search for more cases in and around the household of the passively detected case. RCD is currently used in Zanzibar as part of their elimination strategy. This research aims to evaluate RCD as a surveillance strategy in Zanzibar and consists of a rolling cross-sectional survey and a cost analysis. In the survey, research staff attend RCD investigations to administer a household questionnaire and test all household members for malaria. In the costing component of the study the cost of the system as it currently operates is assessed and compared with hypothetical variants of the system. RDT prevalence was 2.5% amongst index household members and 0.4% amongst member of neighboring households. Logistic regression analyses on current data shows that the odds of being RDT-positive as a member of an index household are 6.3 times that of those in surrounding households (p<0.0001; 95% CI: 3.9-10.3). This heightened odds of infection reflects evidence in support of visiting the index household for the detection of new cases, and demonstrates much lower marginal return to expanding searches beyond the index household.

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PRO-ACTIVE CASE DETECTION IN AN AREA OF ARTEMISININ RESISTANCE: IDENTIFYING ASYMPTOMATIC CARRIERS AND ACCELERATING ELIMINATION EFFORTS

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Additional strategies are needed to accelerate malaria elimination, particularly in areas of artemisinin resistance. Therefore, Médecins Sans Frontières has implemented a novel pro-active case detection approach (ProACD) in Cambodia to detect asymptomatic malaria, complementary to a strengthened passive case detection (PCD). Here, we evaluate the feasibility and output of implementing this strategy and assess the profile of asymptomatic malaria cases, defined as absence of fever in the preceding two days. ProACD involves voluntary screening and treatment rounds in villages and targets at-risk individuals (forest and plantation workers). After a pilot with PCR-based screening, a new ProACD strategy started in Oct 2017 at 22 locations across 2 districts. ProACD is supported by a network of villagers and health promotion activities to sensitise the population. To increase cost-effectiveness, diagnosis is done using a hyper-sensitive rapid diagnostic test (Alere Malaria Ag Pf). *Plasmodium falciparum* (Pf) cases were confirmed with PCR and completed an interviewer-led questionnaire to characterise asymptomatic individuals. ProACD screenings were well accepted and overall 9827 individuals were screened. Of these, 6004 (61%) were unique visits, which gives an overall coverage of 36% (6004/16570). Overall, 62 Pf cases were detected (positivity rate: 0.6%), which were predominately male (52/62, 84%) and aged 15-45 yrs (43/62, 69%). Interviews were completed among 71% (44/62) of whom 20% (9/44) did not report an episode of sickness in the preceding two weeks. This was supported by a statistically significant lower estimated parasitaemia observed among the individuals not reporting any sickness in the preceding two weeks. In contrast, those labelled asymptomatic had a mean parasitaemia of 301 parasites/µL (SD: 599 parasites/µL). In the same period in the same villages we found 543 Pf PCD cases. We demonstrated the feasibility of introducing ProACD. We found that the majority of those labelled asymptomatic have parasitaemia well above submicroscopic levels and likely contribute to onward disease transmission.

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EVALUATION OF A NEW HIGHLY-SENSITIVE RAPID DIAGNOSTIC TEST FOR REACTIVE CASE DETECTION IN RAKHINE STATE, MYANMAR

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Recent malaria surveys revealed unexpectedly high prevalence of asymptomatic malaria infections in Myanmar. Most of these "silent" infections have parasite densities below the limits of detection of conventional rapid diagnostic tests (cRDT) and are detectable only with ultrasensitive PCR (usPCR) methods that have lower limits of detection thousands-fold lower than those of cRDTs. Although usPCR has been used to target elimination interventions such as mass drug administration in Myanmar, the utility and scalability of usPCR is limited by its complexity, cost, and need for centralized testing. A new highly sensitive RDT (hsRDT) that targets *Plasmodium falciparum* HRP-2 offers an alternative point-of-contact test to detect asymptomatic falciparum malaria. A prospective community-based study was conducted in rural Sakhunmaw township of Rakhine State, western Myanmar, to assess the performance of hsRDT for reactive case detection, in comparison with cRDT, using usPCR as gold standard. Individuals with cRDT-confirmed *P. falciparum* malaria were enrolled as index cases. Reactive case detection was done by testing potential contacts from households surrounding the household of each index case. Ten households (average of 4 contacts per household) for each index case were tested, with a target sample size of 50 index cases and approximately 2,000 contacts. Preliminary analyses

after 36 index cases and 1,120 contacts had been enrolled showed that hsRDT identified 3.6-fold more *P. falciparum* infections than cRDT, and approximately 80% of usPCR-positive infections were detected by hsRDT, suggesting that this new point-of-contact test may be useful for targeting elimination interventions such as test-and-treat or mass treatment. Based on final results that will be available in July, 2018, test characteristics of the hsRDT and cRDT in relation to usPCR as a gold standard, including sensitivity, specificity, and positive and negative predictive value, as well as demographic and geospatial analyses of malaria risk, will be presented.

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HOW RELEVANT IS ULTRA-SENSITIVE MALARIA DIAGNOSTICS FOR MALARIA ELIMINATION?

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Sub-microscopic malaria infections are recognized as an important reservoir for mosquito infection. Their extent is underestimated even by standard molecular diagnostics and their relevance for malaria elimination is unclear. Sophisticated sampling of venous blood and ultra-sensitive molecular methods can maximize test sensitivity but are not feasible in routine surveillance. A recently developed ultra-sensitive *Plasmodium falciparum (Pf)* rapid diagnostic test (us-*Pf*RDT) promises high field applicability, but its usefulness in interventions aiming at elimination is disputed. To define the diagnostic sensitivity necessary to guide malaria interventions, venous blood was collected from 300 participants in a community survey in Papua New Guinea. Ultra-sensitive qPCR (us-qPCR) on concentrated high-volume blood samples (2ml) was used as reference to evaluate the sensitivity to detect *Pf* or *P. vivax (Pv)* infections as well as gametocyte carriers in 200µl finger-prick volumes, by the following diagnostics: standard qPCR (st-qPCR), us-qPCR, standard RDT (st-RDT) and us-*Pf*RDT. Relative to the positivity in high blood volumes, st-qPCR and us-qPCR on finger-prick volumes identified 51% and 61% of *Pf* infections and 54% and 61% of *Pv* infections. These constituted 76% and 82% of *Pf* gametocyte carriers and 86% and 91% of *Pv* gametocyte carriers. Sensitivity was much lower for both RDTs. us-*Pf*RDT detected 27% of *Pf* infections (i.e. half of *Pf* infections detectable by st-qPCR) and missed 59% of *Pf* gametocyte carriers, including high gametocyte densities. *Pf* and *Pv* infections undetectable by st-qPCR were evenly distributed in the population. Epidemiological patterns thus corresponded well between st-qPCR and the reference method. Alongside a decrease in *Pv* prevalence with age, the proportion of *Pv* infections undetectable by st-qPCR increased. In conclusion, finger-prick blood volumes suffice to identify virtually all potentially transmitting *Pf* and *Pv* carriers if analysed by molecular methods. Little can be gained from analysing larger blood volumes. Current RDTs cannot replace molecular diagnostics for identifying potential *Pf* transmitters.

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MOLECULAR XENOMONITORING AND ANTIGEN SURVEYS REVEAL STRONG SIGNALS FOR PERSISTENT *WUCHERERIA BANCROFTI* INFECTION IN NORTHERN HAITI AFTER EIGHT ROUNDS OF MASS DRUG ADMINISTRATION

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Lymphatic Filariasis (LF) is a disabling vector-borne disease caused by thread-like filarial worms that affects some 100 million people in developing countries. The Global Program to Eliminate Lymphatic Filariasis is providing annual rounds of mass drug administration (MDA) with antifilarial drugs until specific human infection targets are met. In areas where *Culex* mosquitoes are the primary vector of *Wuchereria bancrofti*, detection of filarial DNA in mosquitoes (molecular xenomonitoring, MX) can identify residual infection in communities. Our group recently conducted a community treatment trial in 10 localities in Northern Haiti (population 12,384) that had persistent LF after 7 rounds of MDA. Localities were randomly assigned to receive treatment with either a 2-drug (diethylcarbamazine and albendazole [DA]) or a 3-drug (ivermectin, diethylcarbamazine, albendazole [IDA]) regimen. One year later, *Cx. quinquefasciatus* mosquitoes were collected using gravid traps placed near randomly selected residences in the 10 localities, and filarial antigen surveys were performed to estimate human infection rates in the same areas. 213 (40.1%) of 531 pools of mosquitoes that were collected from 180 trapping sites were positive for *W. bancrofti* DNA by real-time PCR. Filarial DNA rates in mosquitoes in areas treated with DA or IDA were estimated to be 2.4% (95%CI 1.9-2.9) and 2.7% (95%CI 2.2-3.2) respectively, well above the provisional target for LF elimination (upper 95%CI of the filarial DNA rate in mosquitoes < 1%). After MDA, filarial antigenemia rates in humans in the DA and IDA treatment areas were 10.0% (103/1032, 95%CI 6.3-13.6) and 8.5% (88/1032, 95%CI 0.6-16.5), respectively, well above the elimination target rate of < 2%. A spatial analysis showed similar distributions for positive mosquito trap sites and for houses with at least one infected inhabitant. This study has demonstrated the usefulness of MX to identify and indirectly assess human infection following MDA, and it provides a solid baseline for assessing the impact of future efforts to eliminate LF in this area.

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PAST AND FUTURE SPREAD OF THE ARBOVIRUS VECTORS *AEDES AEGYPTI* AND *AE. ALBOPICTUS*

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Past and future spread of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus* Dengue, yellow fever, chikungunya, and Zika are expanding their respective geographical ranges and causing severe outbreaks in many urban populations. Transmission of these viruses depends largely on the presence of the competent mosquito vectors *Aedes aegypti* and *Ae. albopictus*. The global spread of these two mosquitoes means they are now established on every human-inhabited continent and continuing to spread more widely overland. We show that quantitative estimates of human movement patterns explain the spread of both species in Europe and the United States of America (USA) following their introduction, thereby providing insights with which to predict future movements. We find that the spread of *Ae. aegypti* is characterised by long distance importations, whilst *Ae. albopictus* has expanded more along the fringes of its current distribution. We describe these processes and predict the future distributions of both species in response to accelerating urbanisation, connectivity and climate change. In the next 5-15 years the spread of both species is predicted to occur independently of any environmental changes, as both species continue to realise their newfound anthropogenic niches. *Aedes aegypti* is expected to occupy its niche in the next 5 years and will spread more slowly after 2020. Despite this, the human population living in areas suitable for the mosquitoes is projected to almost double, from ~2.4 billion to ~4 billion by 2050, mostly due to population growth in areas already at-risk. For *Ae. albopictus,* geographic

expansion driven by human introductions is expected to peak by 2050 and the population at risk is projected to increase from ~2.3 billion to ~3.9 billion. Global surveillance and control efforts that aim to mitigate the spread of chikungunya, dengue, yellow fever and Zika viruses must consider the so far unabated spread of these mosquitos. Our maps and predictions offer an opportunity to strategically target surveillance and control and thereby augment efforts to reduce arbovirus burden in human populations globally.

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EXTENSION OF LIFESPAN IN *ANOPHELES COLUZZII* MOSQUITOES BY CLIMATIC MODULATION

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The mechanisms by which *Anopheles* spp. mosquitoes persist through the dry season in Africa remain a critical knowledge gap to our understanding of these malaria vectors. To span this period in locations like the Sahelian zone of Mali, mosquitoes must migrate to areas of permanent water or survive in conditions including high temperatures, low humidity, and an absence of surface water (required for breeding). Adult mosquitoes surviving through this period must dramatically extend their typical lifespan (averaging 2-3 weeks) to 7 months. Previous work found *An. coluzzii* surviving over 200 days in the wild across rainy seasons in a presumed aestivation (hibernation) state, but this state has so far not been replicated in laboratory conditions. Thus, it is currently unknown how aestivation affects malaria vector competence, and other factors. To investigate how environmental conditions change mosquito lifespan (a key indicator of aestivation), we compared survivorship in climate controlled incubators that adjusted humidity (down to 40% RH), temperature (to 18°C), and light conditions (8 hours of light) to normal insectary conditions. These conditions were chosen to mimic the late rainy and dry seasons as well as relevant extremes these mosquitoes may experience during aestivation. We found that by priming mosquitoes in conditions simulating the late wet season in Mali, and keeping mosquitoes in reduced light and temperature, mean mosquito lifespan was increased from 18.34 ± 0.65 to 48.02 ± 2.87 days, median lifespan was increased from 19 (95% CI: 17-21) to 50 days (95% CI: 40-58), and the maximum longevity was increased from 34 to 102 days (p-adj < 0.001). While this increase falls short of the 200+ day survival seen in field mosquitoes, this extension is higher than has been previously reported through environmental or dietary modulation, and is hard to reconcile with states other than aestivation. Future work will expand on these findings, looking to extend the gains in life span while also investigating transcriptional changes in genes affecting aging, metabolism, and epigenetic regulation; hormone and nutrient levels; and vector competence.

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FUNCTIONAL DISSECTION OF A NATURAL RESISTANCE PHENOTYPE AGAINST DENGUE-1 VIRUS INFECTION IN *AEDES AEGYPTI* MOSQUITOES

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Aedes mosquitoes are vectors of several medically significant arthropod-borne viruses (arboviruses), such as dengue viruses (DENV-1, -2, -3 and -4), threatening human health and causing viral epidemics. Natural mosquito populations display substantial variation in their susceptibility to arbovirus infection. Interrogating this natural variation is a powerful approach to understand how mosquitoes acquire and transmit arboviruses, and identify novel host restriction factors. This knowledge is essential to the development of innovative disease control strategies, such as the release of lab-engineered mosquitoes rendered unable to replicate arboviruses. We recently isolated an *Aedes aegypti* mosquito population from Bakoumba (Gabon) that exhibits a strong resistance phenotype to DENV-1 infection. After oral exposure to the same infectious dose, only 50% of Bakoumba mosquitoes become persistently infected with DENV-1, compared to 100% with DENV-3 and 75% with DENV-2. A time-course analysis of viral RNA levels in DENV-1-exposed mosquitoes revealed that resistance occurs during early infection of the midgut within 48 hours after the infectious blood meal. Resistance to DENV-1 is independent of the bacterial microbiota, as the phenotype remained unaffected upon antibiotic treatment of adult mosquitoes. We analyzed the transcriptomes of 'susceptible' (persistently infected) vs. 'resistant' (exposed but uninfected) individuals by RNA sequencing on single midguts, and identified a set of genes that were significantly differentially expressed only in the resistant midguts at 24 or 48 hours post exposure, thus potentially conferring resistance to virus infection. We next tested a shortlist of candidate genes in a functional screen *in vivo* based on RNAi-mediated gene silencing assays followed by oral infection challenge. This led to the identification of a set of 2 novel proviral and 2 novel antiviral genes that are currently undergoing functional characterization by CRISPR/Cas9-mediated transgenesis.

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SUPERINFECTION EXCLUSION BETWEEN DENGUE AND ZIKA VIRUSES IN *AEDES* MOSQUITOES

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Dengue (DENV) and Zika (ZIKV) viruses are emerging Flaviviruses transmitted by *Aedes* mosquitoes. After the 2015 ZIKV epidemic in Brazil, there was a great deal of interest in the ability of mosquitoes, particularly *Ae. aegypti*, to transmit multiple viruses. Subsequent work showed that *Ae. aegypti* is capable of becoming infected with and transmitting ZIKV and DENV after ingesting both viruses in a single blood meal. While human co-infections of DENV and ZIKV have been reported, mosquito co-infections resulting from a single blood meal are likely extremely rare. More commonly, mosquitoes would be exposed to multiple viruses via separate hosts. To assess whether *Ae. aegypti* and *Ae. albopictus* are able to co-transmit ZIKV and DENV, we exposed mosquitoes to both viruses sequentially. Mosquitoes were fed an infectious blood meal containing DENV, an uninfectious blood meal, or were intrathoracically-injected with DENV. On 7dpi, all mosquitoes were fed a second blood meal containing ZIKV. Saliva and bodies were collected on 16 and 23dpi. Individual mosquitoes were dissected, and the saliva, head/thorax, and abdomen were tested for DENV/ZIKV using multiplex qRT-PCR to assess transmission, dissemination, and infection. Mosquitoes that became infected with DENV prior to ZIKV exposure had significantly lower ZIKV dissemination rates than mosquitoes that were fed uninfected blood, or that did not develop DENV infections. Infection rates in the abdomen did not differ between groups, suggesting that DENV either prevented ZIKV escape from the midgut or establishment in secondary infection sites. Transmission rates were very low, regardless of treatment, even at 23dpi. A small number of individuals developed co-infections, but no co-transmission was observed. While it is possible for *Aedes* to co-transmit DENV and ZIKV if both viruses are ingested in a single blood meal, we have shown that co-infections resulting from sequential blood meals are rare. Further research is needed on the environmental, genetic, and behavioral drivers of vector competence that may affect susceptibility to co-infection in the field.

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WOLBACHIA EFFECTS ON ARBOVIRUS INFECTION IN MOSQUITOES ARE VARIABLE AND DEPEND ON THE *WOLBACHIA* STRAIN, THE MOSQUITO SPECIES, AND THE PATHOGEN

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The bacterial symbiont Wolbachia been shown to induce resistance to pathogen infection and transmission in vector arthropods, leading to trials examining Wolbachia for vector-borne disease control. However, some Wolbachia strains have been demonstrated to enhance rather than suppress pathogens in a variety of natural and artificially infected arthropod hosts. Here, we investigated the effects of multiple Wolbachia strains on flavivirus, alphavirus, and bunyavirus infection in Culex and Aedes mosquitoes. We show that depending on the mosquito host, the Wolbachia strain, and the pathogen, Wolbachia may suppress, enhance, or have no effect on virus infection and titer. These data indicate that the suppressive effects of Wolbachia infection on pathogens are not universal, but rather depend on the characteristics of the specific symbiont-vector-pathogen system in question. These results have important implications for the deployment of Wolbachia-infected mosquitoes into field populations for disease control.

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COMPOSITION OF THE *AEDES AEGYPTI* MICROBIOME IS ALTERED BY *WOLBACHIA*, BUT IS NOT CRITICAL TO *WOLBACHIA* BLOCKING OF DENGUE VIRUS

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Dengue virus is primarily transmitted by the mosquito *Aedes aegypti*, and is the causative agent of dengue fever and the more severe dengue hemorrhagic fever/dengue shock syndrome. A method for dengue control being employed globally involves stable transinfection of *Ae. aegypti* with the common insect endosymbiont *Wolbachia*, which mediates an antiviral effect. However, the mechanism by which *Wolbachia* reduces the susceptibility of *Ae. aegypti* to dengue is not fully understood. In this study we considered the impact of *Wolbachia* infection on the intrinsic bacterial population (microbiome) of *Ae. aegypti*, and whether other bacteria that reside in the mosquito might affect the ability of *Wolbachia* to limit dengue virus infection. We used sequencing of the conserved 16S rRNA gene to compare the microbiome profiles of *Ae. aegypti* mosquitoes stably infected with *Wolbachia* to those without *Wolbachia*, collected from field release sites in Australia. We also used antibiotic treatment of laboratory-reared *Ae. aegypti* to shift their microbiome profiles and then assessed their susceptibility to dengue virus. We found that *Wolbachia* had significant interactions with numerous taxa in the field-collected *Ae. aegypti,* causing changes in their relative abundance. The microbiome of the laboratory-reared mosquitoes comprised a much lower number of bacterial species, and the effect of *Wolbachia* was less substantial. Importantly, despite alteration in the microbiome of *Wolbachia*-infected laboratory mosquitoes following antibiotic treatment, we found no change in dengue infection rates. Together these data indicate that *Wolbachia* has some effects on the *Ae. aegypti* microbiome, but it is unlikely that specific resident bacteria are required for the fundamental mechanism by which *Wolbachia* reduces susceptibility to dengue virus.

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A REVERSE VACCINOLOGY APPROACH FOR IDENTIFYING STH VACCINE CANDIDATES

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Novel prophylactic and therapeutic strategies for STH control are needed because resistance has surfaced to all major anthelmintic drugs in parasites of veterinary importance and because this poses a risk to treating parasites of humans. Vaccines are one plausible solution to sustainable control; however, thus far, only parasite vaccines that attenuate rather than prevent infection have been developed. Omics driven, knowledge-based identification of anti-parasite intervention targets is now possible using the vast amounts of 'omics data available for many parasitic nematode species. To identify and prioritize parasite intervention targets, we employed a reverse vaccinology approach that uses bioinformatics to screen parasite omics data for putative immunoreactive antigens for experimental validation. We used a step-wise approach that: (i) identified putative vaccine targets conserved among gastrointestinal nematodes by constructing orthologous protein families from genomes that span the phylum Nematoda; (ii) prioritized OPFs based on taxonomic conservation, life-cycle expression profiles, proteomics data and secretion potential; and (iii) experimentally tested (ELISA) the top prioritized candidates using infection sera as a "proof-of-principle". As an orthogonal approach, we also independently and experimentally identified differentially expressed proteins through immunoblotting (parasite-susceptible vs resistant pig antibodies) for additional candidates. We intersected all putative vaccine targets from both approaches and experimentally evaluated the top candidates. Since 5 of the 6 prioritized candidates tested positive, we move to and will present our results from the more high-throughput screening using protein microarrays of over 200 proteins. This approach identified parasite proteins that activate immunity as potential vaccine targets, experimentally validated a subset of these as immunologically cross-reactive, and utilized high-throughput screening to demonstrate the use of omics data to facilitate sustainable control strategies for human and animal parasitic infections.

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SELECTION AND CHARACTERIZATION OF A NOVEL WHIPWORM VACCINE CANDIDATE THAT INDUCES TYPE 2 PROTECTIVE IMMUNITY

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Trichuris trichiura, the common whipworm, is a leading cause of colitis in the developing world with an estimated 465 million people living with whipworms in their colon. The disease disproportionately affects children, resulting in malnutrition, growth stunting, and cognitive deficits.

Successful control of trichuriasis by mass drug administration has been limited due to the combination of poor drug efficacy, high rates of post-treatment reinfection, and failure to co-implement aggressive sanitation programs. Our long-term solution is to develop a vaccine against *T. trichiura*. There is no established laboratory animal model for this human pathogen, so we have adapted the closely related *Trichuris muris* parasite. Vaccination with *T. muris* excretions and secretions (*Tm*-ES) elicits protection in AKR mice. Using the protective anti-*Tm*-ES sera, we have identified 14 antigens by both immunoscreening the *T. muris* cDNA library and by two-dimensional electrophoretic separation of *Tm*-ES followed by western blotting. The most promising candidate, a recombinant *T. muris* whey acidic protein 49 (r*Tm*-WAP49), led to a significant reduction in larval worm burden. Endpoint ELISA titers revealed a high serum IgG1 to IgG2a ratio in protected groups, reflective of a type 2 humoral response. Similarly, a profound type 2 cellular response (interleukin-4 [IL-4], IL-5, IL-9, and IL-13) was measured in the spleens, mesenteric lymph nodes, and the vaccine-draining lymph nodes of protected mice by 23-plex Luminex analysis. Using immunofluorescent staining with anti-r*Tm*-WAP49 sera, we localized native WAP production to the stichosome of *T. muris.* In pursuit of a 'pan-anthelmintic' vaccine, we have fused a fragment of r*Tm*-WAP49 with the hookworm vaccine *Na*-GST-1, which together led to a similar type 2 protective immunity against whipworm. To determine whether our *T. muris* derived WAP proteins had conserved immunogenicity against *T. trichiura*, we measured the human serological and cellular reactivity to both WAP proteins using biological samples collected from adolescents and adults in Honduras naturally infected with whipworm.

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EOSINOPHIL-DOMINATED PULMONARY TYPE-2 RESPONSE DRIVEN BY ALLERGIC SENSITIZATION INHIBITS LARVAL DEVELOPMENT AND CONTROLS HELMINTH PARASITE BURDEN IN THELUNGS

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Although chronic helminth infection has been associated with diminished allergic reactivity (hygiene hypothesis), the impact of pre-existing allergic sensitization on the outcome of helminth infection is less well-studied. Having previously demonstrated an augmented Th2-dominated parasite-specific $CD4^+$ response in the context of human helminth infection with coincident house dust mite (HDM) allergy, we sought to identify the mechanism driving this response and to understand how allergic sensitization may influence parasite burden at the site of inflammation. Using a murine model of HDM-induced allergic asthma and concomitant *Ascaris* spp. infection, our data show that HDM sensitization prior to infection with *Ascaris* induces a robust Type-2-associated inflammation in the lung characterized by an increase in both IL-5/IL-13 producing innate lymphoid cells (ILC2s; $17.9x10^3$ cells vs $2.4x10^3$ cells, $p<0.01$) and eosinophils (140-fold increase, $p<0.001$) when compared to non-allergic infected mice. This pulmonary Type-2 response led to an IL-4-rich environment (8.3 pg/mL vs 3.3 pg/mL, $p<0.01$) that drove the differentiation of lung macrophages towards the M2 phenotype expressing arginase-1 (17.6-fold increase, $p<0.01$), but not iNOS. When the *Ascaris* larvae migrate from the circulation to the lung tissue in their quest to reach the airways in these HDM-sensitized mice, the Type-2-associated inflammation led to a marked reduction (72%) in the number (20±9 larvae vs. 63±50 larvae; $p=0.044$) and in the development of lung-stage *Ascaris* larvae (size 24816 μM^2 vs 58170 μM^2, $p<0.001$) when compared with non-allergic infected mice. When eosinophil deficient (αdblGATA) mice were infected with *Ascaris* following HDM-induced asthma, the numbers of larvae were no longer reduced ($p=0.18$), nor was there any alteration in larval development when compared to αdblGATA non-allergic infected mice ($p=0.08$). Taken together, our data suggest that allergic sensitization coincident with helminth infection drives an eosinophil-rich pulmonary Type-2 response that limits helminth parasite numbers and also directly hinders their developmental program.

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ASCARIS LARVAL MIGRATION CAUSES LONG-TERM CHANGES IN LUNG STRUCTURE AND FUNCTION

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Ascaris (roundworm) is the most common helminth infection worldwide, infecting over 800 million people and accounting for significant morbidity, particularly in children. Following initial ingestion of *Ascaris* eggs larvae migrate through the host lungs releasing proteinases causing lung injury and hemorrhage. Due to high worm burdens and frequent re-infection, children are at particularly high risk of severe *Ascaris*-induced lung injury. We hypothesize that *Ascaris* larval migration through the lungs causes destruction of the lung extracellular matrix leading to changes in lung function similar to emphysema. BALB/c mice were infected with 2,500 embryonated *Ascaris suum* eggs. µCT imaging was completed to quantify lung volume. Lung compliance was measured using a standardized total pressure-volume (PV) curve based on residual volume and total lung capacity. H&E stained lung tissue was used to measure mean linear intercept (MLI), a standardized evaluation of emphysema-like changes in the lungs, to measure the mean free distance in air space. *Ascaris* infected mice had increased lung volumes measured by µCT at day 12 post-infection (p.i.) (434 vs 485, $p < 0.05$) and 1 month p.i. (458.3 vs 565.6, $p < 0.001$). Additionally, *Ascaris* infected mice had increased lung compliance at Day 12 (0.715 vs 0.846, $p = 0.03$), 1 month (0.9675 vs 1.152, $p <0.005$), and 2 months (0.9525 vs 1.3, $p = 0.01$) p.i. MLI was also increased at 2 months p.i. (33.26 vs 45.91, $p = 0.024$). *Ascaris* larval migration through the lungs causes significant damage to the lung extracellular matrix which impacts lung structure and function. Within the first 2 months p.i., mice develop increased lung volumes, compliance and MLI creating a pulmonary phenotype that resembles human emphysema.

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THE STRUCTURE AND FUNCTION OF EXTRACELLULAR VESICLES SECRETED BY THE GASTROINTESTINAL NEMATODE PARASITE, *ASCARIS SUUM*

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The ability of helminth parasites to modulate or manipulate host biology to both establish and maintain infection is well described. This parasite-derived modulation is often directed towards the host immune response with the goal of suppressing, modifying, or evading the host response. Helminth excretory/secretory products have been the focus of much research effort to identify immunomodulatory molecules, however, specific parasite effectors, and the mechanisms by which they bring about host manipulation, are poorly understood. Extracellular vesicles (EVs) represent a class of newly-identified parasite-derived structures, containing putative protein and small RNA effector molecules. Emerging evidence from our lab, and others, suggest parasite EVs may be important factors operating at the host-parasite interface. We hypothesize that parasitic nematodes release EVs as a mechanism to deliver effector molecules to host tissues and modulate their biology. Here we isolated EVs secreted by adult *Ascaris suum*, a pig gastrointestinal nematode, to study their structure and function. We found that *Ascaris* EVs share the deflated ball morphology reported in the literature with a mean diameter of 168 nM, which is larger than reported in other species. Global proteomic profiling identified 519 proteins from the *Ascaris* EVs, including not only commonly reported

EV markers such as HSP70 and Annexin, but also immune modulation proteins such as macrophage migration inhibitor (MIF-1) and serine protease inhibitor (serpin). *Ascaris* EVs are rapidly internalized (within 24 hours) by primary murine bone marrow-derived macrophages (BMDM) and this uptake was blocked by Cytochalasin D, suggesting phagocytosis or macropinocytosis are possible uptake mechanisms. RNA-seq was used to reveal the small RNA contents of the EVs along with transcriptional changes elicited in BMDM after EV internalization. These data, and the role of *Ascaris* EVs in host immunomodulation, are discussed.

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A REFERENCE *ASCARIS LUMBRICOIDES* GENOME ALLOWS INSIGHTS INTO POPULATION-BASED GENOMIC CHANGES IN SPACE AND TIME

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Human ascariasis is a neglected tropical disease caused primarily by the nematode *Ascaris lumbricoides*. At present, relatively little is known about the molecular makeup and local/global patterns of sequence variation of *Ascaris lumbricoides*. We sequenced germline DNA from a single female worm. Using a reference-based approach resulted in a high quality genome with no unplaced contigs. The *A. lumbricoides* sequences showed >99% sequence similarity with the recently completed *A. suum* genome. We used this new genome to map whole genomes of 77 individual adult Ascaris collected from individuals from 5 closely-separated villages in rural Kenya. Using SNPs from these adult parasites, we demonstrated that parasites clustered by village and that genetic distance between worms was associated with geographic distance between host residences, suggesting that worms in different villages were partially reproductively isolated from each other. Additionally, analysis of mitochondrial genomes, and particularly COX1, allowed for comparison with 88 additional publicly available COX1 sequences from *A. lumbricoides* and *A. suum*. A haplotype network based on a 300bp portion of COX1 from these (88+77) worms showed that the worms collected in Kenya were split between two highly similar worldwide clades; these 2 clades did not split clearly between pig- and human-derived samples. The limited mitochondrial genome diversity suggests a recent (<1000 years) Ascaris population expansion, which could have spread the worms around the world with people or livestock, explaining the similar set of haplotypes present in Kenya and other regions. In fact, there was no association between genetic distances and geographic distances based on the mitochondrial genomes of worms collected across different continents. These data suggest that, while there is some structuring of worm populations locally (which may provide information about transmission patterns), there was no evidence of a selection event or global divergence of haplotypes to differentiate populations from pigs or humans or one continent compared to another.

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IMPROVED MOLECULAR DETECTION OF *ASCARIS LUMBRICOIDES* UTILIZING EMBRYONIC SEQUENCE FOR ASSAY DESIGN

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We have previously described a next generation sequencing (NGS)-based pipeline for the development of improved diagnostic real-time PCR assays based on the identification and targeting of highly repetitive, non-coding genomic DNA sequences. This pipeline has proven effective for improving the sensitivity and specificity of detection for various soil transmitted helminth (STH) species including *Necator americanus*, *Ancylostoma duodenale*, *Ancylostoma ceylanicum*, *Trichuris trichiura*, and *Strongyloides stercoralis*. However, previous attempts to utilize this pipeline for the development of an *Ascaris lumbricoides* assay have proven ineffective. As the aforementioned pipeline has relied upon the analysis of genomic DNA isolated from adult worms, *A. lumbricoides* has been refractory to successful analysis. Such failures have likely occurred due to the phenomenon of chromosome diminution, whereby large portions of the *A. lumbricoides* genome (especially repetitive sequences) are eliminated during embryonic development. As a result, would-be target sequences are not identified during analysis of DNA from adult somatic tissues. Recently, a reference level embryonic genome of *A. lumbricoides* has enabled the analysis of gDNA isolated from immature life stages. As the molecular identification of STHs relies upon the detection of DNA isolated from stool-derived eggs, embryonic targets make ideal assay candidates. Accordingly, utilizing the available embryonic gDNA sequences, we have employed our pipeline to identify a high copy number repeat, greatly improving the sensitivity of parasite detection and reducing Cq values in field-collected samples by as much as ten amplification cycles or more, which corresponds to an increase in sensitivity of approximately 1,000-fold. Given the importance of sensitive and specific detection of parasites for programmatic decision-making efforts, this assay eliminates a critical gap in the STH molecular toolbox.

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ALGORITHMS BASED ON HOST BIOMARKERS TO IDENTIFY FEBRILE PATIENTS AT RISK OF LIFE-THREATENING INFECTIONS IN LOW RESOURCE SETTINGS

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Fever is one of the most common reasons patients seek medical care; however, most infections are self-limited and in the absence of critical illness can be managed conservatively. The inability to identify individuals with acute febrile syndromes at risk of death is a barrier to effective triage and management of severe infections. As endothelial and immune activation contribute to the pathogenesis of life-threatening infections, we hypothesized that measuring biomarkers of these pathways could identify febrile individuals with impending life-threatening infections. Consequently, we assessed biomarkers of these pathways at clinical presentation in emergency departments in two separate cohorts: (i) febrile Ugandan children (n=2,084, 99 deaths), and (ii) febrile Tanzanian adults (n=507, 32 deaths). Lead single endothelial and immune activation biomarkers predicted mortality and were superior to CRP and PCT (P<0.0001). We show sTREM1 to be the top single biomarker for

predicting "all-cause" febrile mortality in Ugandan children (AUROC 0.88, 95% CI 0.84-0.92, negative predictive value [NPV] 99.0%) and Tanzanian adults (AUROC 0.87, 95% CI, NPV 97.9%). sTREM1 displayed non-inferior performance to established clinical disease severity scores (e.g. LODS: AUROC 0.90 95% CI 0.87-0.93, NPV 99.0%; qSOFA: 0.79, 95% CI 0.72-0.87, NPV 97.0%). Combinatorial models adding sTREM1 to either LODS or qSOFA significantly improved their predictive accuracy in both the pediatric (AUROC 0.93, 95% CI 0.90-0.96, NPV 99.5%) and adult (AUROC 0.91, 95% CI 0.88-0.94, NPV 99.7%) cohorts. These data indicate that measuring sTREM1 at clinical presentation, especially when combined with easy-to-measure clinical severity scores, can reliably identify febrile individuals at risk of death. Implementation of biomarker-based algorithms using point-of-care tests could improve the recognition, triage, and outcome of patients with life-threatening infections.

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CUTANEOUS LARVA MIGRANS IN RETURNED CANADIAN TRAVELERS TO THE CARIBBEAN: SURVEILLANCE REPORT FROM CANTRAVNET, JANUARY 2009 — MARCH 2018

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Cutaneous larva migrans (CLM) is one of the most common dermatoses with which travelers to the tropics return. We examined the demographic and travel correlates of Canadian travelers returning from the Caribbean with CLM over a 10-year period to illuminate the recent emergence of this disease in our traveling population. Data on all returned Canadian travelers presenting to a CanTravNet site between January 2009 and March 2018 who were diagnosed with CLM acquired in the Caribbean were analyzed. Of 22,169 travelers in the CanTravNet database over the enrolment period, 299 (1.3%) returned from the Caribbean with CLM. Median age of the returned travelers with CLM was 34 years (range 1 - 73 years), with males accounting for 41% of cases (n=123), and females 59% (n=176). Ninety-five percent (n=284) traveled for tourism. Jamaica was the most well represented source country, accounting for 188 cases (63%), followed by Barbados (n=27, 9%), Dominican Republic (n=25, 8%), Cuba (n=21, 7%), and Saint Lucia (n=11, 4%). In total, 17 different source countries in the Caribbean were represented. Over the first 10-weeks of 2018, 48 travelers with CLM from the Caribbean were evaluated at CanTravNet sites, which represents a 6-fold average increase in cases during those same first 10-weeks in each of the 5 prior years (average 8 cases during the first 10-weeks of each year prior to 2018). Cases in 2018 have been imported predominantly from Jamaica (n=27, 56%), Dominican Republic (n=13, 27%), and Barbados (n=2, 4%), with 1 case (2%) imported from each of Cayman Islands, Cuba, Guadeloupe, Martinique, Saint Lucia, and Saint Martin. Age, sex, and purpose of travel distributions were similar across years. We have documented a large increase in imported cases of CLM originating in the Caribbean in the year 2018, though have not noted any demographic or travel-related aberrations in our returned traveler population to explain this phenomenon.

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ANTIBIOTIC MANAGEMENT OF MODERATE-TO-SEVERE DIARRHEA MAY REDUCE RISK OF LINEAR GROWTH FALTERING IN CHILDREN: A SECONDARY ANALYSIS OF GEMS CASES

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Children with moderate-to-severe diarrhea (MSD) are at particularly high risk of linear growth failure and associated negative health outcomes. However, little is known about whether antibiotic treatment of MSD may protect against linear growth faltering. Using previously collected data from the Global Enterics Multicenter Study (GEMS) of children 0-59 months old with MSD in 7 low- and middle-income countries (LMICs), we conducted a retrospective cohort analysis to assess whether children with MSD who were treated with antibiotics had a lower risk of linear growth faltering in the 60-90 days following the episode. We used relative risk (RR) regression to evaluate loss of ≥1 length/height-for-age z-score [LAZ/HAZ] between enrollment and follow up, and linear regression to assess change in LAZ/HAZ, with propensity score adjustment for variables associated with antibiotic treatment. Of 7659 surviving MSD cases, mean LAZ/HAZ loss during follow up was 0.13, and 3% lost ≥1 LAZ/HAZ. Antibiotics were provided to 81% at presentation. Factors associated with antibiotic treatment included young age, nutritional status, hospitalization, GEMS site, and presence of fever, vomiting, or dysentery. After propensity score adjustment, children provided with antibiotics had a 41% lower risk of losing ≥1 LAZ/HAZ than those who received none (adjusted RR: 0.59; 95% confidence interval [CI]: 0.42, 0.82). Children provided antibiotics in the health center gained 0.06 LAZ/HAZ more than those who were not (95% CI: 0.03, 0.09). Among children ≤ 23 months, those who received antibiotics were less likely to lose ≥1 LAZ/HAZ (aRR: 0.67; 95% CI: 0.48, 0.94), and gained 0.03 LAZ/HAZ more than those who received no antibiotics (95% CI: -0.004, 0.07). Antibiotic treatment of MSD may preserve linear growth in young children in LMICs. Ongoing randomized trials will provide further evidence to evaluate the effectiveness of antibiotics in preventing linear growth faltering in this population. However, any potential benefit of antibiotic management of MSD must be weighed cautiously against negative consequences including antibiotic resistance and microbiome disruption.

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CLINICAL FEATURES AND OUTCOME OF NEONATAL DENGUE AT THE CHILDREN'S HOSPITAL 1, HO CHI MINH, VIETNAM

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Vertical transmission of dengue, a life-threatening disease transmitted by *Aedes* mosquitoes, has been reported in a few case reports. In this context, we tried to determine the clinical features and outcomes of neonatal dengue. We conducted a prospective study on 32 neonates with laboratory-confirmed dengue by positive for either NS1 antigen rapid test or IgM antibody with MAC-ELISA, who were hospitalized at the Children's Hospital 1, Ho Chi Minh, Vietnam from January 2010 to December 2016. Diagnosis of dengue was defined according to the World

Health Organization 2009 classification. The median time to onset of fever was on day 5 (IQR: 4, 8) after birth. The median time for diagnosis in neonates was at 7 (IQR: 6, 10) days of age while the median febrile phase was 3 (IQR: 2, 4) days. The median length of hospital stay was 6 (IQR: 4, 10) days. Petechiae, pharyngeal mucosal hemorrhage, and hepatomegaly were detected in 87.5% (28/32), 6.3% (2/32), and 75% (24/32) of cases respectively. The white blood cell counts in the febrile and critical phase were 7,800±800/mm^3 and 13,400±2,800/mm^3 respectively. The platelet counts in the febrile and critical phase were 97,111±37,826/mm^3 and 30,100±5,749/mm^3 respectively. Hematocrit levels during the febrile and critical phase were 49±1% and 47±1% respectively. Of the 12 patients undergoing abdominal ultrasonography, 2 (16.7%) cases showed gallbladder wall thickening. The C-reactive protein concentration at admission was 1.5±0.7 mg/L. All patients were classified as dengue with no warning signs and had a full recovery. The results of laboratory diagnosis were as follows: 56.3% (18/32) NS1(+) and IgM not performed; 15.6% (5/32) NS1(+) IgM (+); NS1(-) IgM (+), 15.6% (5/32) NS1 not performed and IgM (+). These findings emphasize that early diagnosis of neonatal dengue should be based on a history of maternal illness, NS1 rapid test, petechiae, hepatomegaly and low platelet counts, which tend to decrease significantly in the critical phase. Our study provides additional support for the vertical transmission of dengue, which emphasizes considerable insights into prevention and early detection.

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SAFETY AND IMMUNOGENICITY OF AGS-V, A MOSQUITO SALIVA PEPTIDE VACCINE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 1 TRIAL

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Parasites and viruses carried within mosquito saliva appear to initiate or enhance severity of host infection by taking advantage of saliva-human host interactions. This leads to alteration of the cutaneous environment and modulation of the host's innate and adaptive immune responses, thereby providing a rationale for creating vaccines against mosquito salivary proteins rather than the pathogens contained within the saliva, or a combination of both. AGS-v is a vaccine composed of four salivary peptides isolated from *Anopheles gambiae* salivary glands, but that are common across a number of mosquitoes. In this first-in-human study, we enrolled and randomized 49 healthy adult participants to receive the AGS-v vaccine with and without adjuvant (Montanide ISA 51) versus placebo. Vaccinations occurred at Day 0 and Day 21 followed by an uninfected *Aedes aegypti* mosquito feeding at Day 42. Primary objectives are: 1) to assess safety via incidence of adverse events and 2) to evaluate humoral and cellular immunity by respectively measuring total AGS-v specific immunoglobulins and Th1-associated cytokine release after incubation of peripheral blood mononuclear cells with AGS-v antigens. Secondary objectives are post-mosquito feeding measures of AGS-v specific immunoglobulins and Th1-related cytokine release, mosquito survival and fecundity, as well as the effects of immunized individuals' peripheral blood mononuclear cells on Zika virus after stimulation with *Aedes aegypti* saliva. Results are pending until the data blind is lifted mid-2018, but will be available for presentation at the conference.

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ACUTE KIDNEY INJURY IN SEVERE MALARIA: NEURODEVELOPMENTAL EFFECTS IN UGANDAN CHILDREN

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Acute kidney injury (AKI) is increasingly recognized as an important complication of severe malaria (SM) associated with adverse clinical outcomes. We recently reported that AKI is associated with impaired neurodevelopment in Ugandan children with SM. However, there are currently no data on specific cognitive and behavioral domains impacted by AKI in pediatric SM. We conducted a secondary analysis of a prospective study evaluating development in Ugandan children between 18 months and 12 years of age with cerebral malaria (n=226) or severe malarial anemia (n=209), and healthy community children (n=173). Assessments of cognition, attention, memory, socioemotional function, and executive function were performed at baseline and 6, 12, and 24 months later. Age-adjusted z-scores were computed for each domain using community children's raw scores. AKI was assessed using the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. AKI was defined as a 50% increase in creatinine from estimated baseline. The association between AKI and cognitive and behavioral outcomes across all time points was evaluated using linear mixed effect models adjusted for weight-for-age z-score, child's education, parental education, socioeconomic status, home environment, and severe malaria type (CM or SMA). Children <5 years with AKI on admission had lower scores on overall cognition (β=-0.28 (95% confidence interval, -0.55, -0.00), p=0.048) and attention (β=-0.29 (-0.51, -0.08), p<0.008). Children >5 years with AKI had lower memory scores (β=-0.43 (-0.81, -0.04), p=0.03); diminished executive functioning (β=0.75 (0.03, 1.5), p=0.04); and exhibited more externalizing symptoms (β=0.71 (0.26, 1.2), p<0.002) and more total behavioral problems (β=0.63 (0.18, 1.1), p<0.006). SM with AKI on admission affected multiple cognitive and behavioral domains in our population and may have the largest impact on children >5 years. Prevention, identification, and appropriate management of AKI in SM could help reduce the neurodevelopmental burden of malaria in endemic regions.

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MAPPING DIPHTHERIA-PERTUSSIS-TETANUS VACCINE COVERAGE IN AFRICA, 2000-2016

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Routine childhood vaccination is among the most cost-effective, successful public health interventions available. Amid substantial investments to expand vaccine delivery throughout Africa, most countries still lack robust measures of local routine vaccine coverage and changes in geographic inequalities over time. Here, we leverage data from 183 surveys conducted between 2000 and 2016, including 881,268 children in 49 African countries, to produce annual high spatial resolution (5x5 km^2) diphtheria-pertussis-tetanus (DPT) vaccine coverage estimates for children aged 12-23 months in 52 African countries from 2000-2016, using a Bayesian geostatistical model calibrated to results from the Global Burden of Diseases, Injuries, and Risk Factors Study 2017. Estimated DPT3 coverage increased in 78.7% (95% UI: 71.3 - 83.7%) of second-level administrative units in Africa from 2000-2016, yet substantial geographic inequalities in DPT coverage and dropout remained across and within African countries. In 2016, 10 of 52 countries had at least one local area (second-level administrative unit) with coverage less than half of the national average,

and only Morocco and Swaziland had achieved ≥ 80% DPT3 coverage in all second-level administrative units with high confidence (posterior probability ≥ 95%). Large areas of low DPT3 coverage (≤ 40%) were identified in the Sahel, Somalia and the Ogaden region of Ethiopia, and the Democratic Republic of the Congo (DRC). Low DPT1 coverage (≤ 50%) and high relative dropout rates (≥ 30%) together drove low DPT3 coverage across the Sahel, Somalia and the Ogaden, Central African Republic, Guinea, and Angola. Despite substantial progress in Africa, marked national and subnational inequalities in DPT coverage and dropout persist. These results can help identify areas of low coverage and vaccine delivery system vulnerabilities and ultimately support more precise targeting of resources to improve vaccine coverage and health outcomes for African children.

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CLINICAL TRIAL EXPERIENCE WITH THE MERCK RVSVΔG-ZEBOV-GP EBOLA VACCINE: UPDATED SAFETY, IMMUNOGENICITY, AND EFFICACY

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The 2013-2016 West African Ebola outbreak resulted in >28,600 cases and >11,300 deaths. In conjunction with private and public partners, Merck & Co., Inc., Kenilworth, NJ USA is developing a vaccine that demonstrated efficacy during this outbreak. The vaccine is a live recombinant vesicular stomatitis virus (VSV) containing the Zaire Ebola virus glycoprotein (GP) in place of the VSV GP (rVSVΔG-ZEBOV-GP). Vaccine safety, efficacy, and immunogenicity have been evaluated in 13 Phase 1-3 clinical trials. More than 17,000 subjects have received rVSVΔG-ZEBOV-GP vaccine (clinical dose: ≥$2x10^7$ plaque forming units/mL) in these clinical trials to date. Clinical trial data support a favorable safety profile. Common adverse events included injection-site reactions, fever, fatigue, myalgia, arthralgia, and headache, which were mostly mild to moderate in intensity and of short duration. Arthritis, which was also generally mild to moderate in intensity, has been reported at frequencies <5% in most trials. In a cluster-randomized ring vaccination trial in Guinea, subjects were randomized to immediate or delayed vaccination. As reported by Henao-Restrepo et al. in 2017 (*Lancet*; 389:505-518), while there were no confirmed cases of Ebola virus disease among those vaccinated in the immediate arm 10 days or more after randomization, 10 cases of confirmed disease (in 4 rings) were observed in eligible subjects in the delayed vaccination arm who consented on Day 0 (vaccine efficacy: 100%; 95% CI: 63.5-100%, *P*=0.0471). Immune responses, based on GP antibodies detected by GP-ELISA and neutralizing antibodies detected by plaque reduction neutralization test (PRNT), were detectable at 14 days in most vaccinated subjects, with nearly 100% seroconversion by 28 days. Durability of the anti-GP antibody responses was initially demonstrated through 6 months. Longer term durability of immunogenicity out to 24 months (by both GP-ELISA and PRNT) from a Phase 3 safety and lot consistency trial and updated safety data from across the program will be presented.

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NEUROLOGICAL, COGNITIVE, AND PSYCHOLOGICAL FINDINGS AMONG SURVIVORS OF EBOLA VIRUS DISEASE FROM THE 1995 KIKWIT OUTBREAK IN DEMOCRATIC REPUBLIC OF CONGO: A CROSS-SECTIONAL STUDY

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Without clear mechanisms of disease, except for uveitis, survivorship from Ebola virus disease (EVD) may have a long-term effect on health status. Among a cohort of EVD survivors and close contacts in Kikwit, Democratic Republic of Congo, we sought to describe the prevalence of adverse neurological, cognitive, and psychological findings and to determine the effect of EVD on these outcomes. From August to September 2017, we conducted a cross-sectional study in Kikwit, which is approximately nine hours by road from Kinshasa, the capital city of DRC. We recruited EVD survivors and close contacts. We conducted a physical examination and administered a questionnaire, including culturally adapted versions of Folstein mini-mental status exam (MMSE), Goldberg Anxiety and Depression Scale (GADS), and 7-item EVD-related stigma index. We described the prevalence of EVD sequelae among EVD survivors and among close contacts. Then we estimated the relationship between EVD survivorship (survivors vs. close contacts) and clinical outcomes. Potential confounders including age, sex, educational level, marital status and being a healthcare worker were used in linear regression models. Fourteen EVD survivors and 202 close contacts were enrolled. Among EVD survivors, 3 (21%) reported at least one abnormal neurological symptom and 2 (14%) had an abnormal neurological examination. The MMSE scores were 20.3 (Standard Deviation (SD), 17.1-23.4). Mean GADS scores were 8.3 (SD, 2.6-13.9). Nine survivors (64%) reported experiencing at least one item of EVD-related stigma since the outbreak, but none experienced EVD-related stigma in the six-months prior to the interview. EVD survivors had a statistically significant association with MMSE and GADS as compared to close contacts (MMSE: Adjusted Odds Ratio (AOR), -2.44; 95% Confidence Interval (CI), -4.47 to -0.41; GADS: AOR, 5.45; 95% CI, 2.92 to 7.97). In conclusion, this is the first study to suggest that EVD survivorship can have neurocognitive sequelae and be associated with depressive and anxious symptoms as long as 22 years after an Ebola outbreak.

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DURABILITY OF IMMUNE RESPONSES INDUCED BY THREE LEADING CANDIDATE EBOLA VACCINE REGIMES; RVSV ZEBOV, CHAD3 EBO Z-MVA BN-FILO AND ADHU26.ZEBOV-MVA BN FILO

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Of the vaccines developed in response to the 2014-16 Ebola outbreak in West Africa, three which encoded the Zaire Ebola glycoprotein, showed acceptable reactogenicity and promising immunogenicity in

early-stage clinical trials - a single-dose approach based on a replicating vesicular-stomatitis virus (rVSV ZEBOV) and two heterologous prime-boost combinations using replication-deficient adenoviruses (ChAd3 and AdHu26) to prime and the multivalent MVA BN-Filo to boost. The rVSV ZEBOV vaccine showed 100% vaccine efficacy in a phase III ring vaccination trial in the short-term following exposure to Ebola virus disease (EVD) cases. However, durability of protective efficacy has not been assessed. Although a number of publications reported clinical trials of these vaccines during the outbreak, immunogenicity was not reported using standardised or centralised assays and comparisons of relative immunogenicity could not be made. Clinical trials were undertaken in Oxford of both prime-boost regimes and volunteers have been followed up to determine durability of both humoral and cellular immunity. This revealed that 91% of 43 recipients of AdHu26/MVA vector vaccine recipients had positive glycoprotein specific IgG titres at 2.5 years post immunisation, as did 54% of 13 recipients of Chad3/MVA vectored vaccines. Furthermore, in October 2015, rVSV ZEBOV was administered to 26 contacts of a UK health-care worker who had become infected with EVD in Sierra Leone, recovered and then subsequently relapsed. Using samples from this cohort, we will be able to undertake for the first time comparative immunogenicity measurements using a standardised ELISA and validated ELISPOT assay to determine humoral and cellular immunity against the Zaire ebolavirus glycoprotein induced by these three vaccine regimes. Samples taken 2.5 years post-vaccination will be compared to determine whether differences in quality, quantity and persistence of immunity are observed. These data are important for long-term strategic planning for prophylactic protection of front-line healthcare workers in regions at risk of future outbreaks.

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IMMUNOLOGICAL INSIGHTS BASED ON ANTIBODY BINDING EPITOPES ON THE EBOLA VIRUS GLYCOPROTEIN

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The recent disease outbreaks due to Ebola virus (EBOV) highlight the need to characterize the immune response to such viruses in order to help develop vaccines and therapies. We have mapped the epitopes for over 100 monoclonal antibodies (MAbs) that target the EBOV surface glycoprotein, GP, using extensive GP mutation libraries. A broad variety of MAbs have been mapped, as described in recent publications, including the ZMapp therapeutic MAb cocktail; MAbs from human survivors of EBOV and Bundibugyo ebolavirus infection; cross-neutralizing MAbs targeting the membrane proximal external region (MPER) of GP; a broadly cross-reactive MAb that binds to the GP head region and blocks the interaction of GP with its endosomal receptor Niemann-Pick C1; and MAbs whose synergistic combination transform a non-neutralizing MAb into a potent neutralizer. In partnership with the Viral Hemorrhagic Consortium at Scripps, we have also characterized how *in vitro* neutralization correlates with *in vivo* protection. The epitope maps obtained for different types of MAbs have expanded our understanding of how the immune system recognizes EBOV GP, and correlate MAb epitopes with their neutralizing capabilities to develop anti-EBOV therapeutics and vaccines. This includes identifying mutations that increase the exposure of neutralizing epitopes and that affect EBOV function, which can impact the design of future anti-ebolavirus vaccine strategies. These insights are now being used to design new immunogens that serve as improved vaccines.

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POST-EXPOSURE PROTECTION AGAINST CONTEMPORARY NIGERIAN ISOLATES OF LASSA FEVER VIRUS IN CYNOMOLOGOUS MACAQUES WITH HUMAN MONOCLONAL ANTIBODIES

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Lassa fever is serious viral zoonosis endemic throughout West Africa for which there are currently no regulatory approved medical countermeasures (MCMs) available. To date, most MCMs have been directed against the prototypical Josiah strain of Lassa virus (LASV) isolated from Sierra Leone in the late 1970s. A recent resurgence in cases and fatalities in Nigeria has reinforced the need for MCMs that can account for the genetic diversity of LASV across this region. Here we have characterized 3 contemporary lineage II viral isolates from Edo State, Nigeria in Cynomologous macaques. Two of the three isolates were uniformly lethal in this model and were characterized by hallmark features of Lassa fever including a fever, loss of appetite, weakness, followed by a fulminate vascular leak syndrome, hemorrhagic signs and neurological manifestations. One of these two isolates had shorter mean time to death than the LASV-Josiah. The remaining isolate caused no overt disease in this model despite clear viremia. After establishing the model largely recapitulated human Lassa fever, we assessed the protective efficacy of a monoclonal antibody (mAb) cocktail of 3 antibodies previously shown to protect against LASV-Josiah 8 days after challenge where complete protection was observed 35 days post challenge. In a follow up experiment, this treatment was then reduced to 2 mAbs and, again, complete protection was observed. This work is the first to describe pathogenesis of lineage II LASV and provides a platform for assessment of protective efficacy of a post exposure therapeutic in Cynomologous macaques against currently circulating isolates of LASV from Nigeria.

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GENOMIC EPIDEMIOLOGY OF LASSA VIRUS IN NIGERIA DURING A SEASON OF UNUSUALLY HIGH INCIDENCE

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In early 2018 Nigeria experienced an unprecedented number of Lassa fever cases with widespread geographic distribution. Infection with Lassa virus, the etiological agent of Lassa fever, usually results from contact with infected Mastomys natalensis, though human-to-human transmission has been documented in hospital settings, and remains a priority for public health monitoring. Lassa virus is highly genetically diverse, especially in Nigeria where at least two distinct lineages circulate, posing challenges for the development of sensitive, comprehensive diagnostics. To investigate the epidemiology of Lassa virus in Nigeria and identify recent changes in the virus genome or its transmission route that may account for this increase, we performed genome sequencing on a large cohort of positive cases from 2018 and 2017. We combined this data with an extensive database of Lassa virus genomes spanning a decade of viral surveillance

in Nigeria. Our preliminary results suggest that the high case incidence in 2018 was not linked to a specific variant of the virus and that transmission continues to be sustained by multiple distinct cross-species transmission events. Additionally, we identified clear geographic population structure of the virus, demarcated by major rivers, suggesting the importance of established, local rodent populations in sustaining Lassa virus transmission in Nigeria. Ongoing sequencing of additional samples will further refine and inform these conclusions. Genomic sequencing is a powerful tool to understand the origins, transmission and evolution of Lassa virus and other important human pathogens. This knowledge can further assist in the development of diagnostics and vaccines as well as help to inform public health efforts for this high-priority pathogen.

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DEVELOPMENT OF A TICK-TRANSMISSION MODEL FOR HEARTLAND VIRUS PATHOGENESIS

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Heartland Virus (HRTV; Bunyaviridae: *Phlebovirus*) was initially isolated in 2009 from two patients in Missouri. Since that time there have been over 30 confirmed human cases, including three fatalities, across nine states. The Lone Star tick, *Amblyomma americanum*, has been determined as the arthropod vector. In order to understand disease transmission and pathogenesis it is necessary to develop an animal model which utilizes the natural route of transmission and manifests in a manner similar to documented human cases. In this work, we have attempted to develop a tick transmission model to mimic natural route of HRTV transmission. Our preliminary studies determined that A129 mice are the most appropriate animal model for HRTV pathogenesis. The diverse functional properties of tick saliva in modulating host immune responses to facilitate both feeding and pathogen transmission has been well documented. To initially evaluate the effects of tick saliva on HRTV infection, groups of 3 - 4 week old A129 mice were injected SQ via the foot pad with media, HRTV, or HRTV + tick salivary gland extract (SGE). Clinical observations were mild for both groups which received virus but there was a marked increase in the group which received virus + SGE. Viral load in the blood was detectable until 5 dpi in the virus only group and until study termination at 8 dpi for the virus + SGE group. At 1 and 5 dpi the viral load was similar in both groups but at 3 dpi it was ~1 log higher in the virus + SGE group. During necropsy splenomegaly was observed in both virus only and virus + SGE groups. At 3 dpi virus was detected via rt-qPCR for both groups in the spleen, liver, kidney, heart, lung, brain, stomach, intestine, and testes. The virus + SGE group had significantly higher viral loads in several tissues compared to the virus only group. This experiment clearly demonstrates the impact of tick saliva on HRTV disease severity and the need to further investigate how actual tick feeding will effect transmission and pathogenesis. Further experiments are ongoing to investigate the pathogenesis of HRTV infection acquired through natural route of transmission.

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VESICULAR SYSTEM OF ARTEMISININ RESISTANCE ENHANCES BOTH PARASITE SURVIVAL AND HOST CYTOADHERENCE

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Artemisinin resistance threatens world-wide malaria control and elimination. Elevation of phosphatidylinositol-3-phosphate (PI3P) induces resistance in blood-stages of the human malaria parasite *Plasmodium falciparum*. The parasite unfolded protein response (UPR) has also been implicated, but how PI3P acts and its connection to the UPR are unknown. Our studies show that *P. falciparum* Kelch13 (K13), the marker of artemisinin resistance concentrates at PI3P-tubules/vesicles of the parasite's endoplasmic reticulum (ER), as detected by cryo-immunoelectron microscopy. K13 additionally co-localizes and co-purifies with the major virulence adhesin PfEMP1. The PfEMP1-K13 proteome is comprehensively enriched in multiple proteostatic systems of protein export, quality-control and folding in the ER and cytoplasm including the UPR. Synthetic elevation of PI3P that induces resistance in absence of K13 mutation also yields signatures of proteostasis, UPR and clinical resistance, concluding a key role for PI3P-vesicle amplification as a mechanism of artemisinin resistance. The major resistance mutation K13C580Y quantitatively increased PI3P-vesicles, exporting them throughout the parasite and the red cell. Alterations in PfEMP1 export to the red cell and cytoadherence of infected cells to a host endothelial receptor, are features of multiple K13 mutants. Together the data reveal an amplified PI3P-vesiclur system of artemisinin resistance enhances the parasite's capacity for mitigating artemisinins toxic proteopathy and promoting endothelial adherence, suggesting a strategy by which resistant organisms may persist in host tissues.

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ARTEMISININ RESISTANCE GENE K13 IS LINKED TO DNA REPLICATION AND REPAIR

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Plasmodium falciparum is evolving resistance to treatment with Artemisinin Combination Therapy (ACT) in South East Asia. The further spread of resistance will imperil malaria control efforts and put millions of lives at risk. Understanding the mechanism of resistance to the artemisinin component of ACT will aid in efforts to thwart the evolution to resistance. The gene with the strongest link to artemisinin resistance is K13, which has homology to the human gene Keap1 which regulates the transcription of stress response genes. However, the role K13 plays in *P. falciparum* remains unknown. Since multiple attempts to knockout K13 have failed, it is likely K13 is essential and regulatory mutants are one of the important ways to decipher its function. In this study, we performed RNA-seq on an isogenic K13 regulatory mutant that is more sensitive to arteminsins than the parent wild-type strain NF54. K13 is aberrantly down-regulated during the early ring stage and up-regulated during the early trophozoite stage of the mutant. This K13 dysregulation is associated with strong and consistent shifts in the expression of DNA replication and repair genes, but not other housekeeping pathways that undergo similar rates of transcriptional regulation at these life-cycle stages. The observed effect is specific to the K13 dysregulation and is not present in other *P. falciparum* mutants with the same transposon inserted in other loci. These results are consistent with other reports in the literature linking artemisinin resistance to changes in cell cycle regulation, indicating that specific alterations of DNA replication and repair pathways can significantly alter sensitivity to artemisinins.

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A NOVEL IMMORTALIZED HEPATOCYTE-LIKE CELL LINE (IMHC) SUPPORTS *IN VITRO* LIVER STAGE DEVELOPMENT OF THE HUMAN MALARIAL PARASITE *PLASMODIUM VIVAX*

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Eradication of malaria is difficult because of the ability of hypnozoite, the dormant liver-stage form of *Plasmodium vivax*, to cause relapse in patients. Research efforts to better understand the biology of *P. vivax* hypnozoite and design relapse prevention strategies have been hampered by the lack of a robust and reliable model for *in vitro* culture of liver-stage parasites. Although the HC-04 hepatoma cell line is used for culturing liver-stage forms of *Plasmodium*, these cells proliferate unrestrictedly and detach from the culture dish after several days, which limits their usefulness in a long-term hypnozoite assay. Here, a novel immortalized hepatocyte-like cell line (imHC) was evaluated for the capability to support *P. vivax* sporozoite infection. imHCs maintained major hepatic functions and expressed the essential factors CD81, SR-BI and EphA2, which are required for host entry and development of the parasite in the liver. imHCs could be maintained long-term in a monolayer without overgrowth and thus served as a good, supportive substrate for the invasion and growth of *P. vivax* liver stages, including hypnozoites. The observed high drug metabolism activity and potent responses in liver-stage parasites to primaquine highlight the potential use of this imHC model for antimalarial drug screening. Overall, imHCs constitute an alternative host for *in vitro Plasmodium* liver-stage studies, particularly those addressing the biology of *P. vivax* hypnozoite. Thus, imHCs potentially offer a novel, robust model for screening drugs against liver-stage parasites.

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IT TAKES TWO TO TANGO: THE P52/P36 HEPATOCYTE INVASION COMPLEX

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Following an infected mosquito bite, a limited number of *Plasmodium* sporozoites will make their way to the liver to continue the cycle of infection in the mammalian host. Once in the liver, sporozoites traverse cells searching for a "suitable" hepatocyte, invading these cells through a process that results in the formation of a parasitophorous vacuole (PV), a protective compartment in which the parasite undergoes intracellular replication as a liver stage. Previous studies have established that two members of the *Plasmodium* s48/45 protein family, P36 and P52, are essential for productive invasion of host hepatocytes as their simultaneous deletion results in growth-arrested parasites lacking a PV. Recent studies point towards a pathway of entry possibly involving the interaction of P36 with hepatocyte receptors EphA2, CD81 and SR-B1. However, the relationship between P36 and P52 during invasion remains unknown. Here we show that parasites with a single P52 or P36 gene deletion lack a PV after hepatocyte invasion, thereby each pheno-copying the P52/P36 dual gene deletion parasite line and indicating that both proteins are important in the establishment of a PV and act in the same pathway. We then created a *Plasmodium yoelii* P36^{mCherry} tagged parasite line that allowed us to visualize the subcellular localization of P36 in the sporozoite secretory microneme organelles and found that it co-localizes with P52. Furthermore, through co-immunoprecipitation studies *in vivo*, we determined that P36 and P52 form a protein complex in sporozoites, implying a concerted function for both proteins within the PV formation pathway. In addition, we found that P36 is secreted from the sporozoite. Our results support a model in which glycophosphatidylinositol (GPI)-anchored P52 may serve as a scaffold to facilitate the interaction of secreted P36 with host receptors.

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ROLE OF APICAL SUSHI PROTEIN IN SPOROZOITE INVASION OF SALIVARY GLANDS

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The infective forms of apicomplexan parasites, including *Plasmodium*, have the conserved apical organelles named rhoptries and micronames. Several rhoptry proteins, released during the invasion process, have been revealed crucial for parasite invasion of target cells by forming tight junction between them. *Plasmodium* sporozoites, transmiting malaria disease from mosquito vector to mammalian hosts, firstly invade salivary glands in mosquitoes and then infect hepetocytes in mammals. Recently, we demonstrated that rhoptry neck protein 2 (RON2) has an important role in sporozoite invasion of salivary glands by using sporozoite-stage specific gene silencing system. This raised the possibility that other rhoptry proteins are also involved in sporozoite invasion mechanisms. Apical Sushi Protein (ASP) is known as a rhoptry protein in *Plasmodium* merozoites and *Toxoplasma* tachyzoites with Sushi and GPI-anchor domains. Here, we demonstrate that ASP is predominantly expressed in oocyst-derived sporozoites, that is more than in schizonts, and localized to rhoptries. Applying the conditional knockdown system by promoter swapping, we generated transgenic *Plasmodium berghei* expressing ASP only at schizont-stage. ASP-repressed sporozoites, formed normally in oocysts, failed to invade salivary glands, possibly due to the defect in their attachment and gliding abilities. In addition, RON2 amount in ASP-repressed oocyst sporozoites was reduced to one-tenth of that in control sporozoites, suggesting that ASP may contribute to the stability of RON2. Comprehensive functional analyses of rhoptry proteins in sporozoites might provide clues to elucidate the molecular mechanisms of sporozoite invasion of salivary glands.

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THE IMPACT OF LONG-LASTING INSECTICIDAL (LLIN) NET EXPOSURE ON POST-EXPOSURE LONGEVITY OF PYRETHROID RESISTANT *ANOPHELES COLUZZII* MOSQUITOES IN BURKINA FASO

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Insecticide resistance has the potential to erode recent gains in malaria control. Typically, resistance is determined by evaluating mosquito mortality at 24hrs post-exposure, while sub-lethal or delayed effects that might impact vectorial capacity (e.g. reduced fecundity, impaired feeding, longevity) are rarely documented. In Burkina Faso, we examined the impact of exposure to long-lasting insecticidal nets (LLINs) and untreated controls on the post-exposure longevity of wild pyrethroid-

resistant *Anopheles coluzzii* populations, in WHO cone-bioassays and experimental hut trials with a human-baited LLINs. Wild mosquitoes of unknown age entering huts ("wild") and adult mosquitoes of known age reared from larvae of the same population ("reared") were used in both tests. Reared mosquitoes of different ages (one vs seven-days-old) were also exposed to LLINs. Following exposure, daily mortality was recorded until all mosquitoes were dead. In cone and hut trials, accounting for blood-feeding, LLIN exposure had no effect on longevity of either reared (P=0.843) or wild (P=0.984) mosquitoes. Wild mosquitoes lived significantly longer than reared mosquitoes (P<0.000), and immediate mortality (24hrs) in experimental huts was higher in reared mosquitoes (36% compared to 9%). The method of exposure (i.e. cone vs hut) did not affect longevity (P=0.702). Comparison of one and seven-day-old reared mosquitoes in cone-bioassays, showed significantly higher immediate mortality (2% to 12% mortality) and reduced longevity in seven day-olds (P<0.000). In hut trials, blood-fed mosquitoes lived significantly longer post-exposure than unfed mosquitoes (P<0.000). In contrast to earlier laboratory studies reporting substantial delayed lethal effects in resistant colonies after LLIN exposure, these field experiments found no evidence of LLINs impacting longevity in this highly pyrethroid resistant population in Burkina Faso. The implications of these results for our understanding of the impact of resistance on vector control interventions are considered.

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MAPPING SPATIO-TEMPORAL PATTERNS IN INSECTICIDE RESISTANCE PHENOTYPES IN MALARIA VECTORS ACROSS AFRICA

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The development of insecticide resistance in African malaria vectors threatens the continued efficacy of important vector control methods that rely on a limited set of insecticides. To understand the impact of insecticide resistance on malaria transmission, and develop effective resistance management strategies, we require comprehensive quantification of resistance in field populations to the suite of vector control insecticides. We apply Bayesian geostatistical and machine learning approaches to generate Africa-wide predictive maps of resistance phenotypes for commonly-used insecticides in mosquito species within the *Anopheles gambiae* complex. Our models incorporate data on resistance phenotypes as well as genetic resistance mechanisms in *An. gambiae* complex samples, together with high resolution data on potential environmental drivers of selection for resistance. Our resistance phenotype data set includes results of susceptibility bioassays conducted on 5595 samples across 1183 locations spanning 38 African countries and across insecticide types from four insecticide classes. We augment this with data on the frequency of mutations in the *Vgsc* gene, known to confer resistance to pyrethroid insecticides and DDT, from *An. gambiae* complex samples collected throughout Africa. Environmental covariates include data on the application of insecticide-based vector control interventions as well as potential predictors of mosquito exposure to insecticides used in agriculture. We were able to substantially improve predictive capacity by making use of consistent relationships between resistance phenotypes across different insecticide types, as well as relationships between phenotypic and genetic variation. We also identified environmental and intervention covariates that improve prediction, generating hypotheses about drivers of selection for insecticide resistance. Our maps provide an Africa-wide geostatistical quantification of trends in insecticide resistance, and incorporate measures of prediction uncertainty that highlight regions where field data collection is important.

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JUST BREATHE: USING SIMPLE RESPIROMETRY TO CHARACTERIZE METABOLIC RESISTANCE TO INSECTICIDES IN MOSQUITO VECTORS

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The rapid and widespread adaptation in mosquito populations to chemical insecticides is now compromising vector-borne disease control programs. Tools for monitoring and predicting the evolution of insecticide resistance in the field is paramount to devising successful mosquito control campaigns. However, there are major gaps in our understanding of the resistance mechanisms and their operational impact. Resistance to insecticides like pyrethroids has been associated with increased metabolism by multifunction oxidases and decreased neuronal target site sensitivity (also known as the knock down resistance, kdr). While kdr to pyrethroids seems to have a relatively simple genetic background (few major effect mutations in the insect para gene), metabolic resistance can be a result of alterations in a number of gene families. Identifying genetic architecture of metabolic resistance therefore requires screening of genetic variants across the genome, which is now attainable thanks to a lower cost of next generation sequencing (NGS) and annotated reference genome sequences available for major mosquito vectors. However, no genetic study can have meaningful results without a careful characterization of the phenotype, and we do not have well-established quantitative measures of metabolic resistance. Here we show that a simple method to measure changes in metabolic state of mosquitoes can be used to identify genetic preturbations affecting metabolic resistance. We used inexpensive respirometry technique to measure CO_2 production in *Aedes aegypti* mosquitoes from different strains with varying levels of resistance to deltamethrin. CO_2 output is a suitable indicator of substrate oxidation and energy expenditure that provides information about metabolic state. We tested the hypothesis that the basal metabolic rate is higher in more resistant mosquitoes, and that it is less altered after exposure to deltamethrin. Using NGS of pooled mosquitoes from different phenotypic groups, we show that differences in metabolic state correspond to differences across multifunction oxidase genes.

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INSECTICIDE RESISTANCE IS NOT ASSOCIATED WITH *PLASMODIUM FALCIPARUM* INFECTION IN *ANOPHELES GAMBIAE* S.L. (DIPTERA: CULICIDAE) IN GUINEA

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The threat of insecticide resistance across sub-Saharan Africa is anticipated to have severe implications for the continued effectiveness of insecticide-based vector control interventions. In an area of high malaria transmission in Forecariah Prefecture, Guinea, we investigated the impact of insecticide resistance intensity on *Plasmodium falciparum* infection in *Anopheles gambiae* s.l. CDC bottle bioassays and synergists assays with PBO, were used to assess resistance intensity to carbamates and pyrethroids. A subset of specimens underwent ovarian dissection to determine parity and molecular assays were performed to detect insecticide resistance alleles (*Ace-1*, *kdr* and N1575Y) and *Plasmodium falciparum* infection

in abdomens or head/thoraxes. Pyrethroid resistance was intense as evidenced by mosquito populations that were not only resistant to ten times the insecticide concentration required to kill susceptible individuals, but were also capable of surviving these doses for up to two hours. By comparison, carbamate resistance was lower, with possible resistance restricted to two adjacent villages and significantly associated with the presence of *Ace-1* (p=0.001). Pyrethroid resistance was not associated with *P. falciparum* prevalence, with no significant differences in infection observed between susceptible or resistant vectors (p=0.345 and p=0.148, respectively). Pyrethroid-resistant mosquitoes had significantly lower parity rates, i.e. were younger, than susceptible individuals. However, the small proportion of intensely resistant vectors were more likely to be parous (p=0.042 and p=0.036, for survivors exposed to five and ten times the diagnostic dose of insecticides, respectively). Partial restoration of mosquito susceptibility to pyrethroids following pre-exposure to PBO and the non-association between *kdr*-N1575Y frequency and vector mortality rates indicate that oxidase-based mechanisms are important in driving local pyrethroid resistance. Our findings highlight the need for additional studies, directly examining the impact of insecticide resistance on mosquito fitness.

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INSECTICIDE RESISTANCE MONITORING IN AFRICAN MALARIA MOSQUITOES USING HIGH-THROUGHPUT DIAGNOSTIC MARKERS

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Vector control programs strongly rely on effective insecticides. The African malaria mosquito *Anopheles gambiae* has developed a range of target site and metabolic resistance mechanisms which threatens the sustenance of recent achievements in the fight against malaria. Markers representing the genetic changes responsible for insecticide resistance can be used to monitor their presence in individuals, populations, and geographic areas. Detailed knowledge about the presence, abundance, and spread of insecticide resistance will be a major benefit to vector control strategy design. Recent technological developments greatly advanced both marker discovery and marker detection methods. Many new potential markers have been found in gene clusters associated with insecticide resistance such as cytochrome P450s and glutathione S-transferases. Single-locus genotyping methods such as Taqman assays are now routinely used to screen for established mutations including kdr east and west in VGSC and G119S in Ace1. Fluorescent Locked Nucleotide Acid (LNA) probes allow for more design flexibility at a greatly reduced cost compared to Taqman assays. Our group has designed several LNA assays including a three-dye method which can detect the presence of all three kdr alleles in a single reaction. LNA probes are also routinely used to validate candidate markers found in *Anopheles gambiae* genome projects. Full-scale screening of multiple markers in thousands of mosquitoes requires a high-throughput platform. We successfully genotyped 30 SNPs at once using the IPLEX Mass Array system, though this method is sensitive to background genetic variation and the technology is not widely accessible. Genotyping by sequencing using multiplexed amplicons on the illumina MiSeq allows scalability and flexibility of marker panels and numbers of mosquitoes screened per run. In collaboration with the Sanger Institute, we developed several panels of 25 multiplexed amplicons to allow mass screening of insecticide resistance markers. These panels can be tailored to suit their purpose and can be kept up to date by adding newly discovered insecticide resistance markers.

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INCREASED SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* TO *PLASMODIUM FALCIPARUM* IS NOT ASSOCIATED WITH MARKERS OF INSECTICIDE RESISTANCE IN TORORO, UGANDA

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Insecticide resistance threatens malaria vector control interventions and may impact on the efficiency of transmission of malaria to mosquitoes. West African lab studies showed that *ace-1* 119S and the *Vgsc*-1014F (*kdr-west*) mutations, which are responsible for organophosphate/carbamate resistance and pyrethroid resistance, respectively, increased susceptibility to *P. falciparum* (*Pf*) infection. Similarly, in wild-caught *A. gambiae* in Tanzania, pyrethroid resistant *Vgsc*-1014S (*kdr-east*) homozygotes had increased sporozoite rate compared to WT. We are studying whether similar associations are present in Tororo District, Uganda, where extensive resistance to DDT and pyrethroids, but not to carbamates or organophosphates, has been observed. 132 *Pf*-infected *A. gambiae s.s.* mosquitoes were collected from 100 randomly selected households from 2011 to 2015. Each infected (*Pf*+) mosquito was paired with 3 uninfected (*Pf*-) mosquitoes from the same collection. Taqman assays were used to genotype at *Vgsc*-L1014F/S and at polymorphisms in *Cyp4j5* and *Coeae1d* that were recently associated with pyrethroid resistance in Tororo. PCR was used to genotype the 2La inversion, which is associated with adaptation to aridity, indoor biting, and differential malaria infection. Most mosquitoes were homozygous for *Vgsc*-1014S (97.8% *Pf*- vs. 97.6% *Pf*+), with uncommon L/L homozygotes (1.3% *Pf*- vs. 1.6% *Pf*+), L/S heterozygotes (1.3% *Pf*- vs. 0% *Pf*+) and S/F heterozygotes (0.5% *Pf*- vs 0.8% *Pf*+). For *Cyp4j5*, the prevalence of homozygous resistant, (RR), heterozygous (SR), and homozygous susceptible (SS) genotypes were 19.2%, 38.7%, and 42.0% for *Pf*- mosquitoes and 14.6%, 40.7%, and 44.7% for *Pf*+ mosquitoes. *Coeae1d* prevalences were 28.1% RR, 49.0% SR, and 22.9% SS for *Pf*- mosquitoes and 35.3% RR, 46.7% SR, and 21.0% SS, for *Pf*+ mosquitoes. Prevalences of 2La inversion genotypes were 19.9% $2L+^a/2L+^a$, 28.1% $2L+^a/2La$, and 52.0% 2La/2La in *Pf*- mosquitoes, and 14.2% $2L+^a/2L+^a$, 28.6% $2L+^a/2La$, and 57.1% 2La/2La in *Pf*+ mosquitoes. To date we have found no significant associations between insecticide resistance polymorphisms and risk of mosquito *Pf* infection.

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EFFECT OF PASSIVE METOFLUTHRIN EMANATORS ON LANDING AND MORTALITY OF PYRETHROID-RESISTANT *AEDES AEGYPTI*

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Management of urban *Aedes aegypti* is currently challenged by the evolution of pyrethroid resistance. A tactic with potential for large-scale implementation is indoor deployment of passive emanators (small, 10% metofluthrin-impregnated nets) that are advantageous because they are rapidly installed, require no source of heat, and can act as confusants rather than repellents. We tested whether exposure to metofluthrin emanators installed in experimental houses located in Mérida, Mexico, affected landing and mortality among field-derived strains of *Ae. aegypti* that differed in pyrethroid susceptibility. Experimental houses (n = 8) were rented houses in Mérida where passive emanators could be deployed,

and were modified for experimentation with doubled screed entrances and standardized contents. Mosquitoes were released into paired houses (n = 25 mosquitoes/ house): one house with emanators and one control. Landing counts were performed before, 30 min, 24 hrs, and 72 hrs after emanator deployment. Mortality was measured both 24 and 72 hrs post-mosquito introduction. The experiment was repeated 17 times using different combinations of 5 *Ae. aegypti* strains; a lab insecticide-susceptible strain (New Orleans) (n = 4 paired house reps), 2 locally-derived susceptible strains (n = 11 and 19 reps), and 2 locally-derived pyrethroid-resistant strains (n = 11 and 4 reps). Landing counts did not differ among houses before the introduction of emanators ($F = 3.2$; $p = 0.09$), but were significantly reduced 30 minutes after emanator introduction among all strains in treated houses compared to control houses ($F = 59.8$; $p < 0.0001$). After 24 hrs, mortality was significantly greater in treated compared to control houses across all strains ($F = 21.9$; $p = 0.0002$), but landing counts recovered ($F = 3.42$; $p = 0.043$). After 72 hrs, the majority of susceptible *Ae. aegypti* had died within treated houses (99.5 ± 0.5%). Given the effects on *Ae. aegypti* mortality and landing, metofluthrin emanators could be an important addition to the vector control toolbox, however, additional research is still needed to explore how resistant populations will react to long-term exposure.

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MOLECULAR DIAGNOSTICS OF POTENTIAL PATHOGENS IN THE WILDLIFE TRADE IN LAO PDR

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Wildlife trade is thought to be a major driver of emerging infectious diseases. As part of the Lao PDR-Cambodia One Health Surveillance and Laboratory Network Project (LACANET) we surveyed the presence of zoonotic pathogens in wildlife meat on sale at wet markets across Lao PDR. In total, 717 samples, including urogenital swabs, blood, urine and tissue samples, were collected from 359 animals (encompassing 32 species including squirrels, civet cats, rats and bats) across 11 markets and three Provincial Offices in Lao PDR. Using polymerase chain reaction (PCR) techniques, samples were screened for a variety of zoonotic bacterial and viral pathogens. We identified 69 animals (19.2%) positive for *Leptospira* spp. and 19 animals (5.3%) positive for *Rickettsia* spp., including three confirmed as *R. felis* and one *R. typhi*. Other potential human pathogens identified include *Orientia tsutsugamushi, Ehrlichia* spp. and *Anaplasma* spp. (*A. phagocytophilum, A. bovis*, and *A. marginale*). The most common wildlife meat being traded in markets are from the Sciuridae family (73.3%). Our observations indicate their potential as multiple disease reservoirs, with a total of six different pathogen species being identified, including the discovery of *R. felis*, an emerging rickettsial pathogen, and which is the first reported occurrence in squirrel species. Our results demonstrate that wildlife found for sale at wet markets are able to carry pathogens of public health importance such as *Leptospira* spp., *Rickettsia* spp., *O. tsutsugamushi*, *Ehrlichia* spp. and *Anaplasma* spp. The findings from this investigation highlight that wet markets in Lao PDR are potential sources of emerging infectious zoonotic diseases and thus appropriate measures and mitigation strategies must be taken into account to protect public health, including further ongoing work investigating the risk of transmission of zoonotic pathogens to vendors and buyers.

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INDEX-CLUSTER STUDY OF INTERSPECIES DISEASE TRANSMISSION AT LOLA YA BONOBO SANCTUARY, DEMOCRATIC REPUBLIC OF CONGO

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Lola ya Bonobo (LyB) is a sanctuary for orphaned bonobos (Pan paniscus) outside Kinshasa, Democratic Republic of Congo. Orphaned infants transition to a juvenile group prior to one of three multi-hectare forested enclosures housing adult Bonobos. Animals often come into close contact (<1-2m) with keepers. In cooperation with LyB, we studied the potential for interspecies disease transmission during 2014. We collected baseline specimens from 73 Bonobos during an annual health check, and from LyB staff during enrollment. During the study, specimen collection was initiated by a symptomatic Bonobo (keeper observations) or human (self-reported), with other potentially ill animals or staff for up to five days. Illness would often begin in a living group and spread: Feb. 2014, 4 clusters; May 2014, 3 clusters; Aug. 2014, 2 clusters; single clusters in Sep. and Oct.; and 2 clusters in Nov. 2014. We collected closeout specimens (60 Bonobo and 28 Human) in Oct./Nov. 2015. Nasal/oral samples were analyzed with a Respiratory Pathogen Panel (RPP) which detects 18 viral and 3 bacterial pathogens. At baseline, 9/73 of Bonobos were positive (8 viral, 1 bacterial) and 7/42 of humans were positive via RPP (7 viral). The Feb. clusters were a multi-enclosure outbreak of Respiratory Syncytial Virus A (RSVA), with 29/30 of Bonobos positive. Bonobos in the three May clusters were 10/14 positive for RSVA and 4/14 positive with Rhino/Enterovirus. Of the 8 humans tested in May, only 1 was positive (Parainfluenza 2). The Aug. clusters were positive for Rhino/Enterovirus 10/10 Bonobos, 1/6 Humans. Only 1/6 Bonobos and 0/1 Humans were positive for Rhino/Entero in the Sep.–Oct. clusters. Rhino/Entero was present in the two Nov. clusters with 11/11 Bonobos and 1/5 humans positive. In closeout 5/60 Bonobos and 1/28 Humans positive for virus. This work represents an early analysis of observed contemporaneous respiratory infection of humans and non-human primates in a sanctuary environment. The Bonobos at LyB are at risk from exposure to human respiratory pathogens, however we are currently unable to determine the route of exposure.

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EVIDENCE FOR ASYMPTOMATIC CIRCULATION OF ORTHOPOXVIRUS IN MFOU DISTRICT, CAMEROON

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Monkeypox virus is a zoonotic orthopoxvirus that causes smallpox-like illness in humans. In Cameroon, human monkeypox cases were last confirmed in 1979 and outbreaks in captive chimpanzees occurred in 2014 and 2016. Although no humans had clinical signs or symptoms of monkeypox during recent chimpanzee outbreaks, several were exposed while caring for sick animals, raising questions about the threat of monkeypox to humans in this region. A survey was administered to staff employed at a primate sanctuary (the site of a recent chimpanzee outbreak) and residents from four nearby towns, documenting monkeypox symptoms and contact with wild animals including possible reservoir species, such as Gambian rats and rope squirrels, among others. We also collected one 5mL whole blood specimen from each participant, and performed an ELISA test to detect the presence of anti-orthopoxvirus IgG antibodies. In total, 43 of 125 (34.4%) participants were IgG positive. Among those under the age of smallpox vaccination (<37 years), four (6.3%) were IgG positive (one sanctuary employee and three community members). No one reported past clinical symptoms compatible with monkeypox in their lifetime. Eighty-six participants (68.8%) had recent contact with animals (since August 2016), but primate sanctuary workers were less likely to have frequent forest visits (Fisher's exact test $P<0.001$) and less likely to have contact with possible monkeypox reservoir species, Gambian rats, rope squirrels, sun squirrels, and dormice (Chi-square= 31.8, $P<0.0001$). Participants reported contact with porcupines (n=82), Gambian rats (n=71), sun squirrels (n=35), and rope squirrels (n=33). Among community members, men were significantly more likely than women to hunt or trap Gambian rats (Chi-square=5.9, $P<0.05$). Our results demonstrate evidence for asymptomatic orthopoxvirus infection in Mfou District, Cameroon. Contact with possible monkeypox reservoirs is also common among residents in this region. The present circulation of orthopoxviruses, monkeypox in particular, in an endemic area raises the need for continued surveillance for human and animal disease.

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A MARKOV CHAIN MODEL TO EVALUATE THE HUMAN SAFETY OF ORAL ANIMAL VACCINES FOR WILDLIFE OR NON-ACCESSIBLE ANIMALS: A CASE STUDY OF AN ORAL RABIES VACCINE

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Baits containing oral vaccines aid immunization of animal populations less accessible to parenteral vaccination, but may contain live-attenuated viruses that pose risks of reversion to virulence or residual pathogenicity. Such is the case for first generation oral rabies vaccines, such as SAD-B19, used widely in Europe to manage fox rabies. International health organizations caution against using these vaccines in dogs, proposing instead highly attenuated strains, such as SPBN GASGAS, while requiring detailed risk assessment prior to their use in wildlife and dogs. We mapped pathways by which environmental distribution of oral vaccines may result in its mucosal, subcutaneous, or severe inoculation into people and applied a continuous-time Markov chain to estimate the expected and 95% confidence intervals of the number of severe adverse events (SAEs) under three scenarios. We validated the model by comparing a simulated SAD-B19 vaccination campaign in foxes to prior campaigns in which 250 million baits were dispersed and no SAEs detected. A low risk to humans was predicted with 0.25 deaths per 10 million baits distributed for fox rabies control. However, consistent with international concern, our model predicts that SAD-B19 could result in 7.0 human deaths per 10 million baits distributed if used for dogs. Conversely, our model predicts no human deaths per 10 million baits of SPBN GASGAS for dogs. Among all three scenarios, 98% of vaccine-induced SAEs were due to bites from rabid dogs that had experienced vaccine-induced rabies. Sensitivity analysis using Latin hypercube sampling show that parameters involved in the exposure route involving direct contact with vaccine in the environment most strongly influence outcomes of wildlife campaigns, while parameters involved in the dog bite exposure route most strongly influence dog campaign outcomes. No simulation of SPBN GASGAS resulted in SAEs in sensitivity analysis. This model highlights the safety of SPBN GASGAS and can be used as a standardized approach to inform risk assessments when planning vaccination campaigns of various vaccine types, distribution methods, and animal populations.

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ELEVATED PREVALENCE AND NOVEL HOST INFECTION OF WEST NILE VIRUS AT THE NASHVILLE ZOO, TENNESSEE, USA

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On August 30, 2017, one of 5 bontebok in a mixed species exhibit at the Nashville Zoo at Grassmere exhibited acute hind-limb ataxia and altered demeanor. The 8-year-old male bontebok had no recent history of trauma, toxin ingestion, or travel. Initial examination revealed hyperesthesia, general ataxia, and moderate weakness, suggesting cerebellar/brain stem lesion. Complete blood count displayed lymphopenia and by day 4, ataxia worsened with frequent falling, short-strided gait, and dull mentation. Gross necropsy uncovered lung and liver congestion, mild abomasal ulcerations, and brain hemorrhage. Various tissues were sent to the University of Georgia College of Veterinary Medicine Infectious Disease Laboratory and showed necrotizing meningoencephalitis and spinal myelitis. Fresh brain tissue was submitted to the Tennessee Department of Health (TDH) Vector-Borne Disease (VBD) program for arbovirus testing. Molecular analysis identified West Nile Virus (WNV) as the etiology of illness. Environmental sampling at the zoo was conducted by mosquito capture during September 19 -October 13, 2017. There were a total of 3,998 mosquitoes tested, representing 5 species with the majority (72.06%) being *Culex pipiens*. Only *Cx. pipiens* had detection of WNV as well as St. Louis encephalitis virus (SLEV). The mean weekly maximum likelihood estimate for WNV prevalence among *Cx. pipiens* at the zoo was 9.56 (95% CI 4.13-21.59), or ≈1 WNV infected *Cx. pipiens* per 105 collected. Mosquito data showed a two-fold elevated prevalence of WNV compared to Tennessee. The TDH VBD program and the Nashville Zoo have obtained samples for seroprevalence of WNV from 11 unvaccinated animal species located at the zoo. To our knowledge, this is the first instance of WNV infection among bontebok on public record. Also, the park is centered in the Nashville suburbs and is a venue for large community events. The combined rare incidence of WNV infection in a novel host and the large presence of WNV and SLEV in the zoo mosquito population warrant mosquito control measures implemented in zoo habitats to reduce potential transmission to unvaccinated animal species and human visitors.

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ZOONOTIC AND ENVIRONMENTAL DETERMINANTS OF TUNGIASIS IN A RURAL AREA ADJACENT TO A WILDLIFE RESERVE IN KWALE, KENYA

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Tungiasis is an inflammatory skin disease caused by the ectoparasite *Tunga pentrans*, which burrows into the skin. Tungiasis is associated with intense itching, pain, and secondary bacterial infections and, in severe cases, loss of limbs and death. Tungiasis is a zoonosis common throughout Sub-Saharan Africa, though little is known of the role of wildlife in human tungiasis transmission. Wildlife could become infested with T. penetrans, bring eggs to the vicinity of the household, whereby livestock become infected, thus increasing risk for disease in humans. Through a demographic surveillance system in Kwale, Kenya, located proximally to a large wildlife preserve, we tested associations between human tungiasis and presence of wildlife and other environmental factors. Using a complex survey sampling design, 319 households were selected from three regions. A survey instrument was administered which included questions on livestock assets, reported presence of various species of wildlife around the home, health seeking behaviors, basic demographics and socioeconomics. Humans and livestock were visually assessed for presence of tungiasis. Of the 319 households selected (12.8%) of them had at least one person who had tungiasis. Males were more likely to be infected than females (OR 5.07 (2.52, 10.8)). Increased distance (kms) to the wildlife reserve was found to be associated with decreased odds of infection (OR .90 (.81, .99)). Simple possession of domesticated animals was not found to be associated with tungiasis in humans. However, positive cases of animal tungiasis of all species were found to have positive though non-significant associations with human tungiasis. Out of all wildlife species only presence of stray dogs was found to be associated with an increased risk for human tungiasis (OR 2.37 (1.21, 4.65)). While our research confirmed earlier results that distance to the wildlife reserve was associated with tungiasis in humans, there was only weak evidence that animal tungiasis was associated with tungiasis in humans. More work needs to be done to assess tungiasis transmission between wildlife, livestock and humans.

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SURVEILLANCE AT THE HUMAN-ANIMAL INTERFACE DURING ZIKA VIRUS TRANSMISSION - PERU

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Zika virus has been diagnosed throughout the Americas, reporting over a million human cases (PAHO/WHO, 2018). As transmission wanes, questions remain as to whether a zoonotic cycle of virus maintenance will become established. Some reports found Zika virus in animals within an outbreak zone; however, more data is needed to understand which animal species may become infected during transmission among people. We studied if animals infected during an outbreak could be zoonotic reservoirs of Zika virus. We conducted animal and vector surveillance activities in areas with presence of human cases to determine such infections. Domestic dogs, cats and poultry were sampled, peri-domestic rodents (*Rattus spp*, *Mus musculus*) and bats were trapped and humanly euthanized to obtain biological samples. Other wildlife, such as wild rodents or birds were also trapped and sampled before releasing to their habitat. Non-human primates (NHPs) were sampled from local shelters or breeding facilities located in the vicinity of the areas with human cases. Finally, targeted collections of mosquitoes were performed in the same areas or households where animals were trapped/sampled. In all cases, samplings were performed within a 1 km radius of a reported human Zika case. Samples were obtained from 156 dogs, 18 cats, 153 chickens, 27 ducks, 3 wild rodents (*Proechymis* spp and *Oligoryzomys spp*), 36 *Mus musculus,* and 23 *Rattus sp.* from urban settings, 4 *Mus musculus* and 1 *Rattus rattus* from rural settings, 75 bats from 18 different species, 51 wild birds from 28 different species, and 141 NHPs from 9 different species. Mosquitoes from 62 different species were collected, including 1007 *Aedes spp* (308 males, 613 females, and 86 engorged females) among others. In total, 688 animals were sampled and 21,486 mosquitoes collected. Zika testing is underway, with whole blood and engorged mosquitoes to be tested using RT-PCR as an initial screening tool. Findings will be spatially correlated with the location of human cases, providing a more complete and ecological approach to the understanding of Zika virus transmission, and identifying possible candidates for zoonotic maintenance.

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INTESTINAL PARASITOSIS IN RELATION TO CD4+T CELLS LEVELS AND ANEMIA AMONG HAART INITIATED AND HAART NAÏVE PEDIATRIC HIV PATIENTS IN MODEL ART CENTER, ADDIS ABABA, ETHIOPIA

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In the absence of highly active antiretroviral therapy (HAART), HIV/AIDS patients in developing countries, unfortunately, continue to suffer from the consequences of opportunistic and other intestinal parasites. The aim of the study was to determine the prevalence of intestinal parasites in relation to CD4+ T cells levels and anemia among HAART initiated and HAART naïve pediatric HIV patients in a Model ART center in Addis Ababa, Ethiopia. A cross-sectional study was conducted among pediatric HIV/AIDS patients attending a model ART center a between August 05, 2013 and November 25, 2013. A stool specimen was collected and processed using direct wet mount, formol-ether concentration and modified Ziehl-Neelsen staining techniques. CD4+ T cells and complete blood counts were performed using BD FACScalibur and Cell-Dyn 1800, respectively. The data were analyzed by SPSS version 16 software. The overall prevalence of IPs was 37.8% where 27.8% of HAART initiated and 45.5% of HAART naive pediatric HIV/AIDS patients were infected ($p < 0.05$). *Cryptosporidium* species, *E. histolytica/dispar*, *Hook worm* and *Taenia* species were IPs associated with CD4+ T cell counts <350 cells/μL in HAART naive patients. The overall prevalence of anemia was 10% in HAART and 31.7% in non-HAART groups. *Hookworm*, *S. stercoralis*, and *H. nana* were helminths significantly associated with anemia in non-HAART patients [AOR, 95% CI: 4.5(1.3, 15.2), P< 0.05]. The prevalence of IPs in non-HAART patients was significantly associated with eating unwashed/raw fruit [AOR, 95%CI: 6.3(1.2, 25.6), P<0.05], open field defecation [AOR, 95%CI: 9.3(1.6, 53.6), P<0.05] and diarrhea [AOR, 95%CI: 5.2(1.3, 21.3), P<0.05]. In conclusion. The overall prevalence of intestinal parasites significantly differed by HAART status and *Cryptosporidium* species were found only in HAART naïve patients with low CD4+ T cell counts. Anemia was also more prevalent and significantly associated with IPs in non-HAART patients. This study identified some environmental and associated risk factors for intestinal parasitic infections.

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PERFORMANCE CHARACTERISTICS OF POINT OF CARE LYNX TEST IN EARLY INFANT DIAGNOSIS OF HIV

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Early infant diagnosis of HIV infection (EID) is challenging in rural areas because the currently used assays to detect HIV DNA require transportation of specimens to central laboratories. This leads to substantial delays in return of test results and linkage to care. The developed point of care (POC) diagnostics offer hope to minimize this challenge. This study was done to evaluate the performance characteristics of a POC test based on p24 antigen detection (LYNX) compared to HIV DNA testing for EID in Zambia. This was a prospective study conducted at 3 hospitals and 8 health centers in Southern Zambia from February, 2016 to February, 2018. Infants aged 0 hours to 18 months born to HIV-infected mothers were eligible. At urban sites, infants were enrolled at birth and discharged from the study when the HIV DNA results were issued to caregivers, while at rural sites infants were followed up until 6 weeks after cessation of breastfeeding. Each time an enrolled child had a DBS sample collected for HIV DNA testing at the central laboratory, a LYNX POC test was also conducted for comparison of results. 2181 LYNX POC tests were performed during the study; 1407 tests (65%) were included in the analysis. Of the 1407 test results, 909 (65%) were conducted at birth while 498 (35%) were conducted during routine clinical visits at 6 weeks of age or older. At routine visits, 16 (3.2%) participants were HIV DNA positive. The LYNX test had a sensitivity of 81.3% (CI: 54%, 96%) and specificity of 99.0% (CI: 98%, 99%). At birth, 12 (1.3%) participants were HIV DNA positive. The LYNX test had a sensitivity of 25.0% (95 % CI: 6%, 57%) and specificity of 99.6% (95 % CI: 98%, 99%). The LYNX test was easy to use and had many desirable characteristics for a POC test in a rural setting. However, the LYNX test had a lower sensitivity at 6 weeks of age or older compared to other available POC assays and a very low sensitivity at birth. LYNX POC test has potential to be of value as an EID screening test at routine clinic visits, particularly in rural areas. Its use could improve linkage to care for HIV-infected infants in settings where turnaround times for HIV DNA testing are long.

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HIV+ PREGNANT WOMEN HAVE DIMINISHED TRANSPLACENTAL TRANSFER OF NATURALLY ACQUIRED ANTIMALARIAL ANTIBODIES COMPARED TO HIV- PREGNANT WOMEN IN KENYA

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The transfer of IgG from mother to fetus across the placenta is critical to protect infants during the first few months of life. Maternal infections during pregnancy such as HIV and/or malaria can negatively affect transplacental transfer of antibodies such as those to tetanus and measles. HIV exposed but uninfected (HEU) infants experience increased morbidity and mortality compared to HIV unexposed uninfected (HUU) infants. We hypothesized that *in utero* exposure to HIV affects transplacental transfer of antibodies. 50 HIV- mothers/HUU neonates and 49 HIV+ mothers/HEU neonates were included. All HIV+ women received HAART and were seen by health care workers monthly. We examined in maternal plasma at delivery and paired cord blood plasma 1) the magnitude of antibodies against 4 vaccine antigens (diphtheria, tetanus, hepatitis B, and measles) and 14 *P. falciparum* antigens, and 2) placental transfer of antibodies to neonates. Additional measurements included birth outcome measures and malaria infection prevalence during pregnancy. We found that birth outcomes between HUU and HEU groups were comparable. HIV- mothers had more frequent malaria infections during pregnancy than HIV+ mothers (70% vs 31% p=0.0001). HIV- mothers had higher tetanus antibodies at delivery compared to HIV+ mothers, but antibodies directed against other vaccines were not different between the groups. Vaccine Cord-to-Maternal Ratio (CMR), a measure of placental antibody transfer, were no different between the groups. Anti-malarial antibodies were generally higher in HIV- women than HIV+ women. Interestingly, CMRs were consistently lower in the HIV+/HEU pairs suggesting decreased transplacental antimalarial antibody transfer in HIV+/HEU pairs compared to HIV-/HUU pairs especially to MSP9 ($p = 0.0402$), CSP ($p = 0.0326$), and EBA181 ($p = 0.0166$) which remained statistically significant after adjustment for malaria in pregnancy. While CMRs of vaccine antibodies were not affected by HIV or malaria status, lower CMRs were observed in HIV+/HEU pairs antibodies to naturally acquired malaria infection revealing an important effect even in optimally treated HIV+ women.

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HIV MULTI-CLASS RESISTANCE IN PATIENTS FAILING TO FIRST AND SECOND ART IN RESOURCES LIMITED SETTING, MALI

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Antiretroviral treatment has been widely implemented in resources constraint setting. Second-line ART has become more and more available but the biological monitoring is still limited. In order to achieve the goal of the three 90 of UNAIDS it will be interesting to know the resistance profile of the patients failing to ART. The objective of this study is to determine the prevalence of HIV multi-class resistance viruses and their impact the virological outcome. All patients with virological failure on first or second line ART in our routine genotyping system were included. The pol gene was sequenced by using viroseq or in house ANRS method. We identified 342 patients with genotypes availables, 21 (6%) patients had a wild-type viruses and 321 with multi-class resistant virus. Among this, 273 (79.9%) and 69 (20.1%) patients were respectively on first and second line ART. The median viral load (VL) was 69,740 copies/mm^3 and median CD4 276 cells/mm^3 at failure. The main first line regimen was TDF/3TC/EFV and second-line was AZT/3TC/LPVr. The prevalence of resistance mutations was: M41L (37%), A67G / N (42%), M184V (100%), T215F/Y (68%), K219E / Q (37%) and Q151M (16%) for the nucleoside. For non-nucleoside: K103N (32%), K101E/H/P (11%), Y181C/I/V (37%) and H221Y (21%). For PI: L76V (42%), V82A/F/T/S (21%) and I84V (37%). Patients were resistant to NRTIs in 83%, NNRTIs 94% and PIs 42%. Among the second-line ART failure 19% were resistant to darunavir. After 6 and 12 months of ART 63% and 76% of patients had suppressed HIV RNA less 40 copies/ml. In conclusion, the resistance genotypic testing is crucial to achieve the goal of the three 90 for patient failing to ART in resources limited setting.

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COMPARISON BETWEEN HUMAN IMMUNODEFICIENCY VIRUS (HIV) POSITIVE AND NEGATIVE PATIENTS ADMITTED WITH RESPIRATORY ILLNESSES IN SIAYA COUNTY REFERRAL HOSPITAL (SCRH), WESTERN KENYA, 2014-2016

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Data on etiology of respiratory illnesses (RI) among HIV patients in resource-limited settings are scarce. A population-based surveillance for RI (cough or difficulty breathing) hospitalizations in ≥13 years was set up in Western Kenya. Patients were matched with community controls by HIV status, age and time. Clinical data were obtained, naso/oropharyngeal swabs collected and tested using Taqman Array Cards (TAC) for 15 viruses, 14 bacteria and 1 fungi. Odds ratios (OR) were used to compare characteristics and pathogen-specific distribution by HIV status, and between cases vs controls. A total of 1,572 RI cases were admitted, 728 (46%) were HIV positive of which 474 (65%) were female. Median ages (interquartile range) were: RI cases (35.8, 26.6-55.8) and HIV positive (34.8, 28.8-45.5). HIV positive cases had worse outcome: oxygen supplementation (11.3% vs 7.4%, p=0.036), referral to intensive care unit (7.0% vs 4.0%, p=0.009) and death (21.1% vs 8.8%, p<0.001). A total of 1,004 RI cases (45% HIV positive) were tested using TAC. Comparing cases and controls, more *K. pneumoniae* (11% vs 5%)*, M. tuberculosis* (4% vs 0.3%) and *Rhinovirus* (21% vs 10%) were detected in cases than controls for HIV-positive stratum, while *S. aureus* (17% vs 30%), *S. pneumoniae* (25% vs 48%), *Adenovirus* (0.7% vs 6%), *H. influenza* (18% vs 40%) and *S. pyogenes* (0.7% vs 12%) were the most common pathogens in controls in HIV-negative stratum. It may be challenging to determine specific pathogens causing severe RI among HIV patients. There were not many differences in detection of respiratory pathogens between cases and controls which could reflect asymptomatic carriage among health populations. Identification of more than one potential pathogens in patients also indicate co-infections which warrants further investigation.

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GLOBAL HEALTH ENGAGEMENT VIA THE AFRICAN COHORT STUDY: QUANTIFYING THE PREVALENCE AND INCIDENCE OF HIV-ASSOCIATED CO-INFECTIONS IN SUB-SAHARAN AFRICA

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The Department of Defense Global Health Engagement strategy helps partner nations combat global health threats like HIV, which is responsible for over 1.5 million annual deaths worldwide. We leveraged facilities supported by the President's Emergency Plan for AIDS Relief (PEPFAR) to develop an open-ended prospective cohort that characterizes medical care and clinical outcomes, such as prevalence and incidence of HIV-associated co-infections, in four African countries. The African Cohort Study prospectively enrolls adults at 11 PEPFAR-supported facilities in Uganda, Kenya, Tanzania, and Nigeria. Adults were evaluated at enrollment and every 6 months for tuberculosis (GeneXpert; only HIV-infected), hepatitis B virus (hepatitis B surface antigen; HBV), and syphilis (rapid plasma reagin with a confirmatory treponemal test). Hepatitis C virus was evaluated at enrollment only (anti-HCV antibody assay; HCV). Prevalence and incidence of these infections were compared between participants with and without HIV using Chi-Square and Fisher's exact tests. From January 2013-September 2017, we enrolled 3220 adults (58% female) with a mean age of 38.9 (SD 10.5) years. Of these, 2678 (83%) were HIV-infected, 1793 (67%) were ART-experienced and 1620 (60%) were virally suppressed <1000 copies/mL. At enrollment, tuberculosis was observed among 80 HIV-infected participants (29.9%); HBV among 119 (4.4%) HIV-infected and 22 (4.1%) HIV-uninfected participants (p=0.81); HCV among 43 (1.6%) HIV-infected and 14 (2.6%) HIV-uninfected participants (p=0.09); and syphilis among 291 (10.9%) HIV-infected and 25 (4.6%) HIV-uninfected participants (p=0.63). The incidence of TB was 12.7/1000 person-years (PY). Throughout follow-up, HBV incidence did not differ between participants with and without HIV (6.3/1000PY and 6.1/1000PY; p=0.72), nor did the incidence of syphilis (16.6/1000PY and 13.9/1000PY; p=0.32). This large African cohort demonstrates a high burden of HIV-associated co-infections. Co-infections should be a focus for further capacity building with partner nations.

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TRACKING HIV/AIDS FINANCING AND THE RESPONSE TO DECLINES IN DEVELOPMENT ASSISTANCE FOR HIV/AIDS

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Since 2005, the international community rallied to mobilized a total of 123 billion dollars to fight HIV/AIDS. These investments helped reduce HIV/AIDS incidence and mortality; however, ongoing progress may be at risk as development assistance for HIV/AIDS (DAH-HIV/AIDS) steadily declined by 6.6% since 2012 and calls to reduce future aid spending continue to echo. With this analysis, we track HIV/AIDS spending across financing sources and functions in 188 countries from 2000 to 2015 and estimate governments' responses to reduced DAH-HIV/AIDS. We collected and extracted 5,385 data points from public available reports and databases. We used spatiotemporal Gaussian process regression to estimate a complete times series of HIV/AIDS spending by financing source (government, prepaid private, and out-of-pocket) and function (care and treatment, prevention, and other). We used system generalized method of moments estimation to predict the effects of reductions in DAH spending. In 2015, global HIV/AIDS spending amounted to $48.1 ($45.7-52.1) billion. Of this total, governments financed 62.2% (56.6-66.7%), but in high prevalence countries, DAH financed 76.6% (73.6-79.4%). In low-income countries, for every dollars of DAH-HIV received, governments spent $0.11. We estimated that a 10% reduction in DAH-HIV/AIDS, is associated with a .03% decline in government spending. This effect is stronger for spending on HIV/AIDS care and treatment: a 10% decrease in DAH-HIV/AIDS for care and treatment was associated with a 0.6% increase in domestic HIV/AIDS care and treatment spending. Both domestically and internationally, vast sums of financial resources were mobilized to fight HIV/AIDS since 2000. While governments financed the majority of this fight, 19.3 million people with HIV/AIDS live in countries where DAH-HIV/AIDS finances over 50% HIV/AIDS spending. The lives of these 19.3 million people may at risk as potential reductions in DAH-HIV/AIDS are not likely to be filled domestically. This conclusion jeopardizes the progress made against HIV/AIDS and the hope of an AIDS free generation.

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BEYOND IGG4 ANTIBODIES TO OV-16: *ONCHOCERCA VOLVULUS*-SPECIFIC BIOMARKERS THAT PUSH SENSITIVITIES FOR INFECTIONS ABOVE 95%

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As efforts shift from control to elimination of *Onchocerca volvulus (Ov)*, additional tools to identify those contributing to ongoing *Ov* transmission in low transmission settings will be needed to get beyond the ~80% sensitivity of the IgG4 Ov16 assays (ELISAs, RDTs). By isotype-specific screening of protein arrays developed from ~400 *O. volvulus* proteins, we identified several biomarkers. Two of these biomarkers OVOC10469 and OVOC3261 tested against panel of *Ov*-, *W. bancrofti*-, *L. loa*-infected and healthy controls (~300 samples), in conjunction with Ov16 enhanced the sensitivity for detecting infections from 80% (with Ov16 alone) to ~95% in a luciferase immunoprecipitation system (LIPS) assay with >99% specificity. To understand the kinetics of the response to these 2 "new" antigens, sero-reactivity to OVOC10469 and OVOC3261 were analyzed in archived longitudinally collected sera of 7 chimps experimentally infected with *O. volvulus* and compared to the kinetics of Ov16 antibody. Anti-Ov16 IgG was detectable as early as 100 days of post-infection, while the IgG4 responses coincided with the onset of patency (350-500 days). In contrast to Ov16, the IgG responses to OVOC10469 and OVOC3261 were patency dependent, with IgG4 responses appearing even later. Preliminary analyses with sera from *Ov*-infected patients followed for more than 5-10 years following treatment showed a dramatic drop in anti-OVOC10469 and anti-OVOC3261 IgG levels ($p<0.001$). These data suggest that seroreactivity to these 2 antigens are indicative of the presence of microfilariae (in a surveillance setting). When the prevalence of seroreactivity is sufficient high, this not only suggest likely transmission but may help to develop more informed and refined models. Both OVOC10469 and OVOC3261 have been expressed recombinantly are currently being tested in conjunction with Ov16 for a potential point of care test.

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DEVELOPMENT OF *IN NEW VITRO* CULTURE SYSTEMS FOR THE MAINTENANCE OF MICROFILARIAE AND INFECTIVE LARVAE OF *LOA LOA* FOR DRUG SCREENING

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Co-infection with loiasis remains a potential problem to control programs targeting filarial infections. Suitable *in vitro* culture systems for *Loa* parasite maintenance are needed to foster drug research. This study investigated systems for drug screening against microfilariae (mf) and infective larvae (L3) of *L. loa*, then documented the microfilaricidal effects of 13 anti-parasitic drugs. *In vitro* culture conditions were evaluated by varying three basic media: RPMI-1640, DMEM and IMDM; four sera/proteins: newborn calf serum (NCS), foetal bovine serum (FBS), bovine serum albumin (BSA) and AlbuMax® II (ALB); then co-culture with LLC-MK2 cell line. Motility (T_{90} = mean duration at which 90% of parasites are fully active) and moulting rates of L3 were determined. The effect of antimalarial, anthelmintics, trypanocidal and anticancer agents on *L. loa* mf was evaluated.The media DMEM and IMDM were the most suitable sustaining the maintenance of both parasite stages. Sera and LLC-MK2 significantly improved the survival of parasites (T_{90} of 16.4–19.5 days for *L. loa* mf). The most effective culture systems promoting moulting and maintenance of L3 were DMEM + serum + LLC-MK2. From the GLM analysis, factors that promoted *L. loa* mf viability included feeder cells ($\beta = 0.490$), both IMDM ($\beta = 0.256$) and DMEM ($\beta = 0.198$) media and the protein supplements NCS ($\beta = 0.052$) and FBS ($\beta = 0.022$); while for *L. loa* L3, the protein BSA ($\beta = 0.029$) was included. Inhibition of *Loa loa* motility was seen with mefloquine and amodiaquine (CR_{50} values of 3.87 and 4.05 µg/ml, respectively). Imatinib and SCYX-7158 also induced a concentration-dependent reduction in mf motility. A range of requirements for the *in vitro* maintenance of *Loa loa* stages, suitable for developing an effective platform for drug screening was identified. The considerable action of mefloquine and amodiaquine on *Loa* mf *in vitro* highlights the possibility of repurposing anti-infectious agents for loiasis drug development, while the heterogeneity in the activity of anti-parasitic agents on *Loa loa* mf supports the need for further investigation using *in vivo* models.

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WHOLE BLOOD TRANSCRIPTOME ANALYSIS IDENTIFIES A 7-GENE CLASSIFIER FOR *ONCHOCERCA VOLVULUS* INFECTION

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Identifying the molecular mechanisms controlling the host's response to infection with *Onchocerca volvulus* are important to understand how the human host controls such parasitic infection. Little is known of the cellular immune response upon infection with *O. volvulus*. We performed a transcriptomic study using PAXgene preserved whole blood from 30 nodule positive individuals and 21 non-endemic controls. It was found that of the 45,042 transcripts that were mapped to the human genome, 544 were found to be upregulated and 447 to be down-regulated in nodule positive individuals (adjusted p-value <0.05). Within this set of differentially expressed genes a strong enrichment was found for genes related to translation and ribosomes (corrected p-value <0.05). Mammalian phenotype analysis demonstrated an association with abnormal immune system. Elastic net regression was used to identify the top list of 22 genes that can contribute significantly in the generation of a classifier. For these 22 genes, as well as for 8 reference target genes, validated RT-qPCR assays were developed and used to re-analyze the discovery sample set. These data were used to perform elastic net regularized logistic regression and a panel of 7 genes was found to be the best performing classifier. The resulting algorithm returns a value between 0 and 1, reflecting the predicted probability of being infected. ROC analysis resulted in an area under curve of 0.860 (95% CI: 0.747-0.974). A validation panel of 69 nodule positive individuals and 9 non-endemic controls was used to set a cutoff for positivity and to determine the performance of this classifier. Based on this validation set only, a sensitivity of 60.9% and a specificity of 100% was obtained. When combining the discovery test set and validation set, a sensitivity of 67.7% and a specificity of 93.1% was obtained. Large-scale validation approaches will be necessary to define the intended use for this classifier. Besides the use as marker for infection in MDA efficacy surveys and epidemiological transmission studies, it might also hold potential as pharmacodynamic marker in macrofilaricide clinical trials.

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DIFFERENTIAL MODULATION OF HUMAN INNATE LYMPHOID CELL FUNCTION BY IL-10 AND TGF-B

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Human filarial infections are associated with an expansion of innate lymphoid cell (ILC) subsets that mirror in many ways the CD4+ helper cell (Th) subsets: ILC1s (IFN-γ), ILC2s (IL-5 and IL-13) and ILC3s (IL-17A or IL-22). Chronic filarial infections are also associated with an immune-modulatory environment mediated largely by IL-10 (and to a lesser extent TGF-β), cytokines that alter antigen-specific effector T cell responses. Using multiparameter flow cytometry human ILC subsets can easily be detected in the circulation accounting for only 0.35% (range 0.04-4.51%) of CD45+ leukocytes under homeostatic conditions. Despite the low frequency of ILCs in circulation, *ex vivo* experiments have demonstrated that these ILCs release extremely large per cell quantities of cytokines following activation and, if left unchecked, these ILC-derived cytokines can have deleterious effects on the host. To explore the mechanisms underlying the inhibition of ILC cytokine production, we observed IL-10R and TGF-βRI gene expression in all ILC subsets found in circulation. We examined the expression of IL-10R on ILC subsets and found that ~14-25% of each subset expressed IL-10R and did so at the same per cell intensity (GeoMFI=663) as did human monocytes, known to express IL-10R. To explore the mechanisms that regulate ILC activation, ILC subsets were isolated from the peripheral blood of healthy donors by flow cytometry-based sorting and activated in the presence or absence of IL-10 or TGF-β. ILC2s stimulated in the presence of IL-10 had a marked reduction in IL-5 ($p<0.01$) and IL-13 ($p<0.05$) production when compared to ILC2s activated in the absence of IL-10. A similar cytokine regulation was not observed for ILC1s stimulated in the presence of IL-10. Conversely, TGF-β had an inhibitory effect on ILC1 production of IFN-γ following stimulation but not on ILC2 production of IL-5 and IL-13 following stimulation. Studies are currently underway to explore the downstream effects of IL-10 and TGF-β on ILCs and to identify additional mechanisms that regulate ILC cytokine production.

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IMMUNIZATION WITH RECOMBINANT DIM-1 PROTEIN OF *BRUGIA MALAYI* INDUCE T AND B CELL IMMUNOGENICITY AND PROTECT MICE AGAINST *B. MALAYI* INFECTION

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Lymphatic filariasis, a chronic disease affecting over 120 million people worldwide, endemic in 73 countries. Several attempts (MDA, vector control measures and vaccine development) have been made to control filariasis but there is no licensed vaccine for human use, up to date. The study aims to know the effect of short term (3 dose) or long term (till 3 or 6 weeks) recombinant DIM-1protein of *Brugia malayi* (rDIM-1bm) immunization on modulation of T cell and B cell immunogenicity and its protective efficacy against *B. malayi*. *B. malayi* DIM-1 was successfully cloned, expressed and recombinant DIM-1bm protein (~40 kDa) purified. *Mastomys coucha* were immunized in 3 immunization schedules, 3-dose (3 injections: 0 day, 14 days and 21 days), 3 weeks (Injections at 3 or 4 days interval till 3 weeks) and 6 weeks (Injections at 3 or 4 days interval till 6 weeks) with subsequent inoculation of 100 L_3 after immunization. Of the 3 different immunization schedules, 3-dose immunization was the most effective in protecting *M. coucha* against establishment of L_3 induced infection as inferred by a low recovery of mf in circulation (63%) and parasite burden (52%). The enhanced activity of macrophages, cellular proliferation and NO responses, and elevated levels of specific IgG, IgG1, IgG2a, IgG2b, IgE and IgA, and intense upregulation of both Th1 (IFN- γ, TNF-α and IL-2) and Th2 (IL-4, IL-5, IL-6, IL-10 and IL-13) responses produced by 3-dose rDIM-1bm immunization schedule correlated with parasitological findings. Three or six weeks immunization schedule provided poor protection against L_3 initiated infection and produced mixed immunological responses. These findings suggest that 3-dose schedule protects the host from infection via Th1/Th2 type responses and may be important protein for exploring its vaccine potential. This information would help in evolving effective control and prevention strategies for human lymphatic Filariasis.

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EVALUATION OF EIGHT NOVEL PEPTIDES FOR THE SEROLOGICAL DETECTION OF INFECTIONS WITH *ONCHOCERCA VOLVULUS*

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Current serologic assessments for human exposure to *Onchocerca volvulus* are performed by detection of IgG4 antibodies against the OV-16 antigen. However, additional confirmatory or complementary serological assays are needed to verify exposure/infection status in humans. Eight novel peptides, selected from a proteome scan of linear epitopes of *O. volvulus*, were evaluated for IgG reactivity by ELISA using a panel of 50 sera from persons with responses to OV-16, 26 of whom were also microfilariae positive by skin snip. Samples from 50 OV-16 nonreactive individuals, including people with other filarial infections, were used as controls. Performance characteristics of the peptides (sensitivity, specificity, optimal threshold and area under the curve (AUC)) were determined by receiver operator characteristic (ROC) analyses. Correlation analyses were used to determine the association between the reactivity to OV-16 and the peptides as measured by optical density (OD). Three of the 8 peptides, OvMP-23, OvNMP-17 and OvNMP-18, had ROC AUC values >90%, with 90-92% sensitivity and 80-82% specificity. There was 85-86% agreement when comparing calls from these three peptides to OV-16. In a subset analysis using only the 26 positive samples from people with microfiladermia and excluding 5 OV-16 negative individuals from potentially co-endemic settings, four of the peptides had ROC AUC values >90%. The sensitivity and specificity for these peptides were 88% and 86% for OvMP-1, 88% and 89% for OvMP-23, 85% and 93% for OvNMP-16 and 73% and 100% for OvNMP-18. Analysis of the correlation between OD values for OV-16 and each of the peptides showed no correlation, suggesting that the peptides represent antigens that are independent of OV-16. These preliminary analyses suggest that peptides OvMP-23 and OvNMP-18 may be candidates to complement OV-16 serology. Additional evaluations with expanded serum panels are needed to better define appropriate thresholds and determine if the use of these peptides in tandem with OV-16 will improve serologic detection of onchocerciasis.

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A NEW SYSTEM FOR THE PRODUCTION OF INFECTIVE LARVAE OF *LOA LOA* USING INTRATHORACIC INJECTION OF MICROFILARIAE TO *CHRYSOPS SILACEA*

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Research and drug development for Loiasis is hindered due to the lack of suitable methods of obtaining parasite material. The present work aimed to develop a method for production of *Loa loa* infective larvae (L3) by intrathoracic injection of its vector, *Chrysops silaceae* with microfilariae. Purified *L. loa* mf from baboon blood were injected into the thorax of wild caught *Chrysops* (group 1 = 50mf/fly, group 2 = 100mf/fly). The L3 were

recovered from flies 16 days post injection and quantified per fly section (head, thorax and abdomen). Part of L3 were maintained *in vitro* in DMEM + 10% FBS and feeder layer for 30 days, while recording survival, motility, and moulting. *In vivo* conditions were met by inoculating the other L3 into RAG2IL-2Rγ-deficient C57BL/6 mice and dissecting these 60 days PI. Fly survival differed per group; 55.2% for group 1 and 34.7% for group 2, $p < 0.001$. The L3 yield/fly was higher in group 2 (75.2 %) than in group 1 (50.5%). Parasite survival was > 90% and L3 molting rate was >head (61.3%)>thorax (60.2%)>abodomen (56.4%) ($p = 0.610$). Mice dissection revealed a mean worm recovery rate of 10.7%. Mice infected with L3 from fly heads obtained the highest worm recovery (17.2%), followed by those from thorax (9.8%) and abdomen (8.1 %). Worms recovered measured in average 24.3 mm and 11.4 mm in length and 0.35mm and 0.27 mm in width for females and males respectively. Conclusively, L3 obtained using this new system remained viable (both *in vitro* and *in vivo*). In addition, L3 were able to mature into young adult worms in Rag2IL-2Rγ C57BL/6 mice. This approach presents a novel platform for in-depth research in Loiasis and offers opportunities to unravel mysteries about the biology of this filarial nematode.

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IDENTIFYING A ROLE FOR SETPOINT ANTIBODY TITERS IN DENGUE INFECTION AND DISEASE RISK

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Across infectious diseases, complex host immunity-pathogen interactions shape disease risk. Immunity derived from prior infections or vaccination may positively or negatively affect our ability to fend off infection or disease from related pathogens; however, immune correlates are rarely known. For dengue, this uncertainty is driven by frequent subclinical and therefore unobserved infection coupled with a limited understanding of how serological markers behave over time. We use detailed cohort data from Thailand (3,453 individuals with blood draws every 91 days, 140,612 hemagglutination inhibition antibody titer measurements in all) to develop a framework that simultaneously characterizes antibody dynamics and identifies subclinical infections via Bayesian augmentation. We identify 1,149 infections (95% CI: 1,135-1,163) that were not detected by active surveillance and estimate that 65% of infections are subclinical. Each increase in $\log_2$-titers is associated with a 0.71 times relative risk of infection (95% CI: 0.67-0.76). Further, individuals with pre-existing adjusted $\log_2$-titers of ≤1:40 develop dengue hemorrhagic fever 7.4 (95% CI: 2.5-8.2) times as often than naïve individuals compared to 0.0 times as often for individuals with titers >1:40 (95% CI: 0.0-1.5). PRNT titers ≤1:100 were similarly associated with severe disease. Post infection, individuals develop a stable setpoint antibody load after 1y that places them within or outside a risk window. At this time, we observe a 2.1 times increased risk of infection for those with setpoint antibody loads of ≤1:40 compared to those with greater antibody loads and an 8.9 times increased risk developing hemorrhagic fever. The apparent stability of setpoint antibody loads points to an ability to assess an individual's long-term risk. The current licensed vaccine moves previously naïve individuals into this risk window but also has the potential to move previously infected individuals out of it.

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WANING OF ANTI-DENGUE VIRUS ANTIBODIES FOLLOWING PRIMARY AND SECONDARY DENGUE VIRUS INFECTION AND IMPLICATIONS FOR SEROTYPE TRANSMISSION DYNAMICS

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The mosquito-borne dengue flaviviruses (DENV1-4) are major causes of disease globally. Cohort studies have found that ≥2 years after primary (1°) DENV infection, children are at elevated risk of dengue, which is thought to be due to waning of cross-protective antibodies. In contrast, secondary (2°) DENV infection is thought to induce long-lived, cross-protective antibodies. We measured rates of antibody waning after 1° and 2° DENV infection in the Nicaraguan Pediatric Dengue Cohort Study (PDCS, n=6684) in healthy annual serum samples (n=41,302) over a period of 12 years (2004-2015). Antibody (Ab) titers were measured with the Inhibition ELISA, an assay predictive of both protection from dengue and risk of severe dengue. We used linear mixed-effects models with random effects for individual intercepts (peak) and slopes (waning). The Ab titer half-life after any infection was 4.00 years [95%CI: 3.81-4.20]. However, after 1° infection, initial Ab titer varied by individual but did not wane over time, suggesting individual-level Ab titer "set points". In contrast, Ab titers were higher post-2° infection but waned for many years. Raw post-2° infection Ab titers suggest waning for multiple years with eventual stabilization to a new individual "set-point". We explored the implication of these observations for DENV transmission. A spatiotemporal analysis of RT-PCR-serotyped dengue cases in the PDCS showed that dengue cases were significantly more likely to be of the same serotype if they occur <2 years and <1km of each other but of a different serotype when separated by ≥2 years. We hypothesize that population-level immunity with spatial heterogeneity drives transitions in serotype dominance and are using statistical and mathematical transmission models to explain how spatial and temporal patterns of homotypic vs heterotypic serotypes could account for the interval of protection from symptomatic infection given stable Ab titers after a 1° infection. These findings suggest an alternate model of DENV immunity with important implications for measuring vaccine efficacy in clinical trials and for building mathematical models of DENV transmission.

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FLAVIVIRUS INDUCED T CELL CROSS-REACTIVITY

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Dengue Virus (DENV) is a flavivirus closely related to Zika virus (ZIKV), Japanese encephalitis virus (JEV), West Nile virus (WNV), and Yellow fever virus (YF), all of which are transmitted primarily by mosquitoes. The issue of potential cross-reactivity and how pre-existing T cell immunity to one flavivirus modulates T cell responses to another is of great relevance for

both diagnostic tests and vaccines as these viruses often co-circulate in the same geographical regions. To address this question we have established peptide pools derived from the polyproteins of DENV 1-4, ZIKV, JEV, WNV and YF viruses by generating consensus sequences and predicting MHC binding affinities to HLA alleles most commonly expressed in endemic areas. Subsequently, these pools were tested in blood donors naturally exposed to DENV, ZIKV and WNV or experimentally vaccinated against DENV, JEV and YF for their potential to elicit antigen specific CD4 and CD8 T cell responses as measured by intracellular cytokine staining. Interestingly, T cell cross reactivity could be observed between all flaviviruses tested, albeit to various degrees. Cross-reactivity was more pronounced for MHC II restricted CD4 responses than MHC I CD8 T cell responses for all viruses tested. We are currently characterizing these cross-reactive responses in more detail in terms of T cell memory phenotype and specific activation markers. Knowledge of immunity between related flaviviruses is crucial to understand potential implications for vaccination and diagnostics in highly endemic settings.

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ENDOCYTOSIS OF DENGUE VIRUS NS1 BY HUMAN ENDOTHELIAL CELLS IS REQUIRED FOR NS1-MEDIATED BARRIER DYSFUNCTION AND IS ABOLISHED BY A SINGLE N-GLYCOSYLATION SITE MUTATION

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Dengue virus (DENV) is the most prevalent arbovirus worldwide, and infection with any of the four serotypes leads to a range of outcomes, from inapparent disease to dengue fever to dengue hemorrhagic fever and dengue shock syndrome. Recently, we described a novel role for DENV nonstructural protein 1 (NS1) in triggering hyperpermeability of human endothelial cells and systemic vascular leak *in vivo* via disruption of the endothelial glycocalyx layer (EGL). However, the molecular determinants of NS1-induced endothelial hyperpermeability have not yet been identified. Here, using *in vitro* model systems, we report that a glycosylation site (Asn-207) is required for DENV NS1 internalization by endothelial cells and activation of endothelial cell-intrinsic pathways characterized by degradation of EGL components. This mutation (N207Q) completely prevents (i) NS1-induced hyperpermeability, as measured by trans-endothelial electrical resistance, TEER, and (ii) EGL disruption in human pulmonary microvascular endothelial cells (HPMEC), as determined by activation and/or increased expression of cathepsin-L, heparanase, and sialidases leading to shedding of heparan sulfate and sialic acid at the cell surface. As a potential mechanism to explain this phenomenon, we found that DENV2 NS1 N207Q mutant is less efficiently internalized by HPMEC than the wild-type protein at 37°C as determined by Western blot and colocalization by confocal microscopy using Rab5 as an early endosome marker. However, similar binding levels were found for both proteins at 4°C, suggesting that the N207Q mutation does not affect NS1 interaction with the cell surface but abrogates its internalization by HPMEC. These results describe a new molecular determinant of NS1 required for induction of endothelial hyperpermeability and EGL degradation in HPMEC that relies on its endocytosis. These findings may be crucial for developing antiviral therapies and NS1-based vaccine approaches.

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DENGUE VIRUS NS1 PROTEIN ACTIVATES ENDOTHELIAL CELLS

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Dengue virus (DENV) causes an estimated 390 million infections around the world annually. Severe forms of disease (dengue hemorrhagic fever and shock syndrome) are marked by vascular leakage and increased concentration of vasoactive mediators, such as histamine, VEGF and leukotrienes. Endothelial cells (ECs) are directly responsible for vascular homeostasis and are highly responsive to circulating mediators but are not commonly infected by DENV. DENV encodes 10 proteins; one highly conserved protein (NS1) is secreted from infected cells, correlates with severity and is implicated in increased vascular permeability. We hypothesized that NS1 directly interacts with ECs to promote activation. To address our question, we used a complimentary array of *in vitro* assays with primary ECs aimed to evaluate activation and cellular reorganization. To evaluate the mechanism of action of NS1 we analyzed the ability of NS1 to directly interact with ECs. Confocal immunofluorescence microscopy demonstrated that NS1 binds to the surface of ECs, is internalized rapidly, and accumulates in the cytoplasm over time. RNA-seq and pathway analysis showed a significant upregulation of genes associated with an angiogenic phenotype, including those involved with migration, cellular reorganization and proinflammatory responses. Furthermore, functional significance assessed using *in vitro* migraion and tube formation assays, demonstrated NS1 promoted an increase in the rate of migration and tube formation in ECs, in a dose-dependent manner. Taken together, our results indicate that NS1 directly induces functional and structural changes in ECs that are compatible with an aberrant angiogenic phenotype.

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GENETIC VARIATION BETWEEN DENGUE VIRUS TYPE 4 STRAINS IMPACTS HUMAN ANTIBODY BINDING AND NEUTRALIZATION AFTER INFECTION AND VACCINATION

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One third of the world's population lives in areas with active transmission of dengue viruses (DENVs), the etiological agents of dengue fever and dengue hemorrhagic fever/shock syndrome. There are four antigenically distinct DENV serotypes (DENV1-4). A primary infection with one serotype generates a durable neutralizing and protective antibody response to the serotype of infection only. Within the DENV4 serotype, there are at least five distinct genotypes. The impact of DENV4 genotypic diversity on antigenic variation has not been rigorously examined, nor is it clear whether infection with one DENV4 genotype results in protection against all other DENV4 genotypes, which is crucial for vaccine design. To measure the impact of genetic diversity within DENV4 on the binding and neutralization of human antibodies, we generated an isogenic panel of recombinant viruses containing envelope protein sequences from the different genotypes of DENV4. We characterized the properties of these viruses, including their sensitivity to neutralization by human monoclonal antibodies and polyclonal immune sera. We found that a small number of amino acids within the envelope glycoprotein have disproportionate impacts on virus maturation, growth and ability to infect cells. Additionally, we observed large differences in the ability of DENV4 serotype-specific antibodies and immune sera to neutralize the panel of viruses, suggesting that DENV4 immunity might not be protective against all circulating DENV4 viruses. Individuals vaccinated with a live attenuated DENV4 vaccine displayed greater than 10 fold differences in the ability to neutralize different DENV4 genotypes. Our results have implications for understanding the impact of DENV intra-serotype genotypic variation on natural infection and vaccine performance and suggest that genotypic variation could lead to re-infection by the same serotype or vaccine failure.

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MODEL-BASED ASSESSMENT OF PUBLIC HEALTH IMPACT AND COST-EFFECTIVENESS OF ROUTINE VACCINATION WITH DENGVAXIA® FOLLOWING SCREENING FOR PRIOR DENGUE VIRUS EXPOSURE

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A safe and effective vaccine for dengue could have a major public health impact for billions of people at risk of this mosquito-borne viral disease. Currently, Dengvaxia® from Sanofi Pasteur is the only licensed dengue vaccine, but there are concerns about its safety and effectiveness. In response to new phase-III trial results reported by Sanofi Pasteur in late 2017, the World Health Organization revised its position on Dengvaxia® to recommend that this vaccine only be administered to people known to have prior exposure to dengue virus (DENV), given that the vaccine appears safe and effective only for individuals in that category. Previously, we and seven other modeling groups made projections of the public health impact and cost effectiveness of routine vaccination with Dengvaxia®, but those projections are now obsolete given that they were based on the assumption that children would be vaccinated regardless of whether they have prior exposure to DENV. Using an agent-based model, we made projections of the percentage of symptomatic and hospitalized cases averted after 30 years of routine vaccination at age 9 with prior exposure to DENV confirmed by a diagnostic test with a range of values of sensitivity and specificity. In general, our results suggest that vaccinating individuals who test positive with a highly specific diagnostic (>0.8) can reduce symptomatic disease and hospitalizations at the population level, and that it can do so under a broader range of transmission settings than is possible under vaccination without diagnostic testing. Our results also suggest that the cost effectiveness of vaccination following diagnostic testing depends heavily on who is paying, with cost effectiveness appearing higher from the individual perspective than from that of a healthcare provider. Based on economic cost estimates for Brazil, a highly specific diagnostic would need to cost less than $100 to be cost-effective from the individual perspective and less than $30 from the healthcare provider perspective.

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REGULATORY RESPONSES TO *SCHISOSOMA MANSONI* ARE INITIATED IN THE HUMAN SKIN: SKIN PENETRATING CERCARIAE INDUCE REGULATORY DERMAL APCS

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Following initial invasion of *Schistosoma mansoni* (*Sm*) cercariae, schistosomula reside in the skin for days during which they interact with the dermal immune system. The prolonged interaction of radiation-attenuated cercariae with innate antigen presenting cells (APCs) in the skin is presumed to orchestrate the ensuing protective immunity. To dissect the protective immune responses induced by radiation-attenuated cercariae, we studied the interaction between non-attenuated and radiation-attenuated (RA) *Sm* cercariae and dermal APCs in human skin explants. We exposed human skin explants to either cercariae or RA cercariae and visualized cercarial penetration into human skin using novel imaging technologies. Subsequently, we analysed crawl-out immune cells for their phenotype. Dermal dendritic cell (DDC) subset distribution as well as activation markers in the three-day craw-out compartment was unaltered upon cercarial penetration. However, DDCs showed an increased expression of immune-modulators programmed death ligand (PDL) 1 and 2 and enhanced production of regulatory cytokine interleukin (IL)-10, pro-inflammatory cytokine IL-6 and macrophage inflammatory protein (MIP)-1α/β, primarily after exposure to non-attenuated cercariae. These cytokine responses were absent or decreased after exposure to RA parasites. Coculturing of *ex vivo* primed DDCs with naïve CD4 T cells revealed suppressed T cell secretion of IFNγ and increased IL-10 production, confirming the regulatory potential of these APCs. Using human-monocyte derived dendritic cells (MoDCs) in a transwell model, we showed upregulation of PDL-1 and 2 is mediated by direct contact of MoDCs with cercariae. In conclusion, we are first to investigate human dermal immune responses to *Sm* cercariae in full thickness skin explants and reveal a mixed innate, regulatory response to non-attenuated *Sm* cercariae. Radiation-attenuation of cercariae partially reverses this response. An understanding of the immune suppressive capacity of *Sm* at the site of the human dermis may give clues towards the development of an effective schistosome vaccine.

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FIBROBLAST-SPECIFIC INTEGRIN ALPHA V DIFFERENTIALLY REGULATES TYPE 2 AND TYPE 17 DRIVEN INFLAMMATION AND FIBROSIS IN *SCHISTOSOMA MANSONI* INFECTION

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Fibroproliferative diseases will affect a significant proportion of the world's population. Despite this the core mechanisms that drive organ fibrosis of diverse etiologies remain ill-defined. Recent studies suggest integrin-alpha V (Itgav) serves as a master driver of fibrosis in multiple organs. However, these studies have overlooked a significant population of fibrotic patients, which have a parasitic disease insult and fibrosis driven by type 2 (IL-4/IL-13) rather than type 1 (TGF-β/IFNγ/IL-17) immune responses. To investigate if Itgav is critical for the development of type 2 fibrosis, we analyzed fibroblast-specific Itgav knockout mice (Itgav-KO) during the course of natural *Schistosoma mansoni* infection as well as in models of lung fibrosis and of severe asthma. Surprisingly, while we confirmed a role for Itgav in models of TGF-β driven fibrosis, Itgav was not critical for the development of fibrosis during *Schistosoma mansoni* infection and other type 2 driven disease models as shown by no change in hydroxyproline quantification and collagen gene expression in Itgav-KO animals. Nevertheless, type 17 (IL-17) responses were impaired by Itgav deficiency, independent of its effect on fibrosis (IL-17$^+$CD4$^+$ t-cell frequency: 0.811% vs 0.460% $p < 0.05$). As IL-17 has been found to serve as an important activator of TGF-β-driven fibrosis, the preferential suppression in the IL-17 inflammatory pathway likely explains the critical role of Itgav in TGF-β-dependent but not IL-13-dependent fibrosis seen in a helminth infection. Finally, during a mixed IL-17/IL-13 inflammatory model of severe asthma, Itgav deficiency led to a substantial immune skewing away from type 17 and towards type 2 inflammation in Itgav-KO animals (IL-17$^+$CD4$^+$ t-cell frequency: 6.108% vs 4.218%, $p < 0.05$; IL-13$^+$CD4$^+$ t-cell frequency: 0.769% vs 2.534 %, $p < 0.0001$). Together, these findings have important therapeutic implications, as they suggest targeting both the Itgav/TGF-β and type 2 pathways may be needed to more effectively resolve fibrosis and prevent rebound of opposing pro-fibrotic mechanisms.

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EOSINOPHIL EXTRACELLULAR TRAPS MEDIATE ENTRAPPING OF MICROFILARIAE OF THE RODENT FILARIAL *NEMATODE LITOMOSOIDES SIGMODONTIS*

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During filarial infection eosinophils mediate protection against adult worms and microfilariae (MF), whereas they are only essential against infective L3 larvae following re-infection or vaccination. Similar to neutrophils, eosinophils are able to produce extracellular DNA traps (ETosis), a form of cell death where intracellular DNA is explosively released. The aim of this study was to analyze the impact of eosinophil ETosis in response to MF of the rodent filarial nematode *Litomosoides sigmodontis*. Bone-marrow-derived eosinophils released DNA in response to MF as was shown by scanning electron and confocal microscopy as well as by DNA quantification using a fluorescence assay. Subsequent PCR analysis revealed that the released DNA was of nuclear and mitochondrial content. This eosinophil ETosis reduced MF motility *in vitro* in a DNA- and contact-dependent manner, whereas addition of serum of naïve and infected animals inhibited the ETosis-dependent MF trapping. Stimulation of eosinophils with the small molecule analog 5 (SMA5) of the filarial immunomodulatory excretory-secretory product ES-62 enhanced DNA release and MF trapping by eosinophils. Comparison of the efficacy of bone-marrow-derived eosinophils and eosinophils from the thoracic cavity and gut of *L. sigmodontis*-infected animals further revealed that eosinophils from infected animals are more potent in inhibiting MF motility, which was independent on the local immunomodulation by the adult worms. These results demonstrate that eosinophils impair MF motility in an ETosis-dependent mechanism by releasing nuclear and mitochondrial DNA. Ongoing experiments address the *in vitro* impact of eosinophil ETosis on adult worms and L3 larvae and indicate that eosinophils only trap L3 larvae when specific antibodies are present, as it occurs during filarial re-infection or vaccination. In addition, current *in vivo* experiments are investigating eosinophil ETosis in response to MF and L3 larvae during primary infection and re-infection.

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IL-10 AND ITS RELATED SUPERFAMILY MEMBERS IL-19 AND IL-24 PROVIDE PARALLEL/REDUNDANT IMMUNE-MODULATION IN *LOA LOA* INFECTION

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IL-10 has been implicated as the major cytokine responsible for the modulation of parasite-specific T cell responses in active filarial infections; however, IL-10 belongs to a superfamily of cytokines, and the role of other superfamily members in filarial infection is less well studied. We have previously shown that the induction of the IL-10 superfamily members IL-24 and IL-19 are increased in active (antigen positive) *W. bancrofti* infections and can regulate both Th1/Th17 and Th2 responses. To address the role of IL-10 and its superfamily members (IL-24, IL19, IL-22, IL-28a) in the related filarial infection *Loa loa* and to understand the role played by microfilaria (mf) in their induction, peripheral blood mononuclear cells from 31 patients with loiasis were stimulated with or without filarial antigen, 2 non-parasite antigens (streptolysin O, or tetanus toxoid), or mitogen (PHA), and production of 17 cytokines was assessed using a multiplex Luminex platform for Th1/Th2/Th9/Th17/IL-10 (and IL-10 superfamily)-associated cytokines. All of the patients produced significant levels of IL-10 ($p=0.0414$), IL-13 ($p<0.0001$), IL-5 ($p<0.0001$), IL-4 ($p=0.0094$), IL-9 ($p=0.0016$), IL-2 ($p<0.0001$), and IL-27 ($p=0.0204$) in response to filarial antigen indicating a common infection-driven response. When comparing mf positive (mf+) and mf negative (mf-) patients, there were no significant differences in spontaneous cytokine production nor in parasite driven IL-10, IL-13, IL-5, IL-4, IL-9, Il-2, IL-22, IL-28a, or IL-27 production. In marked contrast, mf+ individuals had significantly increased filarial antigen-driven IL-24 (9.2-fold increase vs. 1.2-fold, $p=0.026$), and IL-19 (2.3-fold increase vs. 1.3-fold increase, $p=0.0497$) compared to mf- subjects. There were no differences in the IL-24 and IL-19 responses to non-parasite antigens and to mitogen between mf+ and mf- patients. These data provide an important link between IL-10 and its related family members IL-19 and IL-24 in the modulation of the immune response in human filarial infections that appears to be driven by microfilaria.

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ABSENCE OF S100A9 INCREASES INFLAMMATORY IMMUNE RESPONSES AGAINST *LITOMOSOIDES SIGMODONTIS* L3 LARVAE AND IMPAIRS LARVAL MIGRATION

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In the *Litomosoides sigmodontis* (*L.s.*) mouse model of filariasis, infective L3 larvae migrate from the site of infection within the skin via the lymphatics and the pulmonary capillaries to the thoracic cavity, where the filariae reside. We previously demonstrated that neutrophils mediate protective immune responses within the skin against invading L3 larvae and contribute to L3-induced lung pathology. S100A9 is a damage-associated protein, which is highly expressed by neutrophils and increasingly found in the lung during the acute phase of *L.s.* infection. In the present study we investigated the impact of S100A9 on *L.s.* infection in S100A9$^{-/-}$ C57BL/6 mice. Following natural infection with *L.s.,* S100A9$^{-/-}$ mice had a significantly reduced worm burden at 12 days post infection that was still observed following subcutaneous infection, which circumvents the first barrier, the skin. The reduced worm burden in S100A9$^{-/-}$ mice correlated with increased frequencies of neutrophils, macrophages and eosinophils as well as increased levels of CXCL1, CXCL2, CXCL5 and neutrophil elastase in the bronchoalveolar and thoracic cavity lavage indicating a pronounced inflammatory response. Furthermore, neutrophils of S100A9$^{-/-}$ mice showed an increased *in vitro* and *ex vivo* activation compared to neutrophils of wild type animals. Pulmonary neutrophils were essential for the protective effect seen in S100A9$^{-/-}$ mice, as depletion of neutrophils by intranasal administration of anti-Ly6G antibodies significantly increased the worm recovery in S100A9$^{-/-}$ mice. Similarly, upon intravenous injection of L3 larvae, S100A9$^{-/-}$ mice had an increased worm burden, suggesting that the simultaneous entrance of the L3 larvae from the pulmonary capillaries into the thoracic cavity reduces inflammatory responses L3 larvae encounter overcoming the protective effects otherwise observed in S100A9$^{-/-}$ mice. These results suggest that S100A9 inhibits L3-induced inflammatory responses in the bronchoalveolar lavage and thoracic cavity, reducing chemokine production and granulocyte recruitment as well as neutrophil activation, facilitating larval migration.

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EVALUATING INTESTINAL PROTEINS OF ADULT FILARIAL WORMS AS POTENTIAL VACCINE AND DRUG TARGETS

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The goal of this study is to identify drug and vaccine targets for filarial diseases such as lymphatic filariasis and onchocerciasis. We conducted proteomic analyses of the *Brugia malayi* adult worm intestinal tract and identified transmembrane intestinal proteins with high homology to other filarial worms, low homology to humans, and a large predicted luminal surface. We hypothesize that such "hidden" antigens would not naturally induce IgE-specific responses in endemic populations but would still be accessible to antibodies ingested by the filarial worms. Using fluorescent microscopy, we observed ingestion of cy3-labeled polyclonal mouse IgG antibodies by filarial adult worms into their intestinal tract. Potential vaccine and drug targets were identified from 9 pre-selected intestinal antigens by observing which proteins were essential for worm survival. After siRNA knockdown of these proteins, adult worms were monitored *in vitro* for motility, microfilaria (mf) release, and metabolism by MTT reduction over 6 days. Knockdowns were confirmed by quantitative PCR and, for proteins showing a robust phenotype, Western blot. Of the 9 candidate proteins, we found two that are essential for worm survival, a UDP-glucoronosyl transferase (UGT) and an IgI-set domain containing protein (IgI-DCP). Knockdown of each protein individually caused a 75% reduction in motility by day 1 and over 70% decrease in mf release by day 6. Additionally, use of FDA-approved commercially available UGT inhibitors *in vitro* resulted in adult worm death. We then performed luciferase immunoprecipitation system assays on serum samples from filarial patients (n=30) and did not detect IgE antibodies against these antigens. These studies have identified two intestinal tract proteins essential for adult filarial worm survival that could be targeted by antibodies but do not naturally induce IgE in infected individuals. Furthermore, demonstration of adult worm killing *in vitro* by currently available medications suggests that UGT is potentially a novel drug target.

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A HUMAN 3D CELL-BASED PLATFORM FOR DRUG DISCOVERY TARGETING *PLASMODIUM* HEPATIC INFECTION

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The liver stage of *Plasmodium* infection is the first step of the parasite's life cycle in the mammalian host and obligatorily precedes the pathological blood stage of infection. Moreover, the liver constitutes the reservoir of dormant forms of *P. vivax* or *P. ovale*, termed hypnozoites, which are responsible for disease relapse in humans. Therefore, hepatic stages of *Plasmodium* infection is an important target to prevent disease, stop malaria transmission, as well as to achieve radical cure. Currently, few of the available therapeutics target the hepatic stage of infection and several have been associated with development of resistance. Furthermore, primaquine, the only marketed drug with activity against hypnozoites once metabolically activated, cannot be widely distributed due to haemolytic effects on 6GPD-deficient individuals, a common trait in malaria endemic areas. These hurdles highlight the demand for *in vitro* models that closely recapitulate the *Plasmodium* hepatic infection and human drug metabolism. Here, we present a 3D human cell-based platform suitable for the discovery of novel drugs targeting *Plasmodium* hepatic infection. The platform relies on human hepatic spheroids generated in bioreactors that sustain crucial hepatic features, such as the cell polarity and long-term metabolic performance. We established and optimized the *Plasmodium* infection procedure in 3D cultures, and extensively characterized the dynamics of infection in this system. The data demonstrates that *P. berghei* infects and develops in the hepatic spheroids, completely maturating into blood-infecting merozoites that effectively infected blood cells *in vivo*. The 3D hepatic infection platform was validated by assessing the activity of antiplasmodial drugs such as atovaquone. Moreover, the long-term 3D hepatic cultures represent a promising tool to target hypnozoite-forming *Plasmodium* parasites. Besides their usefulness for drug discovery, such models could further be applied to unveil aspects of the fundamental biology underlying *Plasmodium* invasion and development in hepatic cells.

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PROTEASOME INHIBITOR-BASED COMBINATION THERAPY POTENTLY INHIBITS ARTEMISININ-RESISTANT *PLASMODIUM FALCIPARUM*

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Malaria remains a significant global health problem. In 2016, there were 216 million cases and ~0.5 million malaria-related deaths. The WHO recommends artemisinin-based combination therapy (ACT) as first line treatment for uncomplicated falciparum malaria. Alarmingly, artemisinin resistance (ART-R) has arisen in Southeast Asia (SEA) and South America and threatens to spread to Africa. Mutations in the propeller domain of Kelch 13 (K13) correlate with ART-R in field isolates, and have been confirmed to confer resistance by *in vitro* gene editing. Compounding the situation, resistance to the ACT partner drug piperaquine is now arising in SEA. Novel drugs that are uncompromised by existing drug resistance are urgently needed. The parasite proteasome is vital for parasite proliferation, and the *P. falciparum*-specific proteasome inhibitors WLL and WLW were recently generated. We sought to determine suitable compounds to be used in combination with WLL/WLW and to characterize WLL/WLW resistance traits. Using K13-isogenic Cambodian parasites that are ART-S or ART-R, we performed isobolograms using WLL/WLW in combination with antimalarials that have distinct targets. We found that regardless of K13 status, WLL and WLW are synergistic with DHA, OZ439, and the deubiquitinase inhibitor b-AP15, and antagonistic with protein translation inhibitors and the 4-aminoquinolines chloroquine and piperaquine. Low-level *in vitro* resistance to WLL and WLW is mediated by mutations in proteasomal subunits. *In silico* modeling revealed that mutations in the β_2 and β_5 subunits are close to inhibitor binding sites. Importantly, WLL-resistant lines retain sensitivity to WLW, and vice versa. This was demonstrated by dose-response curves and a fluorogenic activity-based probe to the proteasome catalytic subunits. Altogether, the data show that WLL and WLW are promising drug candidates and that several antimalarials synergize with parasite-selective proteasome inhibitors. Importantly, these drug combinations potently inhibit ART-R *P. falciparum*.

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ANTIMALARIAL PYRROLIDINAMIDES FAST-ACTING COMPOUNDS WITH A NOVEL MODE OF ACTION

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Malaria continues to be a major global disease still causing impermissible number of deaths. The effectiveness of current antimalarial therapy is under continuous threat through the spread of resistance. Last reports suggest that even the latest class of antimalarials, artemisinins, are also being affected by resistance. Consequently, there is an urgent need to identify novel therapies that overcome resistance issues and offer significant advantages over the current standard of care. Within the TCAM set of phenotypic hits identified at GSK, a pyrrolidinamide chemical series was selected because its chemical novelty offering exciting properties as antimalarial. Pyrrolidinamides exhibit potent *in vitro* activity against asexual stages of both sensitive and multi-drug resistant (MDR) *Plasmodium falciparum* strains including fresh clinical isolates. This chemical series has demonstrated potent oral efficacy in the *P. falciparum* humanised mouse model. Noteworthy, series displays a rate of killing comparable or even superior to that from endoperoxides. Furthermore, pyrrolidinamides display a new mode of action (MoA) based on functional assays and a lack of cross-resistance against a panel of strains with mutations producing resistance to known antimalarial drugs. Parasites exposed to pyrrolidinamides select for resistant mutations that target the acyl CoA synthetase (ACS) pathway. This putative new MoA that shows an outstanding fast rate of killing offers differentiation against all other antimalarials. In this work we will show a detailed parasitological profile of this novel antimalarial series and the promising properties that could be of paramount importance for new combination treatments.

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A RANDOMIZED CONTROLLED TRIAL COMPARING PARASITE CLEARANCE PROFILES AFTER SINGLE DOSE ARTESUNATE AMONG SUBJECTS EXPERIMENTALLY INFECTED WITH ARTEMISININ-RESISTANT OR ARTEMISININ-SENSITIVE *PLASMODIUM FALCIPARUM* PARASITES

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Since its first recognition in 2008, artemisinin resistance has spread across the Greater Mekong Subregion and poses an important threat to malaria control. Novel treatment strategies effective against artemisinin-resistant infections are urgently needed. We recently established an induced blood stage malaria (IBSM) model in which subjects are inoculated with a *P. falciparum* artemisinin-resistant *kelch13* parasites (K13 isolate) carrying the R539T mutation. This model was established to allow investigation and prioritization of novel drugs effective against artemisinin-resistant parasites. The aim of this study was to validate this model by comparing the parasite clearance profiles of K13 to an artemisinin-sensitive isolate (3D7). Healthy subjects were randomized and intravenously inoculated with ~2,800 parasite-infected erythrocytes, either K13 (Day 0, n=10) or 3D7 (Day 1, n=6). Parasitemia was measured by quantitative PCR targeting the *P. falciparum* 18S rRNA gene. On Day 9, all subjects received a single oral dose of artesunate (~2 mg/kg). Recrudescent parasitemia was treated with 960 mg piperaquine. To ensure cure, subjects were treated with atovaquone/proguanil and primaquine before the end of the study. K13 parasites took significantly longer to clear after artesunate treatment: the parasite clearance half-life of K13 was 6.51h (95% CI 6.23-6.82) compared to 2.85h for 3D7 (95% CI 2.68-3.03; $p<0.001$). Adverse events (179) reported were mostly mild (77%; 138/179) and attributed to malaria (74%; 133/179). Two adverse events (1.1%) were attributed to drug treatment (artesunate, n=1; atovaquone/proguanil, n=1). No serious adverse events were reported. Rapid early recrudescence occurred in all K13 subjects (within 48 hrs of treatment), while several days of static low level parasitemia, suggestive of dormancy was observed among 3D7 subjects before recrudescence occurred. These results concur with observations in field studies. This new K13 IBSM model can be used to determine the activity of antimalarial treatments against artemisinin-resistant parasites.

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SJ733, AN ORAL, INHIBITOR OF PFATP4 COMBINED WITH A PHARMACOKINETIC ENHANCER (CYP3A INHIBITOR): A NOVEL APPROACH IN ANTIMALARIAL DRUG DEVELOPMENT

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SJ773 is a novel, orally available inhibitor of *P. falciparum* ATP4 (PfATP4; a critical sodium-proton antiporter in the parasite), and the second in class to enter clinical development. In Phase 1 studies to date, SJ733 demonstrated favourable safety profiles and rapid antiparasitic effect but insufficient duration of antimalarial effect with a single dose. Therefore, the novel approach of inhibiting CYP3A, the dominant enzyme metabolizing SJ733, to increase drug exposure was considered. Specifically, three additional fasting dose cohorts combining SJ733 (75, 300 or 600 mg single dose) with a single dose of the known CYP3A4 inhibitor (pharmacokinetic enhancer or booster) cobicistat (150 mg dose, currently FDA-approved to boost certain antiretrovirals medications), were evaluated in an ongoing Phase 1a First-In-Human Safety and Pharmacokinetic (PK) adult healthy volunteers study (ClinicalTrials.gov: NCT02661373). All 18 male subjects in the cobicistat boosted dose cohorts tolerated SJ733 co-dosed with cobicistat well with no serious adverse events, dose limiting toxicities, Grade 3 or 4 adverse events (AE), or clinically significant ECG/safety laboratory tests findings. All reported AEs were Grade 1, clinically not significant and considered unlikely or unrelated to SJ733. Compared to the equivalently dosed unboosted cohorts, the SJ733 cobicistat-boosted cohorts showed an overall median increase in area under the concentration curve (AUC), maximum concentration (Cmax), and duration above a drug-exposure threshold of 3.7X, 2.5X, 2X respectively and a median, decrease in SJ733 clearance and major N-oxide metabolite SJ506 to parent drug ratio of 3.8X and 4.6X, respectively. In summary, this novel pharmacoboost approach demonstrates a significant increase in drug exposure and duration of a promising new antimalarial SJ733 with no drug toxicity signals noted. This supports further drug development of SJ733 and serves as the first example of a pharmacoboost approach for an antimalarial.

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KAF156 AND LUM-SDF COMBINATION PHASE 2 STUDY PHARMACOKINETIC RUN-IN COHORT RESULTS

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The safety and efficacy of different doses of the combination of KAF156 and Lumefantrine-Solid Dispersion Formulation (LUM-SDF) in patients with acute malaria are currently explored in the Phase 2 clinical study NCT03167242. The protocol for this study includes a PK run-in to exclude a potential pharmacokinetic interaction between the two drugs and the risk of overexposure. In this first part of the study, the pharmacokinetics and safety of the KAF156/LUM-SDF combination was evaluated to understand the impact of LUM-SDF on KAF156 (victim drug) exposure. In the scenario of an increase in KAF156 exposure beyond the acceptable limit, doses in Part A of study NCT03167242 would be adjusted as pre-specified in the protocol. Six male and six female Malian adult/adolescent patients (≥12 years old and ≥35.0 kg) infected with *P. falciparum* were enrolled in Bougoula-Hameau in the PK run-in and given a single dose of 200 mg KAF156 and 960 mg LUM-SDF. The drug-exposure results of these patients confirmed that there is no increase in KAF156 exposure beyond protocol-defined limits when given in combination with LUM-SDF in humans. The combination was well tolerated, and all 12 patients were clear of the initial malaria infection by Day 3 and remained parasite-free at Day 28. Consequently, dosing in study NCT03167242 is proceeding as planned, without any adaptation of the dose of KAF156.

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PHARMACOGENETICS OF THE IVERMAL TRIAL: HUMAN METABOLIC GENES AND MOSQUITO MORTALITY RESPONSE TO HIGH-DOSE IVERMECTIN CO-ADMINISTERED WITH DIHYDROARTEMISININ-PIPERAQUINE

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High-dose ivermectin, co-administered for 3-days with dihydroartemisinin-piperaquine (DP), killed mosquitoes feeding on individuals for at least 28-days post-treatment in a recent trial. Further pharmacokinetic-pharmacodynamic (PK-PD) analysis showed that this prolonged effect-duration could be explained by ivermectin alone, without invoking an unidentified mosquitocidal metabolite or drug-drug-interaction. Ivermectin and piperaquine are both primarily metabolized by *CYP3A4*. The current pharmacogenetic analysis aimed to assess to which degree the inter-individual variability observed for both ivermectin and piperaquine pharmacokinetics, pharmacodynamics, and related adverse events, can be attributed to human metabolism genetics by *CYP3A4* and other enzymes. In the main trial, 3-days ivermectin 0, 300, or 600 mcg/kg/day plus DP was randomly assigned to 141 adults with uncomplicated malaria in Kenya. All participants were genotyped for a total 67 single nucleotide variations (SNV; including five *CYP3A4/5* and three *ABCB1* SNVs) using Taqman allelic discrimination assays. During 28-days follow-up, 1,393 venous and 335 paired capillary plasma samples, 850 mosquito-cluster mortality rates, and 524 QTcF-intervals were collected. Using population modeling, PK and PD parameters were obtained for all dosed participants: ivermectin (n=95) and piperaquine (n=141). The incidence of related adverse events were: 5/45 (11%), 2/48 (4%), and 0/46 (0%) with 600, 300, and 0 mcg/kg/day. Genotypes were correlated with PK-PD parameters and related adverse events. Ivermectin clearance normalized by bodyweight (mean±SD; L/h) differed by *CYP3A4* genotype: *1/*1 (0.0109±0.0035), *1/*1B (0.0091±0.0029), *1B/*1B (0.0125±0.0026) (One-way ANOVA: $F(2,91)=4.14$, $p=0.019$). Similar analysis for piperaquine showed no difference ($F(2,137)=0.10$, $p=0.90$). *CYP3A4* genotype was not associated with adverse events. *CYP3A4* genotype was associated with ivermectin clearance, when co-administered with DP, however there was no association between *CYP3A4* genotype and piperaquine clearance.

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SEVERE MALARIAL ANEMIA AND IN-HOSPITAL MORTALITY IN ZAMBIAN CHILDREN WITH AND WITHOUT BLOOD TRANSFUSION

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Severe malaria is the leading parasitic cause of death, and severe malarial anemia, defined as a hemoglobin (Hb) concentration ≤ 5 g/dl, is the most common clinical presentation. In resource-limited settings where blood stockouts present a challenge to clinical care, distinguishing patients who are most likely to benefit from transfusion can help inform allocation when inventories are scarce. We present preliminary results of a cross-sectional study of hospitalized children (n=329) with severe malarial anemia in a high transmission area of northern Zambia to investigate associations among Hb concentration, blood transfusion, and mortality. Data are from January 2017 to January 2018, with collection ongoing. The median age was 22 mos. (IQR: 12-31 mos.) and 49% were girls. The case fatality ratio was 14%. According to hospital protocol, blood transfusion was indicated for all children. However, due to blood product stockouts, 38 children (12%) did not undergo transfusion. To investigate the interaction between Hb concentration and the survival benefit of blood transfusion, we stratified children by level of anemia (severe: >3 to 5 g/dl, profound: ≤ 3 g/dl). In a preliminary effect-measure modification analysis, children in both strata had similarly increased odds of death if no transfusion was given relative to receiving at least one transfusion (summary OR: 2.84, 95% CI: 1.21-6.68, P=0.016). Previous reports suggest that sub-stratification by degree of anemia might identify patients who are most likely to benefit from transfusion. In contrast, our preliminary analysis shows that children with profound (Hb ≤ 3 g/dl) and severe (Hb >3 to 5 g/dl) malarial anemia gained a similar survival benefit from blood transfusion. Our early results indicate that during periods of looming blood product stockouts, children with malarial anemia should be equally prioritized for blood transfusion regardless of the profundity of anemia.

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PROFILING MALARIA HIGH-RISK GROUPS IN ACEH PROVINCE, INDONESIA: A CASE-CONTROL STUDY

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Malaria transmission in Aceh Province, Indonesia, has been linked to forest activities in young adult males, whose occupations and behaviors increase their exposure to outdoor-biting and forest mosquito vectors. A case-control study was carried out between April 2017 and September 2018 in Aceh Besar and Aceh Jaya districts to evaluate spatial clustering of cases and quantify specific risk factors. All malaria cases identified at health facilities in four subdistricts and confirmed by microscopy, rapid diagnostic test or loop mediated isothermal amplification (LAMP) were included in the study. Two control groups were recruited, one from health facilities and one from neighborhoods of cases. The first control group included febrile patients aged 15 years or older testing negative for

malaria by rapid diagnostic test or microscopy at selected health facilities and recruited in proportion to the expected gender distribution of cases. Community controls were selected from microscopy-negative participants surveyed during malaria screening in neighborhoods of index cases and individually matched to incident cases by neighborhood, age category and gender. All participants were interviewed about potential environmental, socio-demographic, intervention, behavioral and forest-related exposures. As of April 2018, 41 incident malaria cases, 358 health facility controls and 152 neighborhood controls had been recruited into the study. Cases represented all three *Plasmodium* species (*P. falciparum*, *P. vivax* and *P. knowlesi*), were predominantly male (95%), and the majority (78%) aged between 30 and 60 years. Preliminary results suggest that malaria cases were more likely to have a second residence in the forest/forest-fringe and have worked in forest/forest-fringe areas in the past 30 days. Trip duration was positively associated with the odds of malaria, as was reported presence of long-tailed macaques around the primary household. Final results describing spatial patterns of malaria risk and the use of latent class analyses to identify species-specific risk profiles will be presented.

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WHY SOME CHILDREN WITH UNCOMPLICATED MALARIA PROGRESSED TO SEVERE MALARIA IN UGANDA

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We studied associations between social and host biological factors and risk of severe malaria in Ugandan children. We enrolled 325 severe malaria cases and 325 uncomplicated malaria controls matched by age and residence in Jinja District. Patient details and an itinerary of events in response to illness were captured. Caregivers of children with severe malaria were interviewed to explore behavioral factors contributing to delayed care seeking. Conditional logistic regression was used to determine risk factors for severe malaria and delayed care seeking. Independent risk factors for severe malaria included: 1) delayed care seeking (> 24 hours after fever onset; OR 5.88; 95% CI 2.75, 12.5); 2) seeking care at a drug shop as the initial response to illness (OR 3.37; 95% CI 1.66, 6.83); and 3) distance from place of residence to the nearest health center (OR 1.52; 95% CI 1.21, 1.91). Seeking care at a drug shop (OR 3.85, 95% CI 1.19, 12.3) and increasing distance to the nearest health center (OR 1.93, 95%CI 1.21, 3.08) were independent risk factors for delayed care seeking. Drug shops were the most common type of provider (31.6%) initially sought by caregivers of children with severe malaria. However, compared to public health facilities, drug shops offered sub-optimal services, with children less likely to have been examined (46.1% vs. 80.0%; p< 0.001) or referred to another facility (14.6% vs. 68.9%; p<0.001), based on reports from caregivers. Caregivers reported drug shops as convenient, but unable to manage severe illness, contributing to delay. Of host biological factors studied, Hemoglobin S heterozygotes (HbAS; OR 0.48; 95%CI 0.24, 0.96, p=0.038) and alpha thalassemia heterozygotes (_α/αα; OR 0.54; 95% CI 0.36, 0.82, p=0.004) and homozygotes (OR 0.47; 95% CI 0.30, 0.74, p=0.001) were protected against severe malaria. A significant (p=0.05) epistatic effect was noted between _α/αα and HbAS, such that protection was diminished when both mutations were present. Determinants of severe malaria were complex. Key among these was delay in care seeking. Seeking care at drug shops appears to contribute to delay and severe malaria.

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EVALUATION OF MALARIA RECURRENCE IN BRAZIL

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In vivax endemic areas, malaria control must tackle relapses. Although a surveillance system records every single malaria case in Brazil, it does not provide accurate information on recurrences. The aim of this study is to improve the use of the information from the Health Surveillance System, in order to shed light on the actual recurrence epidemiological profile and the effectiveness of the current treatment using chloroquine in combination with a short course of primaquine (7-9 days: total dose 3-4.2 mg/kg) in Brazil. In this observational retrospective open cohort, the data matching was done using bloom filters to deduplicate records of the National System of Malaria Surveillance from 1st July 2014 till 31st May 2015. The impact of baseline characteristics in the frequency of recurrences was tested using Generalized Estimation Equation (GEE). The effectiveness of the current treatment and the synergic effect of primaquine and chloroquine in the blood stage and its causal prophylactic effect were also tested using GEE. Prentice, Willians and Peterson model was used to evaluate the influence of population characteristics in time to recurrence. Among 153,203 malaria cases reported during this year, 24,528 recurrences were identified using the bloom filter matching strategy and the Dice threshold defined for this dataset. The models demonstrated a significant effect of the baseline characteristic and treatment in the relative risk of recurrence. The study demonstrates the feasibility of improving the use of Health Surveillance Systems data. An accurate picture of recurrence may provide evidence to refine the guidelines in Brazil. This study also shed light on the effectiveness of primaquine short course and the synergic effect of its concomitant use with chloroquine. Finally, these results may be used to guide future public health interventions to amplify their effect by targeting high-risk populations.

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IMPACT OF IMPROVED MALARIA CONTROL ON THE FORCE OF INFECTION OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* IN YOUNG PAPUA NEW GUINEAN CHILDREN

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With declining malaria transmission, reliable quantification of ongoing transmission is vital and becomes increasingly difficult using classical entomological measures. Molecular force of blood-stage infection ($_{mol}$FOB), a measure of exposure to new blood-stage infections, has been demonstrated to be a suitable proxy for measuring transmission but is yet to be applied to directly assess the impact of intensified control measures on *Plasmodium falciparum* and *P. vivax* transmission. Using three consecutive longitudinal child cohorts, we investigated the impact of improved control measures on the changing patterns of $_{mol}$FOB, incidence of clinical illness and the cumulative prevalence of infections and to gain insight into how these metrics perform for the two-main species, *P. falciparum* and *P. vivax*. The cohorts included children 1-5 years of age and were conducted before (2006), during (2009) and after scale up of control interventions. Incidence analyses was conducted using the number of clinical episodes and molecularly determined new blood-stage infections in each interval between active case detection (ACD) time-points when

the child was considered at risk. Prevalence analyses was conducted using longitudinal binary outcomes measuring the presence of infection which were measured at each ACD time-point and diagnosed by microscopy and PCR. After scale-up, an immediate reduction in *P. falciparum* $_{mol}$FOB and incidence of clinical episodes, as well as *P. vivax* clinical episodes was observed. There was a delayed impact on *P. vivax* $_{mol}$FOB. After 5 years of intensified control, there was a marked reduction in the incidence of new infections by 73% for *P. falciparum* and 87% for *P. vivax*, and the incidence of clinical episodes by 90% and 88%, respectively. We demonstrate the utility of $_{mol}$FOB in measuring the impact of control interventions on transmission for the first time observing marked differences for *P. falciparum* and *P. vivax.*

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MALARIA IN THE FIRST TRIMESTER OF PREGNANCY IS ASSOCIATED WITH NON-MALARIA FEVER DURING THE FIRST THREE MONTHS OF LIFE IN A BENINESE INFANT POPULATION

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Malaria in pregnancy (MiP) is responsible for maternal anemia, stillbirth, preterm birth and fetal growth restriction. While MiP has also been associated with a higher risk of malaria during infancy, few studies have evaluated its association with non-malaria fever. This study aims to assess the effect of MiP on the risk of non-malaria fever in infants from birth until 3 months of life, especially malaria in the 1st trimester of pregnancy, which is a period of high risk of malaria and of fetal and placental development. From 2014 to 2017, in southern Benin, women were followed from the pre-conception period until delivery (RECIPAL cohort) with monthly detection of malaria using thick blood smear (TBS). Then, a sub-sample of their newborns were followed up from birth until 3 months of age (SEPSIS cohort). Malaria in the 1st trimester of pregnancy was defined as the occurrence of at least one peripheral malaria infection before 15 weeks gestation (ultrasound data). In the child, a non-malaria fever episode was defined as the association of a temperature ≥37.5°C with a negative TBS (performed fortnightly, and anytime during follow-up in case of symptoms). A Poisson regression model was used to assess the effect of MiP in the 1st trimester on the number of child's non-malaria fever episodes. A total of 160 mother-child pairs were included in the analysis. The cumulative incidence of malaria in the 1st trimester of pregnancy was 15.1%. During the first 3 months of life, 20.6% of the children had at least one episode of non-malaria fever. They mainly consisted of moderate respiratory and gastrointestinal infections. After adjustment for potential maternal and infant confounding factors, malaria in the 1st trimester was statistically associated with a higher risk of non-malaria fever (adjusted incidence rate ratio=2.9; 95%CI: 1.5–5.5; p<0.01). Other factors associated with a higher risk of non-malaria fever were prematurity, maternal anemia and a low educational level. These results reinforce the need for the prevention of MiP, especially in early pregnancy, in order to reduce its consequences during infancy.

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MAJOR RESURGENCE OF MALARIA FOLLOWS PREVIOUSLY UNPRECEDENTED DECLINE IN PAPUA NEW GUINEA

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Malaria cases declined worldwide following increased investment in malaria control after the year 2000, but more recent data suggests that this decline has stalled. Papua New Guinea (PNG), long considered to harbor the highest malaria transmission outside of sub-Saharan Africa, made major progress in malaria control following the introduction of long-lasting insecticidal nets (LLIN) and the roll-out of artemisinin-based combination therapy (ACT). Malaria prevalence in the lowlands dropped from 11% in 2008-09 to <1% in 2013-14 and incidence of confirmed cases decreased. A country-wide Malaria Indicator Survey was conducted in 2016-17 to assess intervention coverage and prevalence of malaria in all age groups in comparison with data from previous national surveys (08-09, 10-11, 13-14). The survey was carried out in 102 villages including 2,743 households. A total of 11,358 blood samples were examined for malaria infection by light microscopy. Below 1600 m altitude, 7.1% of the population was infected with malaria parasites, up from 0.9% in 2013-14. Sub-national heterogeneity in prevalence was high. In the Highlands, malaria infections were rare and only found in adults (0.9%), suggesting importation of infections rather than local transmission. Infections with *Plasmodium falciparum* were more common than *P. vivax*. Compared to 2013-14, LLIN ownership (80%) and use (51%) remained largely unchanged and in the malarious lowlands one million people still lack access to an LLIN in their household. Treatment from a health facility was sought for 41% of recent fevers and a diagnostic test was performed in 23% of cases. The first-line ACT was used by 85% of test-positive persons. Malaria control efforts in PNG have suffered a major setback in the last three years. Vector control coverage has plateaued, access to prompt diagnosis and appropriate treatment remains poor and investment in malaria control has decreased. The longer-term goal of malaria elimination from PNG by 2030 is under threat unless malaria control is re-intensified without delay, inclusive of the provision of sufficient funding for interventions and supporting operational research.

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THE EFFECT OF PHYSIOLOGICAL STATE ON POWASSAN VIRUS INFECTION OF SALIVARY GLAND CULTURES FROM FEMALE *IXODES SCAPULARIS* (BLACK-LEGGED TICK): A POTENTIAL ROLE FOR A PUTATIVE TICK TRANSCRIPT

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The tick, *Ixodes scapularis*, transmits a number of pathogens, including *Borrelia burgdorferi* and tick-borne flaviviruses (TBFVs). TBFV infections cause thousands of human encephalitis cases worldwide each year. In the US, confirmed human infections with Powassan virus (POWV) have a fatality rate of 10-30% and are increasing. The attenuated Langat virus (LGTV) is often used as a convenient experimental TBFV model.

The tick salivary glands (SGs) serve as the primary organ barrier to TBFV transmission. Recent optimization of TBFV growth in *ex vivo* tick organ cultures allows a deeper understanding of virus biology in viable organs. Information on host proteins and cellular processes involved in TBFV infection of ticks is limited. Transcript knockdown of a putative tick gene, H576, decreased LGTV infection of *I. scapularis* ISE6 embryonic cells, suggesting a potential role for this hypothetical gene. The goals of this study were to advance these findings by using SG cultures with the pathogenic POWV and to compare the following in SG cultures from blood-fed and unfed ticks: (1) viability, (2) infectious TBFV replication, and (3) effect of transcript knockdown of H576 on POWV infection. SGs from blood-fed ticks were viable for three days less than SGs from unfed ticks. Infectious TBFV replication was higher in SGs from blood-fed than SGs from unfed ticks. This suggested that the tick's physiological state can alter TBFV replication. Transcript knockdown of H576 decreased infectious replication in POWV-infected SG cultures from both blood-fed and unfed ticks. Future research may include similar studies in tick midgut cultures and in TBFV-infected ticks to determine effect on TBFV infection and transmission. This research helps to identify novel host proteins as potential targets for countermeasure development against TBFV infection and transmission. In addition, this system may prove useful as a translational tool for identifying potential tick transcripts/proteins that can be used as targets for TBFV therapeutics. This research was supported by the Intramural Research Program of the NIH, NIAID.

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THE ROLE OF DEER TICK SALIVARY MICRORNAS IN REGULATING POWASSAN VIRUS INFECTION

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Powassan virus (POWV) is a North American tick-borne flavivirus that is the causative agent of a severe neuroinvasive illness. POWV is transmitted to humans by infected ticks, and transmission of POWV can occur within three hours of *Ixodes scapularis* attachment. Successful tick feeding is facilitated by an assortment of bioactive salivary factors which are secreted into the feeding pool that the tick creates on the vertebrate host. It was previously shown that *I. scapularis* saliva facilitates POWV infection and influences the disease outcome for mice inoculated with a low dose of POWV; however, the specific tick salivary components responsible for enhancing POWV transmission have yet to be identified. The objective of this research is to identify and characterize exogenous *I. scapularis* salivary microRNAs (miRNAs) and their potential role in POWV infection. Distinct salivary gland miRNA expression profiles are related to tick blood feeding, and this research will be the first to evaluate specific tick salivary miRNAs for their role in regulating the host immune response and establishment of a pathogen. We hypothesize that the exogenous miRNAs in POWV-infected *I. scapularis* salivary glands regulate the host immune response to create an immunologically privileged microenvironment that facilitates the establishment of POWV infection. For the present study, we fed POWV-infected and uninfected *I. scapularis* females on naïve mice for 1, 3, and 6 hours (1 tick per mouse, n=6), and dissected the tick salivary glands at each time point. miRNAs were extracted from each tick salivary gland and submitted for next generation sequencing (NGS). After aligning the NGS reads from the 6 hour fed *I. scapularis* salivary glands to the *I. scapularis* miRNA database, 40 distinct known miRNAs were identified in both uninfected and POWV-infected tick salivary glands. NGS analysis is currently underway to identify known and novel exogenous salivary miRNAs that are differentially expressed upon POWV infection. Select *I. scapularis* salivary miRNAs will be examined for their potential to enhance POWV replication and the host immunologic response *in vitro*.

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RISK FACTOR COMPARISON OF HUMAN-TICK ENCOUNTERS BETWEEN LYME DISEASE ENDEMIC AREAS OF THE NORTHEAST AND MIDWEST UNITED STATES

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Disease dynamics and prevention strategies are often extrapolated beyond the area, state or country studied in an effort to find a one size fits all control method. However, the dynamics of zoonotic vector-borne diseases are determined by a complex set of parameters including human behavior that may vary within socio-ecological contexts. In the case of Lyme disease, anthropogenic factors affect the enzootic transmission cycle of *Borrelia burgdorferi* and the populations of its vector, *Ixodes scapularis*, including changes in land use and habitat, which in turn affect the likelihood of tick-human contact and human infection. Here we compare the behavioral risk factors associated with human-tick encounters between highly endemic areas for tick-borne diseases in the Northeast and Midwest of the United States, with different landscape configurations, and compare the relative importance of peridomestic versus recreational risk between both regions. We also hypothesize that human behavior will vary between the two areas, especially in regards to self-protection and interactions with animal hosts; for example, practicing rodent control and implementing deer deterrents versus attracting small mammal reservoirs and baiting deer. Using a novel smartphone application, The Tick App, as a survey tool, we collected fine temporal and spatial data regarding daily outdoor activities, tick encounters and protection methods. During spring and summer of 2018, The Tick App was made available to the public, and implemented in two communities in each region, where participants were actively recruited to use the application in combination with an acarological assessment of their backyards. Within each region, the selected sites vary in levels of Lyme and babesiosis endemicity from emerging to endemic, demographics, and land cover/land use, from urban (Staten Island, NY) to rural. The comparison of environmental and human risk factors for encountering ticks, and by extension tick-borne diseases, between two highly endemic regions will help inform regionally tailored prevention tool development and public health messaging.

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ECOLOGICAL FACTORS DRIVING THE EMERGENCE OF BABESIOSIS IN THE UNITED STATES: THE ROLE OF CONFECTION AND ALTERNATIVE TRANSMISSION PATHWAYS

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Babesiosis continues to increase in incidence as well as expand geographically on the heels of the Lyme disease epidemic. The incidence of babesiosis is significantly lower than that of Lyme disease (~1,700 cases/year compared to ~30,000 Lyme disease cases/year). However, increased infection of the shared blacklegged tick vector with *Babesia microti* in some regions suggests future increases in human disease or the presence of significant underdiagnosis and underreporting. We hypothesize two non-exclusive mechanism could enhance *B. microti* transmission in the natural cycle: a positive effect of *B. burgdorferi* coinfection on *B. microti* in mice - as we previously demonstrated in a laboratory setting - or a 'cryptic' transmission pathway. To evaluate these alternatives, we conducted a 3-year (2004-16) longitudinal study at six sites in Connecticut and Block Island, RI. Between May and August each year, small mammals were trapped biweekly; 1,245 unique individuals were trapped. We a suite of used mixed effects models to inform spatio-temporal and

demographic covariate choice and multi-state markov models to infer interactions and transmission. We detected a potential facilitative effect between pathogens, mediated by demographic structure. In addition, the higher prevalence of *B. microti* suggested alternative, non-vector mediated transmission pathways, consistent with our previous detection of transplacental transmission of *B. microti*. Our results indicate both mechanisms are partially implicated in the increased tick infection with *B. microti* and consequent increased risk of babesiosis. At the regional level, these mechanisms may facilitate the invasion and persistence of *B. microti* in previously unoccupied locations or areas with reduced tick populations.

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COME RAIN OR COME SHINE. DIFFERENTIAL SPATIOTEMPORAL DYNAMICS OF SCRUB TYPHUS AND MURINE TYPHUS IN THE LAO PDR

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Scrub typhus and murine typhus are endemic to South and South East Asia and are a major causes of treatable febrile illness. In this study we analysed the annual patterns of incidence of scrub typhus and murine typhus in Lao PDR (Laos) and the spatiotemporal dynamics from reported patients. Serology and PCR data from consenting febrile patients admitted to Mahosot Hospital between 2004 and 2016 with suspected typhus were analysed with demographic, clinical, weather and geographical data. These data were then tested against equivalent data from three Lao provincial hospitals. Over the 12 year period, 820 scrub typhus and 559 murine typhus patients were identified. The median days of fever was 7 days (range 0-60 days) and the median age was 30 years (range 1.8- 80 years). The proportion of scrub typhus patients with an eschar identified was 19%. The majority of patients came from Vientiane Capital Province (71%) followed by Vientiane Province (16%). There was a peak in number of patients with murine typhus in 2009 and 2010 while there was a dramatic increase in scrub typhus cases in 2011 from 56 cases in 2010 to 174 in 2011. Scrub typhus patient presentations peaked in the wet season months of July and August while murine typhus was most frequent in the hot, dry months of March to May. The full differential spatiotemporal results for both murine typhus and scrub typhus will be presented in relation to weather and habitat and tested against data from Lao provincial hospitals. This is the first study to look at the dynamics of geography and temporal conditions for the prevalence of scrub and murine typhus in Lao PDR. With increasing concern of the global spread of scrub typhus and of the focal importance of murine typhus, knowledge of temporal and spatial patterns will inform targeted public health interventions for environments and seasons of high burden.

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DIAGNOSIS OF SPOTTED FEVER GROUP RICKETTSIOSES IN UNITED STATES TRAVELERS RETURNING FROM AFRICA, 2007—2016

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Spotted fever group rickettsioses (SFGR), such as African tick bite fever (ATBF), are among the most commonly diagnosed diseases for ill travelers returning from southern Africa. We summarized demographic, clinical, and diagnostic features of imported SFGR cases in U.S. travelers returning from Africa that had laboratory specimens submitted to the Centers for Disease Control and Prevention. Diagnosis of SFGR was performed by indirect immunofluorescence antibody assay, immunohistochemical staining, PCR, or culture. Cases defined as probable SFGR, confirmed SFGR, or confirmed ATBF. Clinical and epidemiological categorical variables were described as counts and proportions; continuous variables were described using geometric mean titers (GMT), median, and range. 127 patients satisfied laboratory criteria for confirmed or probable SFGR. Fever was the most common symptom (n = 88; 69%), followed by ≥ 1 eschars (n = 70; 55%). Paired serums were submitted for 36 patients (28%); 12 patients (33%) had non-reactive initial serum sample, but converted to a titer ≥ 64 with the convalescent sample. 27 patients (21%) had infection with *R. africae* based on PCR analysis of eschar swab (n = 8) or biopsy (n = 23). 15 patients had eschar biopsy or swab samples and serum sample(s) submitted together; 9 (60%) had PCR-positive eschar results and non-reactive acute serology. Healthcare providers should consider SFGR when evaluating patients for a febrile illness with eschar and compatible foreign travel history. PCR testing of eschar biopsies or swabs provides a confirmed diagnosis in early stages of disease; eschar swabs or biopsies are an underutilized diagnostic technique.

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TRANSCRIPTIONAL RESPONSE OF THE RAT FLEA, *XENOPSYLLA CHEOPIS*, TO BLOOD FEEDING AND INFECTION WITH *YERSINIA PESTIS*

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Yersinia pestis, the causative agent of plague, is a highly lethal pathogen transmitted by the bite of infected fleas. *Xenopsylla cheopis*, the rat flea, is a vector of several zoonotic bacterial pathogens that in addition to *Y. pestis,* includes *Bartonella* spp. and *Rickettsia typhi*. Once ingested by a flea, these pathogens must overcome host immunity, establish a replicative niche in the gut, and alter gene expression to promote survival and transmission. However, the rat flea genome has not been sequenced and the nature and kinetics of the gut immune response to bacterial infection remains poorly characterized. To address this, groups of *X. cheopis* fleas were fed sterile Brown Norway rat blood, or rat blood containing $5x10^8$ CFU/ml *Y. pestis* KIM6+ using an artificial membrane feeding device. The midgut and proventriculus was dissected from 10 fleas from each group 4h after feeding, and from a third group of fleas that had not fed for 5 days. RNA was extracted from pooled digestive tracts and the transcriptional profiles were determined by next-generation RNA sequencing and *de novo* transcriptome assembly. Analysis of differentially expressed transcripts indicates that expression of antimicrobial peptides (attacin and coleoptericin), chitin-binding proteins (peritrophins), and a serine protease (chymotrypsin) is upregulated in digestive tract epithelial cells in response to infection with *Y. pestis*. Interestingly, fleas are not thought to produce a peritrophic matrix (PM); thus, the upregulation of peritrophin genes that are frequently integral to PM formation was unexpected. Upcoming studies will use histochemistry and confocal microscopy to image the *X. cheopis* midgut and determine whether a PM is produced. Cumulatively, the data indicate that the immune deficiency pathway (IMD) regulates early flea immune responses to infection. RT-qPCR studies are underway to determine expression kinetics of peritrophin and IMD-regulated transcripts during the first 24h of infection. Mechanistic understanding of flea gut immunity and physiology may lead to unique strategies to block transmission of flea-borne pathogens.

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WORMS AND WELLBEING: 15 YEAR ECONOMIC IMPACTS FROM KENYA

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Estimating the impact of child health investments on adult living standards faces several major methodological challenges, including the paucity of experimental interventions for which it is feasible to locate participants many years later, as well as difficulties in accurately measuring economic outcomes. This study exploits a randomized school health intervention that provided deworming treatment to Kenyan children. We estimate impacts on their living standards and wellbeing 15 years later, at which point the effective respondent tracking rate was 85%. In our main finding, we show that deworming beneficiaries experience a large and persistent 15% gain in total adult earnings. Treatment group individuals also have significantly higher total consumption expenditures, are more likely to live in urban areas, and are more likely to state that they are "very happy" in surveys. Given deworming's extremely low cost, a conservative estimate of deworming's annualized social internal rate of return is 42.5%.

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RISK FACTORS FOR INFECTION WITH SOIL TRANSMITTED HELMINTHS INTIMOR-LESTE: A LONGITUDINAL ANALYSIS DURING A COMMUNITY INTEGRATED WASH ANDDEWORMING INTERVENTION

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Ascaris spp., hookworm (*Necator americanus, Ancylostoma duodenale* and *A. ceylanicum*) and *Trichuris trichiura* are the most common soil transmitted helminths (STHs). Given their faecal-oral route of transmission and direct penetration of the skin by hookworm, STHs are common in poor communities. Factors previously shown to be associated with infection are poor sanitation, lack of water, deficient hygiene and low socio-economic index. In the WASH for WORMS cluster randomised controlled trial, 23 communities (clusters) received a deworming intervention consisting of albendazole administered to all eligible residents every 6 months for two years. Additionally, 11 intervention clusters received a WASH programme that included improving water access, increasing improved household sanitation, and promoting hand-washing with soap. We have previously published a cross-sectional analysis reporting on WASH risk factors associated with STH infection at trial baseline. Here we report the results of a WASH risk factor analysis including all four time-points of the RCT after intervention implementation, spanning a two-year period. Surprisingly, for *Ascaris* spp., no associations were found between STH infection and individual sanitation or hygiene variables nor household sanitation variables. Disposal of household garbage by digging or burying resulted in higher odds of infection. For school-aged children, using a toilet at school was associated with higher odds of infection. For *N. americanus*, practising open defecation at the previous time point was a risk factor, while wearing shoes indoors was protective and there were no significant associations with household or school sanitation variables. For both *Ascaris* spp. and *N. americanus*, using an unprotected spring or dugwell or surface water was associated with higher odds of infection, while using a water source more than 15 minutes away resulted in lower odds. This study adds to the body of evidence exploring the links between WASH related conditions and behaviours and the risk of STH infection.

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ULTRASENSITIVE DETECTION OF *TOXOCARA CANIS* EXCRETORY-SECRETORY ANTIGENS BY A NANOBODY ELECTROCHEMICAL MAGNETOSENSOR ASSAY

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Human Toxocariasis (HT) is a zoonotic disease caused by the migration of the larval stage of the roundworm *Toxocara canis* in the human host. Despite of being the most cosmopolitan helminthiasis worldwide, its diagnosis is elusive. Currently, the detection of specific immunoglobulins IgG against the *Toxocara* Excretory-Secretory Antigens (TES), combined with clinical and epidemiological criteria is the only strategy to diagnose HT. Cross-reactivity with other parasites and the inability to distinguish between past and active infections are the main limitations of this approach. Here, we present a sensitive and specific novel strategy to detect and quantify TES, aiming to identify active cases of HT. High specificity is achieved by making use of nanobodies (Nbs), recombinant single variable domain antibodies obtained from camelids, that due to their small molecular size (15kDa) can recognize hidden epitopes not accessible to conventional antibodies. High sensitivity is attained by the design of an electrochemical magnetosensor with an amperometric read-out with all components of the assay mixed in one single step. Through this strategy, 10-fold higher sensitivity than a conventional sandwich ELISA was achieved. The assay reached a limit of detection of 2 and15 pg/ml in PBST20 0.05% or serum, spiked with TES, respectively. These limits of detection are sufficient to detect clinically relevant toxocaral infections. Furthermore, our nanobodies showed no cross-reactivity with antigens from *Ascaris lumbricoides* or *Ascaris suum*. This is to our knowledge, the most sensitive method to detect and quantify TES so far, and has great potential to significantly improve diagnosis of HT. Moreover, the characteristics of our electrochemical assay are promising for the development of point of care diagnostic systems using nanobodies as a versatile and innovative alternative to antibodies. The next step will be the validation of the assay in clinical and epidemiological contexts.

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LONGITUDINAL CHANGES IN RISK AND INTENSITY OF INFECTION WITH SOIL-TRANSMITTED HELMINTHS AFTER COMMUNITY-WIDE MASS DRUG ADMINISTRATION IN RURAL MYANMAR

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Mass drug administration (MDA) targeted at school-aged children is the method recommended by the WHO for soil-transmitted helminth (STH) control in endemic countries. However, MDA alone does not prevent reinfection between treatments. In endemic countries that have been providing MDA for many years, such as Myanmar, the levels of reinfection will determine how effective MDA is in suppressing infection in the long-term. Longitudinal data from three STH epidemiology surveys, conducted between June 2015 and June 2016 in the delta region of Myanmar, were

analysed to determine the risks of STH infection in the whole community, including two MDA rounds. Risk ratios (RRs) were calculated to compare risks of infection with each STH between different time points. Mean change in eggs per gram of faeces (EPG) were calculated for each age group. Overall STH prevalence fell by 8.99% from the first to the last survey. However, EPG only significantly decreased for hookworm. RRs for any STH infection were 0.68 for survey 1 to 2 (four months reinfection) and 1.0 for survey 2 to 3 (six months reinfection). Age and sex stratified RRs for the four-month reinfection period were generally below one, whereas RRs for the six-month reinfection period were above one, indicating that more people were infected after six months reinfection post-MDA. Mean EPG generally decreased between the surveys in all age groups. However, *Ascaris lumbricoides* and *Trichuris trichiura* EPG increased between surveys 2 and 3 in the 5-14 age group. This analysis demonstrates that there is a significant increase in risk of STH reinfection after six months compared to four months. Whilst STH prevalence decreased over the course of the study, EPG did not significantly decrease for *A. lumbricoides* and *T. trichiura*. This potential increase in infection risk is important and requires further investigation, especially as MDA programmes are known to experience delays in treatment due to competing priorities, lack of staff/resources, delay in drug delivery, etc. MDA in Myanmar may need to be either expanded or the frequency increased to prevent reinfection and achieve sustainable reductions in STH infection.

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GLOBAL STATE OF INEQUITY IN DEWORMING COVERAGE IN LOW-INCOME AND MIDDLE-INCOME COUNTRIES: A SPATIOTEMPORAL STUDY OF HOUSEHOLD HEALTH SURVEYS

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Over the past 15 years, deworming programs for soil-transmitted helminths have scaled up across many low and middle-income countries. Deworming has become a major focus of global public health programs and is designed to be "pro-poor." This study quantifies inequities in receipt of deworming across and within countries over time to guide future policy and investment. We used nationally representative, geospatial survey data from pre-school age children (age 1-4 years) contained in the Demographic and Health Surveys from 2003-2016. Deworming was measured using the mother's report of whether her child received drugs for intestinal parasites in the previous 6 months. We used the mother's location to map geospatial variation in deworming coverage. We computed predictors of receipt of deworming, and assessed inequity by disaggregating deworming by wealth quintile and gender, computing a country-level wealth concentration index (measure of inequity), and examining trends across space and time. We included 372,050 pre-school children from 45 endemic countries. Of these, 46.5% were reported by their mothers to have received deworming during the previous 6 months. Deworming coverage varied by country, from 8.1% in Zimbabwe (2010) to 91.1% in Rwanda (2014), and varied substantially within countries and over time. Dewormed children were more likely to be older, wealthier, and have mothers that were older and more educated; rural residence was only weakly predictive of deworming. Globally, deworming increased monotonically with family wealth, ranging from 42.8% in the lowest quintile to 51.9% in the highest. We found a positive wealth concentration index (deworming concentrated in the wealthy) in 41 of 45 countries. Deworming equity improved over time in the majority of countries and deworming was equitable by gender. Although deworming pre-school age children is considered "pro-poor", these data suggest that, in most countries, deworming coverage is lowest among the poorest children. These inequities appear to be improving over time and we found no evidence of gender-related inequity. This study can be used to monitor deworming equity over time.

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MIGRATION AND LOCAL MOVEMENT CAN IMPEDE ELIMINATION EFFORTS OF SOIL-TRANSMITTED HELMINTHS (STH) BY MASS DRUG ADMINISTRATION (MDA)

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Soil-transmitted helminth (STH) infections affect predominantly socio-economically disadvantaged populations in sub-Saharan Africa, East Asia and the Americas. One of the WHO 2020 goals is the elimination of STH as a public health problem, defined as reducing the prevalence of medium-to-heavy STH infection among school-aged children (SAC) to less than 1%. WHO guidelines recommend 75% coverage among pre-SAC and SAC to achieve this goal. Previous epidemiological studies, in part based on mathematical models, have evaluated optimal intervention strategies of mass drug administration (MDA) to clusters of villages. These studies assumed that villages are closed independent units with no movement of people in or out of communities. Here we examine how local and regional migration, for example, of seasonal migrant labourers, and local movement among villages within the same regional cluster affect the outcome of MDA programmes. We derive migration rates from the Tumikia trial dataset that includes records of the movements of individuals and GPS mapping of households in the region. We show that even if on average 80% of the entire resident population within a village cluster are treated, an inward migration rate of defined sizes of infected individuals per treatment cycle can prevent STH elimination in this cluster. We detail calculations of these migration rates that prohibit elimination of transmission for low, medium and high transmission settings. We also show that local movements, for example, children's daily trips to school, can reduce the effectiveness of MDA programmes if schools are located outside of treated clusters or represent transmission hotspots with high amounts of contaminated infectious material in the environment within treated clusters. In general, if STH prevalences in villages within a regional treatment cluster are very heterogeneous, frequent movements among villages within a cluster may postpone the time when elimination is reached during MDA programmes. We discuss what measures can mitigate these migration inputs that can sustaining transmission.

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HLA-G EXPRESSION DURING HOOKWORM INFECTION IN PREGNANT WOMEN

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HLA-G plays a key role on immune tolerance. Pathogens can induce soluble HLA-G (sHLA-G) production to down-regulate the host immune response, creating a tolerogenic environment favorablefor their dissemination. To our knowledge, no study has yet been conducted to assess the relationship between sHLA-G and geohelminth infections. The study was conducted in Allada, Southeastern Benin, from 2011-2014. The study population encompassed 400 pregnant women, included before the end of the 28th weekof gestation and followed-up until delivery. At two antenatal care visits and at delivery, stool and blood samples were collected. Helminths were diagnosed by means of the Kato-Katz concentration technique. We used quantile regression to analyze the association between helminth infections and sHLA-G levels during pregnancy. sHLA-G levels gradually increased during pregnancy and reachedmaximal levels at delivery. Prevalence of helminth infections was low, with a majority of hookworm infections. We found significantly more hookworm-infected women above the 80th quantile (Q80) of the distribution of the mean sHLA-G level ($p<0.03$, multivariate quantile regression). Considering only women above the Q80 percentile, the mean sHLA-G level was significantly higher in hookworm-infected compared to uninfected women ($p=0.04$). In our study, we found that high levels of sHLA-G were associated with hookworm infection in pregnant women. This result is consistent with the potential involvement of sHLA-G in immune tolerance induced by helminths during pregnancy.

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THE LIVE ATTENUATED TETRAVALENT DENGUE VACCINE TV003 AFFORDS EARLY PROTECTION AGAINST DENV-2 AND DENV-3

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Over the past several decades dengue has become hyper-endemic in all tropical and sub-tropical regions of the world. Current estimates report nearly 100 million symptomatic and 300 million asymptomatic dengue infections annually. We have evaluated the live attenuated tetravalent (LATV) candidate dengue vaccines TV003 and TV005 in hundreds of dengue-naïve and dengue-exposed individuals in the United States, Thailand, and Bangladesh. A single dose of either vaccine was 100% efficacious against challenge with DENV-2 administered 6 months after vaccination in a controlled human infection model (CHIM). TV003 is currently in Phase 3 clinical trial enrolling 17,000 participants in Brazil. In the current clinical trial, we sought to evaluate the early efficacy of TV003 against DENV-2 and DENV-3 using our dengue CHIM. We enrolled 60 subjects in a randomized, double-blind, placebo-controlled trial. At study day 0, 40 subjects received TV003 and 20 subjects received placebo. At study day 28, 30 subjects received 10^3 PFU of DENV-2 and 30 subjects received 10^3 PFU DENV-3. Subjects were evaluated approximately every other day in the clinic through Day 16 post-vaccination and then on Study days 21 and 28 (Day of challenge). The returned to the clinic approximately every other day through day 16 post-challenge and 21, 28, 56, 90, and 180 days post-challenged. Blood was drawn for clinical laboratory assays and for the recovery of vaccine or challenge virus through Study day 21 post-vaccination and post-challenge. Blood was drawn for immunology and several time-points post-vaccination and post-challenge. The primary efficacy endpoint was protection against viremia induced by either DENV-2 or DENV-3. Secondary endpoints included protection against rash and neutropenia. Clinical, virologic, and serologic data post-vaccination and post-challenge and the efficacy against DENV-2 and DENV-3 will be reported. Results from this study will be informative to better understanding the early development of a protective immune response to the usefulness of this candidate vaccine for travelers.

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UTILIZING THE DENV HUMAN CHALLENGE MODEL TO DEFINE THE MOLECULAR DETERMINANTS OF THE MEMORY B CELL RESPONSE FOLLOWING HETEROLOGOUS DENV INFECTIONS

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Dengue virus (DENV) is a mosquito-borne flavivivirus responsible for the syndromes dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Vaccine efforts have been complicated by varying protection against all 4 serotypes of the virus and poor performance in DENV naïve individuals. Individuals who have experienced naturally occurring secondary infections develop DENV serotype cross-reactive strongly neutralizing antibodies (Abs), but it remains unclear how these responses are formed, what targets they recognize, and how they are maintained over time. In this study we utilize a human challenge model, in which subjects were infected with an attenuated DENV1 (n = 6) strain or placebo (n = 2), followed by an attenuated DENV2 strain 9 months later. Plasma and PBMC samples were collected at various time-points before and after the second infection. To examine how the DENV serotype cross-reactive (CR) and type-specific (TS) neutralizing Ab responses evolve following sequential DENV infections, we used antibody depletion assays, and neutralization assays with recombinant chimeric DENVs. Following the primary DENV1 infection, the majority of the neutralizing Ab response (79.7%) was directed TS epitopes on DENV1. In 4/6 subjects, the DENV1 TS neutralizing Ab response mapped to an antigenic site on domain I of the envelope protein defined by human MAb 1F4. Following the secondary DENV2 infection, individuals developed high levels of neutralizing Abs to all 4 serotypes. The majority of the DENV1 (76.8%), DENV2 (86%), DENV3 (98.7%) and DENV4 (97.8%) neutralizing antibodies recognized epitopes that were conserved (cross-reactive) between serotypes. Studies to map the conserved epitopes recognized by serum neutralizing Abs and memory B cells are ongoing. Our results provide valuable information about how the memory B cell response develops following heterologous DENV infections to provide cross-reactive and protective immunity.

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A RANDOMIZED, CONTROLLED AGE DE-ESCALATION TRIAL DEMONSTRATES THAT THE TETRAVALENT DENGUE VACCINE TV005 IS SAFE AND IMMUNOGENIC IN DENGUE-ENDEMIC BANGLADESH

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Over half the world's population is at risk of dengue virus infection. Each of the four dengue serotypes can cause the full spectrum of disease ranging from asymptomatic infection to life-threatening illness characterized by plasma leakage and vascular collapse. Dengue was first reported in Dhaka, Bangladesh in the 1960s and our data from a recent seroprevalence study in Dhaka demonstrates that 68% of adults have evidence of exposure to one or more serotypes. The NIH live attenuated tetravalent TV005 vaccine has been examined extensively in US-based studies, but evaluation in both naïve and previously exposed endemic populations is critical. As such, we sought to evaluate the safety and immunogenicity of TV005 in Dhaka. We performed a placebo-controlled, double-blind, age de-escalation study in Dhaka and enrolled 192 participants with 48 participants in each of four age cohorts (36 TV005, 12 placebos). Following dosing, participants were re-evaluated on days 7, 14, 28, 56, 180, 360, 720 and 1080. Adverse events were recorded through day 28. Viremia (days 7 and 14) and immunogenicity (days 0, 28, 56, and 180) were evaluated. All cohorts have completed one year of follow-up; surveillance for dengue infection remains ongoing through the 3-year study follow-up. TV005 was well-tolerated; no differences were observed in fever, headache, or safety labs (WBC, ANC, platelets, ALT, or PT/PTT) between treatment arms. An asymptomatic rash occurred more frequently in TV005 recipients ($p = 0.04$). Using culture-based methods, six (4.2%) vaccine recipients were viremic by day 14, with a mean peak titer of 0.5 $\log_{10}$ PFU/mL. In the youngest two age cohorts, 95% (5-10 years of age) and 97% (1-5 years) had a trivalent or better response. A tetravalent response was seen in 64% and 72% of these same cohorts, respectively. Serologic data on the older cohorts is forthcoming. In this endemic region, data demonstrate that TV005 is well-tolerated and elicits a balanced serologic response in children. For the NIH tetravalent dengue vaccine, these data contribute to the understanding of safety and immunogenicity in naïve or partially-immune subjects in endemic regions.

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SAFETY AND IMMUNOGENICITY OF DIFFERENT FORMULATIONS OF A TETRAVALENT DENGUE PURIFIED INACTIVATED VACCINE (DPIV) IN HEALTHY ADULTS FROM PUERTO RICO THROUGH THREE YEARS OF FOLLOW-UP

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We report here the final results after a 3-year follow-up of a Phase 1, observer-blind study (NCT01702857) aimed to assess the safety and immunogenicity of 2 doses of an investigational tetravalent DPIV in Puerto Rico. One-hundred healthy adults (predominantly dengue virus [DENV]-primed) were randomized 1:1:1:1:1 to receive saline placebo or 1 of 4 DPIV formulations (1µg per DENV type adjuvanted with aluminum hydroxide [Alum], $AS01_E$ or $AS03_B$, or 4µg per DENV type adjuvanted with Alum) at day (D) 0 and D28. Serious adverse events (SAEs), potential immune-mediated diseases (pIMDs), medically-attended AEs (MAEs), and clinical DENV cases were recorded up to study end (year [Y] 3 post-vaccination). Functional antibody responses were determined by microneutralization assay at various time points. Two participants in the 4µg+Alum group reported 4 SAEs, and 2 in the placebo group reported 5 SAEs up to month (M) 14; all were considered unrelated to vaccination. No SAEs were reported during Y2 and Y3 follow-up. Two cases of auto-immune thyroiditis (placebo: 1; 1µg+$AS01_E$: 1) and worsening of pre-existing rheumatoid arthritis (1µg+$AS03_B$) were reported up to M14, and 1 case of rheumatoid arthritis (1µg+$AS03_B$) and reactive arthritis (1µg+Alum) up to Y3; no pIMDs were considered vaccination-related. Forty-eight participants reported MAEs up to M14, without notable differences across groups, and 2 in the Y2 and Y3 follow-up. Fourteen cases of suspected dengue disease, all negative by RT-qPCR, were reported by 9 participants. The Y3 per-protocol immunogenicity cohort included 79 participants (76 seropositive for ≥1 DENV type). Geometric mean titers (GMTs) against DENV types 1-4 at Y2 and Y3 post-vaccination waned slightly from M13 levels, but remained mostly higher than pre-vaccination, with highest GMTs for 1µg+$AS03_B$ (1220, 921, 819, 941 at Y2; 1329, 1169, 1220, 719 at Y3 for DENV types 1-4, respectively). Increased GMTs were observed at Y3 post-vaccination for placebo, consistent with seasonal exposure to dengue or other flavivirus infections. All DPIV formulations were well tolerated and no safety signals were identified during the 3-year follow-up.

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CELL-MEDIATED IMMUNITY GENERATED BY TAKEDA'S LIVE-ATTENUATED DENGUE VACCINE (TDV)

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A Phase II study (DEN-205) was performed to test the impact of dosing and sero-status on the performance of Takeda's tetravalent dengue vaccine candidate (TDV) in a dengue endemic location. Here we report that potent cell-mediated immunity (CMI) was generated after a single dose of the vaccine, independent of vaccine formulation and baseline sero-status. For testing, PBMC collected pre-vaccination (day 1) and at months 1, 6 and 12 post-vaccination were assessed for vaccine-induced responses using an IFNγ ELISpot assay. Peptide pools matching all vaccine components - the complete DENV-2 proteome (C, prM/E, NS1, NS2, NS3, NS4, and NS5) and prM/E from DENV-1, -3 and -4 – were used as antigens for testing. In addition, peptide pools matching the NS1, NS3 and NS5 proteins from DENV-1, -3 and -4 were used to assess serotype-cross-reactivity of vaccine induced responses. Potent IFNγ ELISpot responses were detected at month 1 and demonstrated durability out to 12 months post-vaccination. Response rates of >90% were detected in the TDV vaccine formulation recipients at 6 and 12 months post-vaccination and were comparable between sero-negative and sero-positive subjects. Response rates generated by the high-dose formulation (HD-TDV) did not exceed those of the TDV formulation and were in fact lower at all comparable time-points. Median response magnitudes among the positive responders exceeded 1000 SFC/10^6 PBMC for the TDV formulation, were maintained at these high levels at the 12 month post-vaccination time-point, and were not significantly different when baseline sero-status was considered. Response magnitudes for HD-TDV did not exceed those for TDV and were often lower. Importantly, response magnitudes for DENV-1, -3 and -4 derived peptide pools were high, but response rates were ~50% lower than for the DENV-2 responses; however, these response rates were well maintained out to 12 months post-vaccination. NS3 and NS5 were majority targets of the response regardless of vaccine formulation or baseline sero-status. Overall, the vaccine was highly immunogenic and induced potent and durable CMI independent of formulation and baseline sero-status.

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EFFICACY PROFILE OF THE CYD-TDV DENGUE VACCINE: BAYESIAN SURVIVAL ANALYSIS OF INDIVIDUAL PHASE III TRIAL DATA

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Dengue infection confers lifelong type-specific immunity, but enhances risk of severe disease upon secondary infection. Phase II and III trials of Sanofi Pasteur's CYD-TDV dengue vaccine showed an overall protective effect, varying by serotype and prior exposure. To characterize CYD-TDV efficacy, we employ a survival model with time varying hazards including subjects' age, country and baseline serostatus. We consider vaccination to give temporal cross-immunity and alter risk of disease associated with prior exposure, by acting as a silent, disease-free infection. The model is fitted to three years of individual level phase III trial data for >30,000 children aged 2-16 years. Using data augmentation to predict participants' serostatus, we estimate vaccine efficacy by prior exposure and serotype. The effects of age upon vaccine efficacy, duration of protection, and infection are examined. Our model reproduces observed age-specific Kaplan-Meier survival curves and attack rates well. We find an overall beneficial effect of vaccine, and support for our hypothesized mechanism of vaccine action. Cross immunity is significantly positive and long lived in seropositive vaccinees, but non-significant in seronegative vaccinees. Both the magnitude and duration of cross immunity are higher in seropositives and increase with age, independently of baseline serostatus. Cross immunity further varies by serotype. Among seropositive recipients, cross immunity is significantly positive for each serotype and is highest in serotypes 3 and 4. For seronegative recipients, cross immunity is significantly greater than zero for serotype 4 only. A sensitivity analysis of a model without explicit age effects gives an inferior but reasonable fit, though it fails to reproduce attack rates observed in the first year of passive surveillance. Our model refines existing efficacy estimates and demonstrates that the 'silent infection' hypothesis of vaccine action largely explains the complex and heterogeneous vaccine efficacy profile. Funding: DJL, NMF - Bill & Melinda Gates Foundation. ID - Imperial College JRF scheme. RS, NJ & LC employed by Sanofi Pasteur.

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COMPREHENSIVE ANALYSIS OF THE IMMUNE RESPONSE ELICITED BY TAKEDA'S TETRAVALENT DENGUE VACCINE CANDIDATE

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Dengue viruses cause a global disease burden of approximately 100 million apparent infections annually. Takeda Vaccines is developing a live attenuated tetravalent dengue vaccine candidate (TDV) based on a recombinant attenuated dengue serotype 2 virus (TDV-2). For dengue virus serotypes 1, 3, and 4, the pre-membrane (prM) and envelope (E) genes replace the corresponding DENV-2 genes in the attenuated backbone to form chimeric viruses (TDV-1, TDV-3, and TDV-4). The safety and efficacy of TDV is currently being evaluated in a double-blinded phase III controlled study being conducted in eight dengue-endemic countries in Latin America and Asia. Dengue vaccine development has been hampered by an incomplete understanding of the host immune response following vaccination and the lack of clear immune correlates of protection. To address these issues, we have taken a systematic approach towards detailed characterization of humoral, cellular and innate immune responses elicited by TDV. Data will be presented on characteristics of neutralizing antibody responses, magnitude, functionality and specificity of T cell responses, innate immune responses and characterization of anti-NS1 antibody responses following vaccination. Currently, pre- and post-vaccination clinical samples from early and late timepoints from children and adults participating in phase 2 randomised double-blind trials conducted in dengue-endemic countries are being used to fully characterize the immune response to TDV. We will present our progress on the comprehensive analysis of the immune response elicited by TDV, and opportunities to compare immune responses elicited by tetravalent vaccination with immune responses following natural infection. Development of a panel of immunological assays may be applied to case-controlled analysis of breakthrough dengue infections in the on-going efficacy trial to identify immune correlates or surrogates of protection against dengue.

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IMPACT OF MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS ON *STRONGYLOIDES STERCORALIS* AND HOOKWORM INFECTION IN PAPUA NEW GUINEA

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Strongyloides stercoralis and hookworm are intestinal nematodes that often co-infect individuals with lymphatic filariasis. Currently, annual mass drug administration with diethylcarbamazine (DEC) + albendazole (ALB) is used for elimination of lymphatic filariasis, but new evidence has shown that adding ivermectin (IVM) to DEC + ALB markedly enhanced anti-filarial efficacy and potentially accelerate reducing the prevalence and intensity of *Strongyloides* and hookworm infections. Stool and dried blood spot samples were collected as part of an open label, randomized clinical trial conducted in 2017/18 to assess the community safety and effectiveness of 2-drug (DEC/ALB) versus 3-drug therapy (IVM/DEC/ALB) for lymphatic filariasis in the Bogia District of Papua New Guinea. Samples were collected prior to administration of therapy (n=267), 4 weeks after therapy (n=141) and the collection of 1 year follow up samples is currently ongoing. In 2017, 58 % of participants were male with a mean age 28 ± 16 years. To assess the prevalence and intensity of *Strongyloides* and hookworm, we performed Kato katz, duplex quantitative PCR in stool, and ELISA using NIE recombinant antigen for *S. stercoralis* in dried blood spots elute. On kato katz, 78% of samples were positive for hookworm eggs with a geomean of 227 (0-4,012) ova/gm and PCR positivity of 95% at baseline. *Strongyloides* was present in 3% of samples by PCR and 10.5 % positive by NIE ELISA at baseline. No other intestinal parasites were observed. Prevalence of *Strongyloides* and hookworm were similar between the two treatment arms at baseline. Four weeks following therapy mean egg reduction rate for hookworm was 97% in both arms. The samples size at 4 weeks was too small to see a meaningful difference in *Strongyloides*. Changes in the prevalence and antibody levels by NIE ELISA one year following treatment are being evaluated. Hookworm and *Strongyloides spp* are endemic in Papua New Guinea, with almost 100% infection rate for hookworm in our study population. The impact of DEC/ALB versus IVM/DEC/ALB on *Strongyloides* and hookworm in communities at one year following treatment will be discussed.

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INTEGRATED BURDEN ESTIMATION SURVEY OF SKIN-PRESENTING CASE MANAGEMENT NTDS (BURULI ULCER, LEPROSY, LYMPHATIC FILARIASIS AND YAWS) IN MARYLAND, LIBERIA

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Successful control of case management neglected tropical diseases (CM-NTDs) requires provision of significant resources and skilled healthcare staff among affected communities. Understanding the true burden of these diseases represents the first step towards developing a rational control strategy and achieving 2020 elimination targets. As CM-NTDs are focally distributed and population prevalence can be less than 1 per 10,000, this presents distinct economic and logistical challenges to measure disease burden and no validated strategies for an integrated approach currently exist. By exploiting the shared clinical characteristics of Buruli ulcer, leprosy, lymphatic filariasis-associated morbidity and yaws, we have developed a novel integrated two-stage cluster randomised survey to concomitantly estimate the burden of all four conditions within existing health infrastructure. This group of CM-NTDs are conducive to an integrated approach as all four share primary clinical presentation via the skin. Our method capitalises on these characteristics through door-to-door screening surveys conducted by community health workers trained to identify prevalent cases using visual aids that display common CM-NTD skin lesions. An expert team trained in the diagnosis of CM-NTDs visiting the home of every suspected case subsequently confirm or refute diagnosis and all stages undergo stringent quality control assessment. This protocol and the novel suite of tools developed for implementation have been tested across three pilot studies in Nimba County, Liberia and we present the results of all survey stages - screening, verification and quality control - from all pilot investigations and a population-level proof-of-principle survey conducted in Maryland County, Liberia (population 167,340).

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ESTIMATING NEGLECTED TROPICAL DISEASES AND ENVIRONMENTAL RISK FACTORS WITH SPATIO-TEMPORAL GAUSSIAN PROCESS REGRESSION

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The Institute for Health Metrics and Evaluation (IHME) create estimates of disease burden at the national and subnational level worldwide through its Global Burden of Disease study. IHME seeks to utilize all existing, high quality data, to create time-series estimates of health-related risk factors and diseases from 1980-2017. The abundance of data strengthens the validity of our estimates, yet creates its own challenges in data synthesis. In order to synthesize large quantities of spatially, temporally, and age-correlated data, IHME developed Spatio-Temporal Gaussian Process Regression (ST-GPR) for risk factor and disease modeling. ST-GPR is currently used to model many of the risk factors, vaccine-preventable diseases, and neglected tropical diseases for the Global Burden of Disease study. ST-GPR involves three stages: first, a linear mixed effects model incorporates covariate information to create a full time-series for the risk or disease being estimated. We then calculate a weights matrix across space, time, and age, to capture residual variability missed by the linear stage, and add the weighted residuals back in to the model to create a non-parametric second stage (spacetime) estimate. Lastly, we run a Gaussian process regression on the data and our mean function, the stage 2 estimate. We have recently made significant improvements to this modeling strategy. We created new methods of quantifying variance on our stage 2 estimate, allowing for more accurate uncertainty in model estimates. We are exploring techniques to create consistent prevalence estimates, modeled in logit space, within nested location hierarchies. Finally, we incorporated space-time interactions in our weights matrix to capture more subtleties in spatial and temporal trends.

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INTEGRATED MAPPING ASSESSMENT FOR TREATMENT DECISION-MAKING IN AREAS SUSPECTED TO BE CO-ENDEMIC FOR ONCHOCERCIASIS, LYMPHATIC FILARIASIS AND LOIASIS: A CASE STUDY OF TWO LGAS IN RIVERS STATE, NIGERIA

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Many areas co-endemic for onchocerciasis (OV) and loiasis have been excluded from mass administration of medicine with ivermectin because treatment with ivermectin can cause serious adverse events among individuals with high-intensity *Loa loa* infection. In the context of OV elimination, all areas with ongoing transmission require treatment; consequently, rigorous mapping of untreated areas suspected to be endemic for loiasis is needed to determine whether ivermectin treatment is both necessary and safe. The present study used probability proportionate to estimated size to select 30 communities in each of two local government areas (LGAs), Andoni and Bonny, in Rivers State, Nigeria. Both LGAs are ivermectin-naive and have communities with >40% RAPLOA prevalence. In each selected community, 100 individuals ages 16 and older were randomly selected and tested between 10 AM and 4 PM for Loa intensity with CellScope Loa, lymphatic filariasis (LF) antigenemia with the filariasis test strip (FTS), and antibodies against OV and LF via the Ov16/Wb123 Biplex rapid diagnostic test (RDT). Dried blood spots were also taken for confirmatory testing. No evidence of OV transmission was found in either Andoni or Bonny, with prevalence of 0.04% and 0.0% by Ov16 RDT respectively. Results from Ov16 ELISA analysis are pending. In both Andoni and Bonny, evidence of pockets of LF transmission was found, with antigenemia prevalence of 1.8% (95% CI: 1.1-2.7%, range: 0.0%-9.6%) and 1.2% (95% CI: 0.01-2.3%, range: 0.0%-12.8%) respectively. No individuals with Loa intensity of greater than 20,000 mf/ml were identified in either LGA. Biannual administration of albendazole is recommended for LF treatment in areas co-endemic for Loiasis Mapping surveys that include Ov16 as well as CellScope testing have the potential to resolve uncertainty about both the need for and safety of ivermectin treatment, potentially reducing the map of areas for which ivermectin treatment is necessary but cannot be given.

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COMPARISON BETWEEN PAPER-BASED AND M-HEALTH TOOLS FOR COLLATING AND REPORTING CLINICAL CASES OF LYMPHATIC FILARIASIS AND PODOCONIOSIS IN ETHIOPIA

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Lymphatic filariasis (LF) and podoconiosis are disabling diseases, endemic in Ethiopia. The main clinical manifestations include lymphoedema (LF and podoconiosis) and hydrocele (LF only). To ensure access to morbidity management and disability prevention (MMDP) services, data on patient numbers in each implementation unit is required. House-to-house census is considered the gold standard for estimating patient numbers, and data are usually collated and reported using paper-based methods. However, often there are delays in data reaching the regional and central level,

which leads to subsequent delays in rolling out and prioritising MMDP services. The increase in mobile phone mHealth tools offer an alternative, potentially more rapid and cost-effective, approach. As part of a LF and podoconiosis burden assessment conducted in Hawella Tula and Bensa districts in Ethiopia, this study compared the standard paper-based methods with the new *MeasureSMS-Morbidity* tool for clinical cases data collation and reporting. Health extension workers (HEWs) were trained on both methods. Comparisons were made on patient information; age, gender, location (*i.e. kebele)*, condition, severity of condition and acute attacks. Data were analysed for trends, including the differences in ranking the villages in each district based on the highest to lowest number of cases. In addition, financial and human resource requirements were compared. In total, 59 HEWs (19 from Hawella Tula; 40 from Bensa) collated and reported a similar number of cases by paper-based (n=2377) and SMS (n=2372) methods. Significant correlations were found between the two methods for all cases and lymphoedema cases in both districts, and for hydrocele cases in Bensa district only. The total cost of paper-based reporting was 13.7% more expensive than SMS reporting due to costs associated with data collection and entry. The rank correlation showed the same villages would be prioritised for delivery of MMDP services, with time and cost-savings observed using SMS reporting, suggesting it is an effective and efficient alternative tool to help facilitate care to those who need it most.

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MEASURING PROGRESS OF CONTROL AND ELIMINATION PREVENTIVE CHEMOTHERAPY NEGLECTED TROPICAL DISEASES IN ETHIOPIA

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Ethiopia has one of the highest burden of Neglected Tropical Diseases (NTDs) in Africa. The national NTD program targets schistosomiasis, trachoma, lymphatic filariasis(LF), onchocerciasis, and soil transmitted helminthiasis(STH) through community and school-based mass drug administration (MDA) of preventive chemotherapy. The national program conducted a desk review of program progress and assessment reports to evaluate the achievements of the national program toward the global and national strategic goals for NTD control and elimination for the period 2013 to 2018. By 2013, the geographical coverage for disease specific preventive chemotherapy was 47% for LF, 81% for onchocerciasis, 79% for schistosomiasis, 92% for STH, and 58% for trachoma. Through increased donor support and government commitment, the national NTD program reached 100% geographic coverage for all PCT diseases. In 2017 and the national program coverage was 88% for LF, 77% for STH, 76.5% for schistosomiasis and 86% for trachoma. The national program started carrying out LF transmission assessment surveys (TAS) and trachoma impact surveys. By 2017, 5 districts had reached the criteria for stopping MDA for LF and 66 districts for trachoma. This progress means that 1.1 million people are no longer at risk of LF and 5.6 million people for trachoma. Based on both epidemiological and entomological survey 7 onchocerciasis endemic districts have achieved the criteria to stop MDA. STH and schistosomiasis are considered endemic throughout Ethiopia, however data from sentinel site and spot check sites shows that prevalence in many districts is declining year by year. In conclusion the national program has made remarkable progress toward the 2020 global target for control and elimination of NTDs. However, further efforts are needed to address disease specific inadequate MDA coverage in the hard to reach districts, and address water, sanitation and hygiene interventions to control disease and interrupt transmission.

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AN INTEGRATED APPROACH FOR THE CONTROL OF INTESTINAL PARASITES, SOUTH-CENTRAL CÔTE D'IVOIRE

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Hundreds of millions of people are infected with helminths and intestinal protozoa, particularly children in low- and middle-income countries. Preventive chemotherapy is the main strategy to control helminthiases. However, rapid re-infection occurs in settings where there is a lack of clean water, sanitation and hygiene. From August to September 2014, a cross-sectional epidemiological survey was conducted in 56 communities of three departments Djékanou, Toumodi and Taabo in south-central Côte d'Ivoire. Each study participant was invited to provide a stool and urine sample. Stool samples were examined for intestinal helminth and protozoa infections using the Kato-Katz and formalin-ether concentration methods, respectively, and *Schistosoma haematobium* eggs detected in urine by a filtration technique. Socio-demographic characteristics and knowledge, attitude, practices and beliefs with regard to hygiene, sanitation and intestinal parasitic diseases were collected using a questionnaire administered to household heads. Multivariable logistic regression models were used to analyse the association between parasitic infections and potential risk factors. A total of 4305 participants had complete parasitological and questionnaire data. The prevalence of hookworm was 35.3%, 34.2% and 10.9% in the departments of Djékanou, Toumodi and Taabo, respectively. Other helminth species (*Schistosoma mansoni*, *S. haematobium*, *Ascaris lumbricoides* and *Trichuris trichiura*) were found with prevalences below 10%. Intestinal protozoa, e.g. *Entamoeba histolytica/dispar* and *Giardia lamblia* were similarly prevalent in the three departments. Hookworm infection was associated with open defecation, sex and age. *E. coli* infection was negatively associated with the use of tap water in households (OR = 0.66; $p = 0.032$). Garbage deposit near the household was positively associated with *G. lamblia* infection (OR = 1.30; $p = 0.015$). Our results will serve as baseline to monitor the effect of a package of interventions, including preventive chemotherapy, sanitation and health education on re-infection with helminths and intestinal protozoa.

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EFFECTS OF WATER, SANITATION, HANDWASHING, AND NUTRITIONAL INTERVENTIONS ON ENVIRONMENTAL ENTERIC DYSFUNCTION IN YOUNG CHILDREN: A CLUSTER-RANDOMIZED CONTROLLED TRIAL IN RURAL KENYA

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Nutrition-specific interventions have typically achieved modest reductions in childhood stunting. Recurrent enteric infections leading to chronic gut inflammation and structural changes may contribute to early life growth faltering. Within a cluster-randomized trial in rural Kenya that enrolled pregnant women and followed their children (ClinicalTrials.gov, NCT01704105), we evaluated whether improvements to drinking water quality (W), sanitation (S), handwashing (H), and nutrition (N) would prevent or reduce gut inflammation and permeability. This substudy included children in four arms of the trial: combined WSH; N, which consisted of lipid-based nutrient supplementation from 6-24 months and age-appropriate feeding counseling; N+WSH; control. We measured biomarkers of gut inflammation (myeloperoxidase and neopterin) and permeability (alpha-1-antitrypsin, lactulose, and mannitol) at ages 6, 17, and 22 months. Analysis was intention-to-treat. We evaluated 1485 children. At age 6 months, compared to controls, urinary lactulose was higher in the nutrition (+0.45 log mmol/L, CI 0.23, 0.67) and N+WSH (+0.27 log mmol/L, CI 0.05, 0.49) arms. Similarly, urinary mannitol was higher in the nutrition (+0.46 log mmol/L, CI 0.23, 0.69) and N+WSH arms (+0.25 log mmol/L, CI 0.02, 0.47). At age 17 months, compared to controls, lactulose and mannitol remained elevated in the N+WSH arm only (+0.32 log mmol/L, CI 0.02, 0.62 and +0.25 log mmol/L, CI 0.01, 0.49 respectively). At 22 months, there were no differences between the arms. At all three time points, none of the interventions impacted the urinary lactulose to mannitol ratio (L:M). Although child linear growth was improved in the N and N+WSH arms, elevated lactulose and mannitol in these arms suggest that the nutrition intervention increased gut permeability. These unexpected results highlight the importance of understanding the pathophysiology of gut inflammation and permeability and the mechanism underlying the intervention effects. Fecal myeloperoxidase, neopterin, and alpha-1-antitrypsin results are forthcoming.

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THE IMPACT OF HOUSEHOLD CLUSTERING ON ENTERIC PATHOGEN INFECTION IN RURAL LAOTIAN COMMUNITIES

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Recent large-scale studies that focused on household-, rather than community-level WASH improvements found limited impact on health. WASH conditions may influence both intra- and inter-household transmission of pathogens, but there is limited evidence on the relative importance of these different transmission pathways across pathogens. We conducted a variance decomposition analysis to quantify the extent to which an individual's log odds of enteric infections were explained by WASH access (household sanitation, improved water, and soap) versus household clustering. We surveyed 25 randomly selected households in 50 randomly selected villages in Saravane Province, Laos. Stool samples were collected from a primary school-aged child, their sibling <5 years old, and their parent, and analyzed for 25 enteropathogens using the TaqMan Array Card, a qPCR-based diagnostic assay. All 3 samples were returned from 297 households, for a total of 891 samples. TAC analysis will be complete in June 2018; to date, 291 (33%) samples have been analyzed. Initial results indicate that giardia (77%), hookworm (71%), EPEC (51%), and rotavirus (42%) were the most prevalent pathogens. WASH covariates explained 13% of the total variance in log odds of giardia infection; of the variability not explained by WASH covariates, 18% was due to household clustering. WASH covariates explained 9% of the total variance in log odds of hookworm infection; 47% of the remaining variability came from household clustering. WASH covariates explained 29% of the total variance in log odds of EPEC infection, while >99% of the remaining variability was the effect of household clustering. Lastly, WASH covariates explained 3% of the total variance in log odds of rotavirus infection, whereas 33% of the remaining variability was due to household clustering. The relative influence of WASH covariates (3-29% of variability) and household clustering (18-99% of variability) on log odds of enteric infection differed by pathogen. A more nuanced understanding of pathogen transmission within and between households and variations by pathogen could help better target WASH improvements.

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THE EFFICACY OF CONSUMER HANDWASHING AGENTS FOR REMOVAL OF DIARRHEAL AND RESPIRATORY PATHOGENS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF LABORATORY BASED STUDIES

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Respiratory and diarrheal diseases are leading causes of morbidity worldwide, and handwashing is commonly recommended to prevent disease transmission. Despite increasing availability of various consumer handwashing products, to our knowledge the existing evidence on product efficacy has not been summarized. The objective of this study was to evaluate the efficacy of consumer handwashing products against diarrheal and respiratory pathogens. A systematic review was performed by developing inclusion criteria, searching and selecting papers, assessing quality, and extracting and summarizing the results of included studies on handwashing efficacy. Studies were included if they reported an experiment on human hands showing the percent or log reduction value (LRV) of bacterial or viral diarrheal/respiratory pathogens after washing with a consumer product. A Mantel-Haenszel random effects meta-analysis was used to generate a summary LRV describing the reduction in contamination on hands. Overall, 31 papers met inclusion criteria, describing efficacy for hand rubs, soaps, and water only against two bacteria and eight viruses. For bacteria, hand rubs resulted in a 3.4 LRV (95% CI 3.2-3.7), soap a 2.2 LRV (2.0-2.4), and water only a 1.8 LRV (1.5-2.2). For viruses, hand rubs resulted in a 1.0 LRV (0.8-1.1), soap a 1.3 LRV (0.8-1.7), and water only a 1.0 LRV (0.7-1.4). Results for bacteria were consistent across studies; virus results had higher heterogeneity. Findings were robust to removal of lower quality studies. Both hand rubs and soaps meet United States regulatory requirements of ≥ 2-log reduction; no product met this standard for viruses, although information on viral pathogens was limited compared to bacteria. Thus, for bacterial pathogens both soaps and antiseptic rubs are likely to be effective for handwashing, and the most readily available option should be used to maintain good hygiene. However, as many emerging infectious diseases are viral respiratory pathogens, we recommend further research on the efficacy of products against viruses to develop appropriate recommendations for handwashing for existing and emerging diseases.

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PREDICTED SHORT AND LONG-TERM IMPACT OF DEWORMING AND WASH ON TRANSMISSION OF SOIL-TRANSMITTED HELMINTHS

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Regular preventive chemotherapy (PCT) targeting high-risk populations is a highly effective way to control STH in the short term, but sustainable long-term STH control is expected to require improved access to water, sanitation, and hygiene (WASH). However, experimental studies conducted so far have not always been able to demonstrate that WASH prevents STH (re-)infections. The aim of the current study was to investigate the

impact of WASH on STH infections during and after a PCT programme using a mathematical model. We use the individual-based transmission model WORMSIM to predict the short and long-term impact of WASH on roundworm, whipworm, and hookworm transmission in contexts with and without PCT. In the model, we distinguish two WASH modalities: sanitation, which reduces individuals' contributions to environmental contamination; and hygiene, which reduces individuals' exposure to the environmental reservoir. We simulate the impact of varying levels of population-level uptake and individual-level effectiveness (reduction in contribution and/or exposure) of the two WASH modalities, as well as their combined impact. We predict that WASH has little observable short-term impact on STH infections levels in the context of PCT programmes, but is pivotal to sustain control or eliminate infection levels after scaling down or stopping PCT. The impact of hygiene interventions is determined more by the effectiveness of the intervention at the individual level than population-level uptake, whereas the impact of sanitation interventions depends more directly on the product of uptake and the effectiveness. In conclusion, there is a clear added benefit of WASH to sustain the gains made by PCT in the long term, although its impact can be difficult to measure in the context of deworming programmes. Because the impact of WASH is expected to vary with chosen modality, level of uptake, and quality of execution, trials should disentangle exposure and contribution-related interventions, provide detailed reports on access to and use of each modality, and should focus on the long-term impact on transmission and risk of recrudescence after stopping PCT.

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ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI* AND *KLEBSIELLA* SPP. ISOLATES FROM HOUSEHOLD WATER, FOOD PREPARATION SURFACES, AND SOIL IN COMPOUNDS IN MAPUTO, MOZAMBIQUE: POTENTIAL SOURCES OF ENVIRONMENTAL TRANSMISSION OUTSIDE THE CLINIC

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Environmental conditions may be important drivers of antibiotic resistant (AR) infections, especially in low-income, pathogen-rich environments. We examined potential environmental reservoirs of AR bacteria in households in a controlled before-and-after study of a sanitation intervention in Maputo, Mozambique. Samples of household source and stored water, food preparation surfaces, household soil, and soil at the latrine doorstep were collected in 30 intervention and 30 control compounds 1 year after the intervention. Samples were cultured in mTEC broth and confirmed as *E. coli* or *Klebsiella* spp., followed by disk diffusion to assess antibiotic susceptibility. From each sample, 1-2 isolates were tested for the 12 National Antibiotic Resistance Monitoring System for Enteric Bacteria antibiotics of interest for *E. coli*. To-date, 227/505 (45%) isolates have been cultured, with 35 (15%) confirmed as *E. coli* and 63 as *Klebsiella* spp. (28%, though only 36 tested to-date for AR). Approximately 55% have been culturable, with remaining non-culturable isolates likely due to long storage time at -80°. After completing AR testing in May, we will compare isolate AR results by environmental conditions (i.e. WASH infrastructure) assessed at concurrent household surveys. To-date, 19/35 *E. coli* isolates were resistant to ampicillin, including isolates within all sample types. Resistance to cefoxitin, nalidixic acid, streptomycin, tetracycline, and sulfamethoxazole-trimethoprim (SXT) was also observed. Most *Klebsiella* spp. isolates (34/36) were also resistant to ampicillin, with resistance to amoxicillin-clavulanic acid, azithromycin, cefoxitin, gentamicin, streptomycin, tetracycline, and SXT detected as well. Although only about half of household environmental isolates have been tested, we have already observed high levels of resistance to ampicillin - a first-line drug for pediatric enteric infections - among other AR across all types of environmental samples. These data provide early evidence of the potential importance of contaminated environments as sources of exposure to AR bacteria in dense, low-income household settings.

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DEFINING DIARRHEA: A POPULATION-BASED VALIDATION STUDY OF CAREGIVER-REPORTED STOOL CONSISTENCY IN THE AMHARA REGION OF ETHIOPIA

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Diarrhea is a leading cause of death among children aged less than five years globally. Most studies of pediatric diarrhea rely on caregiver-reported stool consistency and frequency to define the disease. Research on the validity of caregiver-reported diarrhea is sparse. We collected stool samples from 2,398 children participating in two clinical trials in the Amhara region of Ethiopia. The consistency of each stool sample was graded by the child's caregiver and two trained laboratory technicians according to an illustrated stool consistency scale. We assessed the reliability of graded stool consistency among the technicians, and then compared the caregiver's grade with the technician's grade. We also tested if the illustrated stool consistency scale could improve the validity of caregiver's report. The weighted kappa measuring the agreement between the two laboratory technicians reached 0.90 after 500 stool samples were graded. The sensitivity of caregiver-reported loose or watery stool was 15.5% (95% confidence interval [CI]: 9.7, 24.2) and the specificity was 98.4% (95% CI 97.1, 99.1). With the illustrated scale, the sensitivity was 68.5% (95% CI: 58.5, 77.1) and the specificity was 86.1% (95% CI: 79.3, 90.9). The results indicate that caregiver-reported stool consistency using the terms "loose or watery" does not accurately describe stool consistency as graded by trained laboratory technicians. Given the predominance of using caregiver-reported stool consistency to define diarrheal disease, the low sensitivity identified in this study suggests that the burden of diarrheal disease may be underestimated and intervention effects could be biased. The illustrated scale is a potential low-lost tool to improve the validity of caregiver-reported stool consistency.

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HOUSEHOLD AIR POLLUTION EXPOSURE AND MITOCHONDRIAL DNA COPY NUMBER IN CORD BLOOD: IDENTIFYING SENSITIVE WINDOWS AND SEX-SPECIFIC EFFECTS

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Household air pollution (HAP) exposure during sensitive windows of development, beginning *in utero*, may program future disease risk through oxidative stress (OS) pathways. Mitochondria may respond to OS by increasing DNA copy number (mtDNAcn), however when compensatory mechanisms are overwhelmed mtDNAcn may be decreased. Prior studies suggest that females may be more adaptive to *in utero* OS. The Ghana Randomized Air Pollution and Health Study (GRAPHS) recruited nonsmoking pregnant women and randomized them to one of two cookstove interventions or control. Four, 72- hour carbon monoxide (CO) personal air pollution exposure measurements were performed over pregnancy and interpolated to estimate weekly prenatal CO exposure. Venous umbilical cord blood was collected, cord blood mononuclear cells isolated, and DNA extracted in 164 samples. Multiplex quantitative real-time polymerase chain reaction (qPCR) was used to determine mtDNAcn. Distributed lag models (DLMs) estimated the time-varying association between mtDNAcn and CO exposures. Models were adjusted for maternal age, body mass index (BMI), marital status, socioeconomic status, child sex, gestational age at delivery. Sex-stratified analyses were performed. Median prenatal CO exposure was 0.91ppm (0.51-1.48). Overall, DLMs identified a significant association between a 1ppm increase in prenatal CO exposure and increased mtDNAcn in cord blood from 5-10 weeks gestation. Sex-stratified analyses identified a significant association between a 1ppm increase in prenatal CO exposure and decreased mtDNAcn in boys from 5-10 weeks gestation but increased mtDNAcn in girls from 0-10 weeks gestation. These findings suggest that *in utero* HAP exposure, as measured by CO exposure, alters mitochondrial function and that sex may modify the effect. An increase in mtDNAcn, as seen in girls, may reflect a compensatory mechanism for cell survival. Conversely, mtDNA damage secondary to OS may limit mitochondrial replication thus decreasing mtDNAcn, as seen in boys. These data support prior work demonstrating sex-specific responses to *in utero* OS, with females being more resilient.

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"MAD DOGS AND ENGLISHMEN: THE "SEASONING" OF THE MILITARY, MISSIONARIES, AND MAGISTRATES IN 19TH CENTURY COLONIAL OUTPOSTS

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The first wave of European colonialism into Africa and the Far East occurred between about 1450 and 1600. By the 19th century, Britain (and other countries) had established a significant foothold in territories throughout Africa and the Indian subcontinent. Once military forces had stabilized these regions, ranks of missionaries and civil servants soon followed. What they found, however, were conditions that were extremely different from those that they had left behind in the United Kingdom. In short, they were completely unprepared for what they found. This presentation examines the process of "seasoning" (known more commonly today as acclimatization) on British arrivals to the tropical reaches of Africa and the Indian subcontinent. Drawing on diaries and medical literature of the 19th century, the authors will show how imperfectly these ex-patriots appreciated what awaited them in the far corners of the Empire. Advice on becoming "seasoned" to the tropical climate--offered by many commentators who had never been abroad—often proved as useless as it was potentially dangerous.

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DATA QUALITY IN THE ERA OF "LIVE DATA": CHAMPS DATA QUALITY MONITORING SYSTEM

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The Child Health and Mortality Prevention Surveillance (CHAMPS) Network seeks to identify definitive causes of stillbirth and under-5 deaths in countries with high child mortality. The network is comprised of 7 sites in Africa and South Asia that are using post-mortem and pathology techniques with the goal of determining cause of death via a panel of experts. Through a consistent set of data specifications defined by the CHAMPS Program Office (PO), sites primarily capture data via a survey web application (REDCap) and transmit weekly to the PO, where the cumulative data are stored in SQL servers. The requirement to make data rapidly publically available according to the open access policies of the study sponsor, the need for validated data at the point of expert panel review, combined with challenges of managing transactional data from ongoing data collection and transmissions, require unique strategies to data quality evaluation and resolution at the PO and site levels. The PO developed a data quality monitoring and feedback system that works on multiple fronts: site-level capacity strengthening through REDCap rules implementation and dynamic resolutions at the point of data entry, data validation of the raw csv files at the point of data upload and processing into the SQL warehouse, and development of matching SQL based data quality algorithms with metadata to manage the SQL and REDCap data quality rules. The PO has defined 61 REDCap data quality rules applying to demographic, laboratory, and clinical data which can be implemented at a site-level, with an additional 6 rules applying across REDCap projects that are implemented at a PO level. When an incongruous set of data is identified, a new data quality rule is developed through the gathering of requirements (both logic and resolution language). Rules are developed and released in batches, typically every 2 weeks, and documentation supports versions of the data quality rules through the agile lifecycle. This multi-faceted approach has established a robust data quality monitoring system, capable of rapid error identification and resolution to support sharing valid data across the network.

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HEALTH, NUTRITION AND FOOD SECURITY AMONG ADOLESCENT REFUGEES

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The rights of all people to the highest standards of physical and mental health, as well as freedom from hunger and malnutrition, underlie a human rights based approach to health policy. This applies equally to refugees who have been forced to flee their homelands. However, maintaining basic health standards for refugee populations remains a challenge. While existing research tends to focus on maternal and child health, relatively little is known about the health of adolescents in refugee settings. Using quantitative data collected by the authors in 2018 from over 5,000 adolescents in two diverse refugee settings—Syrian refugees in Jordan and Rohingya refugees in Bangladesh—we describe the multitude of health and nutrition challenges faced by adolescent refugees. We explore physical and mental health, as well as dietary diversity and food security. We look at variation by gender, age, and location of the refugee population (camp vs. non-camp). In addition, we look at the role of programs (emergency relief and social protection) and health infrastructure in improving health outcomes for this population. As increasing numbers

of refugees cross international boundaries, understanding the health problems they face is critical in ensuring appropriate health care, in particular for adolescents who are on the verge of entering their prime fertility and labor productivity years.

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A QUALITATIVE STUDY TO UNDERSTAND POPULATION'S PERCEPTION ON PLAGUE AFTER THE 2017 EPIDEMIC, ANTANANARIVO, MADAGASCAR

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Yersinia pestis continues to threaten the Malagasy population with around 300 reported cases/year during September - April. In 2017, Madagascar experienced a large urban epidemic of Pneumonic Plague (PP). We are conducting a study to explore the knowledge, attitudes, perceptions and practices (KAPP) regarding plague of the population living in Antananarivo, the capital city. Here, we present the results of initial interviews among a sample of residents living near treatment centers. We used the epidemic database from the Institut Pasteur de Madagascar (IPM) to identify three Fokontany (smallest administrative unit) close to two plague treatment centers and conducted individual and group interviews among recovered plague patients, health care professionals and residents living nearby the treatment center. Interviews were conducted by a multidisciplinary team composed of health geographers and anthropologists using semi-structured questionnaires and the photovoice methodology. Photovoice is a qualitative process by which individuals can represent their reality using photographic technique. We also collected information on antibiotic usage and health care utilization. Five photovoice and group interviews were conducted among 21 participants. Participants where aged between 18 to 40 years old. Men's and women's groups have been separated. A camera was provided to each participant. Each participant was asked to take three photos and afterwards to describe the reasons why they took such photos. The discussion from these collective interviews highlighted gaps and confusion regarding plague, including the mode of propagation (air and/or water), in all the investigated fokontany. The proximity of individual's location to the plague treatment center may influence their knowledge and perceptions regarding plague. Understanding the spatial and social features related to plague could improve next plague outbreak/epidemic preparedness. This work will support health community communication during sensitization campaigns and reduce the knowledge gaps for the general population.

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IS GOVERNMENT VECTOR CONTROL PERCEIVED AS EFFECTIVE? A BINATIONAL COMPARISON BETWEEN TWO US AND TWO MEXICAN CITIES WITH DISPARATE LEVELS OF TRANSMISSION

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As has been evidenced during multiple vector-borne outbreaks across the world, perceptions about the government can greatly influence vector-control success. In turn, the success of vector-control programs will influence the perceived effectiveness of those programs. These perceptions may influence community uptake of recommended vector control strategies. Preliminary evidence from Hermosillo, SN suggested that community awareness of government insecticidal spraying may cause a "false sense of security" and reduce household mosquito avoidance behavior. In this work, we investigated perceptions toward the effectiveness of government-led vector control strategies, knowledge of the activities conducted and the association between perceived effectiveness and uptake of generally recommended public health strategies. Cross-sectional knowledge, attitudes, and practice (KAP) surveys were conducted in Hermosillo, Sonora; Nogales, Sonora; and Tucson, Arizona; and Key West, Florida. Responses pertaining to demographics, knowledge of mosquito-borne diseases, attitudes towards government vector-control, and household mosquito avoidance practices were assessed. Multivariable regression models were used to characterize participants who perceived government activities to be effective. In terms of a Likert scale (1-5), more people in Hermosillo (mean = 3.15, sd = 1.01) and Tucson (mean = 2.91, sd = 1.11) reported that government programs are "somewhat effective", those in Nogales reported that government programs are "hardly effective" (mean = 1.83, sd = 1.19), while Key West considered government activities to be "very effective" (mean = 3.72, sd = 1.04). Separate regression models for each city indicate associations between government effectiveness and the amount of exposure to mosquito-borne disease prevention information, as well as history of Dengue infection. More studies should take into account perceptions towards government activities when conducting surveys to understand the dynamics of uptake of household level vector control activities.

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CLINICAL CAPACITY BUILDING TO ADDRESS DIABETES PREVENTION AND CONTROL IN A COMMUNITY HEALTH CENTER IN JALISCO, MEXICO

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Diabetes is the most costly illness for the Mexican healthcare system, and the government has declared the epidemic a national emergency. In response, the state of Jalisco created the Overweight, Obesity, and Diabetes Prevention and Control Strategy. The strategy highlights working in interdisciplinary teams in primary care settings to deliver a comprehensive approach to diabetes prevention, screening, care and control at the population level. Community clinics outside of the public health care system are designated to play an important role, although most lack the operational system to plan and implement targeted public health programming. This abstract describes a capacity-building initiative led by University of California San Francisco (UCSF) advanced practice nurses and public health practitioners to build clinical systems that contribute to a comprehensive diabetes prevention and control program at Tiopa Community Clinic in Autlán, Jalisco, Mexico. The "Tiopa Sustainability Collaboration" embeds UCSF advanced practice nurses with Mexican national registered nurses to form a clinical operations management team at Tiopa. Initial assessments of facility staffing, operations, and services identified necessary system changes to 1) improve delivery of quality diabetes care, and 2) align facility services with the state's strategy for diabetes prevention and control. Specifically, the program engages interprofessional clinical staff in continuing education, population health program development, and quality improvement initiatives. To date, activities carried out by the nurse led clinical operations management team include: (1) data collection and analysis of a community health assessment; (2) initiation of quality improvement committee projects; (3) systemization of clinical documentation procedures to improve case identification; and (4) interdisciplinary case conferences for end-stage renal disease patients. The ultimate goal of this project is to

provide a comprehensive, cost-effective, community-level diabetes care model aligned with government strategies for diabetes screening and treatment.

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A MULTI-YEAR PROJECT IMPLEMENTING KANGAROO MOTHER CARE IN RURAL TANZANIA

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WHO has recommended Kangaroo Mother Care (KMC) as routine care for all infants. As a result, implementation plans are underway in many less developed countries around the world. This paper discusses a multi-year project begun in 2014 to implement KMC with low birth weight (LBW) and high risk newborns at a rural district hospital in Tanzania. The research involves the use of de-identified retrospective medical records (analysis of 116 KMC mother-baby dyads, all LBW with 45% at or under 1500 gr.) and case studies supplemented by clinical observations. Data collected include infant weight, infant body temperature, breast milk (BM) production, survival period, and cause of death if death occurred. Results from 2014 indicate that even with KMC, many LBW neonates die due to insufficient BM and introduction of cow's milk substitutes. As a result, the research team and program introduced manual breast pumps and a training-feeding protocol for mothers to enhance breast milk extraction and the use of Kangaroo (KMC) for LBW and high risk newborns. Existing hospital feeding protocols have been used to guide feeding and mothers self-monitor and record their breast milk production. With the new protocol, neonatal temperature stability, breast milk output, feeding success, weight gain, and survival have increased in our case study group. An analysis of KMC log records indicates 74% survival rate in this LBW group. Only one baby weighing less than 1000 gr. weight has survived (25%), but this baby was part of our KMC intensive education case study group in 2016. Prematurity and respiratory distress, hypothermia, and infection are frequent causes of death with hypothermia accounting for 29% of the deaths. Efforts for education in 2018 will accentuate this factor in addressing deaths. Our study indicates that KMC is very effective in enhancing survival, but that protocol enhancements may be necessary with LBW babies and that intensive support and education of new mothers are also crucial to success.

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CHALLENGES FOR IVERMECTIN UPTAKE TO PREVENT NODDING SYNDROME IN ONCHOCERCIASIS HYPER-ENDEMIC AREAS OF CAMEROON, TANZANIA AND UGANDA

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Evidence suggests an association between onchocerciasis and epilepsy, including Nodding Syndrome. Community-directed treatment with Ivermectin (CDTI) to control onchocerciasis has been on-going in Cameroon since 1998, in Tanzania since 1997 and in Uganda since 2009. Our aim was to assess social factors limiting ivermectin (IVM) uptake in areas hyper-endemic to onchocerciasis and with high prevalence of epilepsy. Ethnographic research was carried out between 2015 and 2017 in Cameroon, Tanzania and Uganda. Data from participant observation, informal conversations, in-depth interviews and focus group discussions were triangulated and compared and contrasted across the three countries. Sampling was theoretical and retroductive data analysis was conducted using NVivo 10. Common barriers to IVM uptake included lack of motivation for community directed implementors (CDI). CDIs needed more time and support in reaching remote or people absent during distribution period. Supportive supervision for CDI was limited and inadequate. Some eligible individuals were excluded due to alcohol consumption, having seizures, being migrants or living in remote parts of the village. Fear of side effects that limit ability to work, prohibition of alcohol consumption, distrust in the drug and distribution system and a lack of perceived benefits to individuals feeling healthy, were the main barriers to adherence. Respondents mentioned that radio programs were effective in improving community attitudes towards IVM and suggested that non-financial contributions from the community members themselves, like free labour on farms of CDIs, may help incentivize CDIs. Continued community sensitisation, monitoring of IVM distribution, improved training of CDIs, creation of audit cycles to efficiently address suspected adverse effects and rumours, alternative options of IVM administration for missed / unforeseen people is necessary in onchocerciasis hyper-endemic areas of Cameroon, Tanzania and Uganda. These findings are in line with earlier claims in literature that reported coverage of ivermectin may be overestimated currently and could be improved.

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WHAT IS THE SOCIO-ECONOMIC IMPACT OF LEG LYMPHOEDEMA ON PATIENT CAREGIVERS IN A PODOCONIOSIS AND LYMPHATIC FILARIASIS CO-ENDEMIC DISTRICT OF ETHIOPIA?

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Lymphatic filariasis (LF) and podoconiosis are neglected tropical diseases (NTDs) endemic in Ethiopia. Both diseases cause painful and disabling lymphoedema of the lower limbs, and people affected require a range of morbidity management and disability prevention measures. Lymphoedema patients are often subject to a complex range of physical, social and economic hardships. These hardships are likely to extend beyond the patient, to the people who care for them. However, little is known about the caregivers; who they are - how they assist - what impact it has on their own lives. Further, it is not known if the different stages of lymphoedema (i.e. mild, moderate, severe) have a corresponding impact on caregivers. The aim of this study was to determine the socio-economic impact of leg lymphoedema on patient caregivers in the Simada District, Amhara Region of Ethiopia where over 1000 cases of leg lymphoedema have been reported. The objectives were to 1) identify lymphoedema patients with different stages of severity and to examine the potential impact on their caregivers and family unit; 2) determine the characteristics and role of primary caregivers; and 3) quantify the quality of life and economic impact on the primary caregivers. In total, 90 lymphoedema patients, grouped by the stage of their lymphoedema (30 mild, 30 moderate, 30 severe) were identified. Their primary caregiver was interviewed using a semi-structured questionnaire including a range of information e.g. type of assistance provided, frequency and duration of tasks, and number of days off work or school, and comparisons made between the different patient caregiver groups. The study is ongoing and the findings will be presented to provide a better understanding of the role of LF and podoconiosis caregivers, which has previously been unrecognised, and is crucial in making progress towards Sustainable Development Goal 5, in recognising and valuing unpaid care and domestic work.

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IMPROVING HEALTHCARE DECISION MAKING IN RURAL BANGLADESH THROUGH A PHYSICIAN CALL CENTER

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Despite progress in many health indicators, maternal and child mortality in Bangladesh remain unacceptably high. Delays in recognizing danger signs and seeking care contribute to these deaths. Mobile phones are widely available in Bangladesh and over 87% of households own a cell phone. As part of a study to understand etiology of child mortality, we aimed to enhance patient access to physician consultation and ability to make informed healthcare seeking decisions using a physician call center. From September 2017 to February 2018, a private company provided toll-free access to a physician by phone 24/7 for a population of 203,000, living in 52,295 households of rural Bangladesh. Physicians screened calls for danger signs that required quick referral and advised referral to six designated facilities. Since September 2017, 1568 calls were received and 560 (36%) were regarding illnesses among children under-5; diarrhoea and cough were the most common complaints. A total of 172 children were referred to the facilities and among them 103 were referred with danger signs. The key reasons for overall referral were diarrhoea (23%), feeding difficulty (19%) and respiratory illness (16%). Among 172 referred cases, we were able to follow up with 99 children and only 21% of those sought care. For pregnancy and post-partum complications, 129 calls were made and 24 (19%) were referred. Major causes for referral were vaginal bleeding (21%) and premature rupture of membrane (17%). In the first 6 months this call center functioned, about 1 in every 50 households called to avail services. A total of 303 callers (57% under-5, 8% pregnant/post-partum, 35% from others category) received referral to aid in healthcare seeking, suggesting that this system has the potential to have a positive impact on maternal and child health. Yet, the low proportion of people who sought care following advice to do so warrants further inquiry. Future work to learn if the most vulnerable groups within this population can access the call center would be beneficial to describe its potential as an intervention and could be favorable for broader public health improvement interventions.

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MENTAL DISORDERS AND PATHWAYS TO CARE IN RURAL UGANDA

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Integrating mental health into public health systems has become a global priority, with mental health and well-being now part of the Sustainable Development Goals. In the aim to provide good quality care for mental disorders, understanding patients' pathways to biomedical care as well as alternate therapy choices is key. As part of a larger mixed-methods study in eastern rural Uganda, the present qualitative study inquired into the health seeking itineraries of patients who attended the outpatient mental health clinic of Iganga district hospital. Out of 28 in-depth interviews with patient caregivers, four case-studies were selected for further analysis to assess differences in pathways to care at the intra-household level, where several family members suffered from similar mental disorders. Data analysis was based on emergent and thematic content analyses using NVivo 11. Health seeking itineraries were largely pluralistic, combining and alternating between traditional healing practices and biomedical care, regardless of the specific mental disorder. Intra-household differences in care seeking pathways - e.g. where one patient received traditional help or no care at all, while the other received biomedical care - depended on the caregiver's perceived explanatory illness model for each patient. If interpreted as a form of bewitchment, traditional medicine and healing was the first form of care sought, while the mental health clinic was seen as a recourse to "free" care. Patients who showed visible improvements once stabilized on psychotropic medication provided motivation towards continued biomedical care. However, stock-outs of the free psychotropic medication at the clinic led to dissatisfaction with services due to out-of-pocket expenses, and eventually a return to alternative therapy choices. Various combinations of cultural, structural and socioeconomic factors affect caregivers' choices of pathways to care for mental health patients. These cumulative processes, which ultimately impact patient treatment outcomes, need to be first addressed if we are to promote more inclusive and effective public mental health systems.

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EFFECTIVENESS OF USING COMMUNITY HEALTH WORKERS TO CONDUCT HEALTH EDUCATION ON ECHINOCOCCOSIS PREVENTION AMONG TIBETAN VILLAGERS IN WESTERN SICHUAN PROVINCE, CHINA, 2015-2016

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Echinococcosis is a potentially severe zoonotic parasitic infection and highly endemic in some communities in western Sichuan province, China where dogs and wild animals serve as intermediate hosts. Human infections occur in both children and adults and range from asymptomatic to disabling or fatal. There are not enough local health department staff and resources in this region to focus on echinococcosis awareness and prevention. In this project, we explored the effectiveness of using locally trained community health workers (CHWs), equipped with digital audio players, to conduct a 12-month (September 2015 to August 2016) echinococcosis prevention health education intervention in a Tibetan village located in Ganzi Tibetan autonomous prefecture in Sichuan Province. CHWs provided information on disease transmission and prevention approaches during household visits and at community gatherings. We evaluated this approach using pre- and post-intervention surveys and focus group interviews; villagers were recruited through convenience sampling. Following the intervention, 98.4% of 64 post-survey villagers indicated awareness of both the importance of not feeding offal to their dogs and of not drinking river water directly, compared with 56.1% and 61.4% of 57 pre-survey villagers, respectively. During focus group interviews, the majority of 22 participating villagers indicated that the CHW health education interventions improved their health behavior practice, including drinking only boiled water and avoiding consumption of raw meat. Ten of 12 CHWs indicated that the audio player was an effective tool for communicating echinococcosis messages. Although the intervention appeared to have increased villagers' knowledge about echinococcosis transmission and prevention and their health practice, follow-up assessments are needed to confirm this finding. We recommend using CHWs to conduct similar echinococcosis interventions in other high-risk communities along with additional evaluations aimed estimating impact on behavioral change.

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AWARENESS ABOUT APPROPRIATE ANTIBIOTIC USE IN A RURAL DISTRICT IN SUB-SAHARAN AFRICA: WHERE IS THE STARTING POINT FOR PREVENTION OF ANTIBIOTIC RESISTANCE?

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Misuse of antibiotics and antibiotic resistance (ABR) are global concerns particularly affecting low-and middle income countries. In Mozambique, the community awareness of antibiotics and ABR has not yet been explored. This study aimed to describe community understanding of antibiotics and ABR, as a means of providing an empirical basis for message development and positioning. The study was conducted in Manhiça, a rural district of Southern Mozambique, from 2016 to 2018, and followed a qualitative research design. 16 in-depth interviews and 4 focus group discussions were conducted among community members. The data was analyzed following a grounded theory approach. Half of the participants recognized the term antibiotic, even though their definition was rarely aligned with the biomedical. Participants associated antibiotics to colors, shapes and diseases. The majority of participants did not recognize the term ABR and only two had an understanding of it. Limited knowledge about the biomedical terms and definition of antibiotics and ABR was the main finding of this study. However, community members were willing to build constructs around these terms. Such constructs were built from own experience, exposure to antibiotics, contact with different formal and informal sources and social interactions. Our findings support the need for educational interventions based on the current local knowledge, perceptions and experience to increase antibiotic awareness.

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AN OPEN SOURCE PLATFORM FOR THE DEVELOPMENT OF CLINICAL TRIAL PROTOCOLS FOR POVERTY-RELATED DISEASES

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The most important preparation for successful clinical trials is the conception, development and refinement of a clinical trial protocol that is of high quality and integrity. Protocols are typically being conceived, reviewed and finalised within relatively small groups of researchers. With the support of The Global Health Network (TGHN), The European & Developing Countries Clinical Trials Partnership (EDCTP) aims to develop an open source platform for designing and refining protocols for clinical trials that will be performed in resource-limited settings on medicinal products such as drugs, vaccines, microbicides and medical diagnostics for poverty-related infectious diseases (PRDs). The goal of the platform is to provide guidance for researchers from low- and middle-income countries, who want to develop clinical trial protocols using input from limited, closed groups or the entire scientific community ("open source") for the optimisation of the final protocol. First, we will conduct a landscape analysis of existing resources and a gap analysis of resources, tools and repositories for clinical protocol building. Second, we will create a repository of resources and tools about data repositories and guidelines related to clinical protocol development and develop appropriate training material applicable to trials in resource-limited settings for PRDs. Third, we will develop an online, interactive toolkit to assist researchers in protocol planning and development and allows any interested party to make comments and suggest improvements to the protocol. This will include a dynamic web space to host the web tools and will combine a community of practice made up of researchers in low- and middle-income countries, with resource areas, eLearning courses, examples of well-written protocols etc. This toolkit will be linked with the EDCTP data sharing resource platform (accessible via 'EDCTP Knowledge Hub' currently developed by TGHN) to support researchers to think about data sharing from the outset of clinical trial planning.

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DEVELOPING AN EARLY WARNING SYSTEM TO SCREEN FOR GONORRHEA INFECTION IN GHANA USING COST SENSITIVE CLASSIFICATION AND REGRESSION TREE

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Statistical regression models, such as logistic regression, have been widely employed to analyse gonorrhea occurrence. However, this model has its own assumptions between dependent and independent variables which when violated, can lead to erroneous estimation of gonorrhea occurrence likelihood. To overcome this challenge, a non-parametric model such as Classification and Regression tree (CART) can be used. CART has been employed in business administration, industry, engineering and Medical diagnosis. It is a powerful tool for dealing with prediction and classification. However, most researchers who have used this method in medical diagnosis do not integrate misclassification cost in their model development process. Instead, they focus to improve prediction accuracy assuming equal misclassification cost i.e that the cost of false positives is equal to the cost of false negatives.. This work seeks to develop an early warning system using Cost Sensitive Classification and Regression Tree (CS-CART) which would reduce the false negative (Type II error) prediction when classifying gonorrhea infection status. Gonorrhea surveillance data from five Health facilities in Ghana and spanning the years 2012 to 2016 included 906 patients; 254 positive and 652 negative gonorrhea cases diagnosed by Nucleic Acid Amplification Test (NAAT) were analyzed. The dataset was partitioned into two; training (637 patients) and testing (269 patients) data.A CART model assuming equal and unequal misclassification cost was developed to classify gonorrhea infection status. The results indicate that false negative rate of the CART and CS-CART was 57% and 0% respectively for the training data while for the testing data, the false negative rate for CART and CS-CART was 82% and 26%, respectively. CS-CART can be used as an early warning system in which most individuals who are not at risk of gonorrhoea would likely be identified and those who are at risk of gonorrhoea would be confirmed using laboratory techniques. This could help save treatment cost and lessen the potential burden of antimicrobial resistance in Ghana.

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BURNOUT AND WHO'S WHO IN PEDIATRIC GLOBAL HEALTH: A LOOK AT NATIONAL TRENDS

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Burnout affects up to 50% of residents, yet little is known about the relationship between participation in global health (GH) and burnout. Secondary analysis of a national cross-sectional electronic survey,

developed by the Pediatric Resident Burnout and Resilience Study Consortium (PRBRSC). Pediatric and IM/peds residents from 34 member institutions were eligible to participate. Chi-squared analyses compared burnout, empathy, spirituality, resilience, and mindfulness in GH and non-GH residents. GH involvement was measured in terms of track participation and also recent/current GH elective. Measurement tools included Davis Empathy Scales including Empathy concern (IRLEC) and Perspective taking (IRLPT), Hatch Spirituality scale, Smith's Brief Resilience Scale, Maslach Burnout Inventory, and Cognitive and Affective Mindfulness Scale. P-values ≤ 0.05 were considered significant. In 2016-2017, 3830 surveys were completed, with 486 (12%) residents reporting GH involvement (GH track or recent/current elective). Residents involved in GH were less likely to be married or have children, and more likely to be IM/Peds residents, when compared to those not involved in GH ($p \leq 0.05$). Residents in GH tracks had higher IRLEC and Hatch scores ($p \leq 0.05$). However, track participants who had completed a recent GH elective (current or last rotation) scored no differently than their GH peers. In conclusion, although GH involvement can enhance personal and professional development and is associated with higher resident empathy and spirituality, the data suggest that it may not offer protection against burnout. Future work should focus on preparing residents for GH with effective pre-departure training, adjusting workloads, and encouraging use of mental health resources to diminish burnout in GH participants.

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THE EBOLA DATA PLATFORM: A NOVEL COLLABORATION FOR TRAINING AND RESEARCH IN EMERGING INFECTIONS

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Despite the potential public health gains of enabling access to patient-level data on emerging infections, the launch of a centralised, international platform to deliver on this remit has not been achieved to date. Barriers include concerns over retention of national data ownership, patient privacy, appropriate consent, loss of academic recognition, criticism or exploitation of the data generators, perceived data misuse and the challenges of sharing benefits with communities where data is generated. Opinions on how to address these barriers vary among the many stakeholders implicated in outbreaks and response, yet the success of a central platform relies on surmounting all of them. In the aftermath of the West African Ebola outbreak, significant collaborative efforts by the affected governments, funders, public health authorities, NGOs and academic institutions have resulted in the establishment of a governance framework to collate and enable access to emerging infections data, beginning with the data from the outbreak. Resources have been invested in developing software, hardware, standards as well as technical expertise in Ebola-affected countries to ensure robust data management and to deliver a secure and consolidated database. The inclusion of scientific and statistical training in the remit of the platform promotes use of the data by the health and research communities in West Africa. The Ebola Data Platform aims to enable ethical, equitable and rapid access to data and information on Ebola and, in the future, other emerging pathogens in resource-limited settings. The platform ultimately seeks to improve patient outcomes, public health preparedness and outbreak response by establishing a new paradigm in data access that can change the global response to public health emergencies.

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ATTITUDES TOWARDS MATERNAL VACCINATION IN PERU

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The Peruvian national expanded program on immunization currently includes Tetanus-diptheria and influenza vaccination for pregnant women. Td coverage was reported as 56.1% for two or more doses in 2012, resulting in 82.1% of women's most recent births being covered against tetanus. Peru recommended influenza vaccination for pregnant women during the 2009-10 H1N1 vaccination campaigns, reaching only 9.1% of pregnant women. They have continued reporting providing seasonal influenza vaccine for pregnant women. Factors associated with maternal vaccination uptake are not well understood in Peru, but a 1995 missed opportunities study found that among 2,031 women of childbearing age, 47% had missed opportunities to vaccinate, most commonly for the first dose of tetanus toxoid. Reasons for missed opportunities included policy in the clinics, such as when to open a vial (31%), personal attitudes of women (30%), logistical challenges of the clinic (15%), false contraindications (20%), and attitude of health workers (4%). Given that 98.4% of women reported attending at least one prenatal care visit, the opportunity to vaccinate women is high. In order to better understand perception of vaccination during pregnancy, we will conduct in-depth interviews with 10 pregnant women. Adult pregnant women will be identified and recruited from a peri-urban community outside of Lima by community health workers. Sociodemographic data such as age, education level, religion, marital status and occupation will be collected. Women will also be scored with the Parent Attitudes About Childhood Vaccines (PACV) questionnaire to identify vaccine hesitant women. The in-depth interview guide includes knowledge of vaccines, antenatal care experience, comfort level discussing vaccination topics with healthcare personnel, information from peers about maternal vaccines, and motivating factors for vaccination. The results will be stratified by vaccine hesitancy according to the PACV. A preliminary codebook will be used, based on previous research in Latin America but will be updated as needed. Final results will be available August 2018.

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COMMUNITY SUPPORT TO DELIVER INTEGRATED COMMUNITY CASE MANAGEMENT SERVICES IN NIGER STATE, NIGERIA

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Despite strong evidence showing integrated community case management (iCCM) as a proven intervention for reducing childhood mortality, sustainability remains a challenge. Community ownership and contribution are important factors in sustainability. The RAcE-funded iCCM project in Niger state (2014-2018) aimed at improving coverage of diagnostic, treatment, and referral services for malaria, pneumonia, and diarrhoea in children 2-59 months through trained community-oriented resource persons (CORPs). The project's community engagement strategy involved CORPs and social mobilizers (SMs) to increase community demand and support for iCCM services. SMs sensitized community members on appropriate health seeking behaviour and mobilized community engagement in iCCM. CORPs' needs were discussed in community dialogues, decisions taken to address them, and follow-up actions jointly implemented by community members, SMs and state iCCM team. SMs documented community support provided to CORPs in cash or kind and validated these during CORP review meetings. The value of the support

was estimated in US$ using current market rates and actual costs as appropriate. Over the project period, the value of all community support to CORPs was estimated at $122,786. Of this, 433 CORPs received cash assistance of about $18,131; 12 received community contributions for building their houses estimated at $12,376; 302 received chemicals and other farming support worth $67,790; while 301 received farm products and gifts. 47 CORPs received fuel or transport fare for drug collection from supervising health facilities totalling $1,437; five CORPs received motorcycles worth $984 and one a bicycle ($30). Best practices shared at local review meetings encouraged other communities to replicate achievements. Community contributions documented demonstrate the acceptability of iCCM in beneficiary communities and their willingness to contribute to ensure sustained services. Every community possesses unique resources to support iCCM implementation when explored through meaningful and transparent engagement.

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TEACHING GLOBAL HEALTH COMPETENCIES TO MEDICAL STUDENTS THROUGH EXPERIENTIAL LEARNING WITH LOW-WAGE MIGRANT WORKERS IN SINGAPORE

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Global health competencies are recognised as an important, if not essential part of medical education, including cross-cultural skills, an understanding of migrant health, and primary health care in diverse settings, but not necessarily in an overseas context. This paper therefore reports on a novel global health education intervention spearheaded by the National University of Singapore School of Medicine, in partnership with HealthServe, a local non-governmental organisation that provides low cost healthcare services, casework and social assistance to migrant workers. While male low-wage migrant workers constitute 13% of Singapore's population, they have limited social contact with and are often poorly understood by the local population, and are often vulnerable due to limited medical, financial, legal and social support. The experiential learning occurs during the third year Family Medicine placement in a five-year undergraduate curriculum, where students interact with migrant workers in different settings over four evenings: at HealthServe's low-cost primary healthcare clinic, as guests at a migrant workers' dormitory, and informal social interactions in districts frequented by migrant workers in Singapore. The selective was designed to allow students to experience and reflect on the complexities of the psycho-social-cultural, political and global context of health and medicine within this vulnerable population. Over 500 reflective essays were collected between 2013 to 2018. In this paper, the results of a thematic analysis of these student reflections will be presented; emerging themes include students gaining more understanding and empathy for migrant workers' circumstances and disparate access to healthcare, gratitude for their own circumstances, and realising that they as privileged Singaporeans are not very different from low-wage migrant workers who come from other Asian countries to undertake the unpleasant work that Singaporeans don't wish to do. The analysis suggests that the learning experience resulted in the students' transformational learning and gaining of global health competencies.

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REACHING THE HARD TO REACH: EARLY WARNING ALERT AND RESPONSE NETWORK AS A CRITICAL TOOL FOR REAL-TIME SURVEILLANCE DURING EMERGENCIES IN IRAQ

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The early warning alert and response network (eWARN) is a surveillance system that was designed and tested in complex emergency settings. The main objective of eWARN is to rapidly detect and respond to signals that may suggest outbreaks and/or clusters of epidemic-prone diseases when other surveillance systems may be disrupted, overwhelmed, or unavailable. First established as a paper-based surveillance in Iraq in 2013, eWARN was upgraded using a real-time electronic tool in 2015 to accommodate a growing humanitarian crisis and an increasing vulnerability. This transformation process and supporting results give rise to a descriptive analysis using key indicator variables for eWARN's performance, including the number of continuously expanding reporting sites, alerts and consultations, with the goal of examining eWARN's effectiveness during the crisis in Iraq. In June 2015, the electronic version of eWARN had 90 reporting sites. This number grew to 214 by December 2017. While eWARN covered 11 out of 18 governorates in the country, Ninewa, Anbar and Dahuk had the highest number of consultations, reflecting the most severely affected areas by the emergency. In total, there were 8,995,097 consultations from health cluster partners between 2015 and 2017: 1,070,015 in 2015, 2,137,606 in 2016, and 5,787,476 in 2017. The top five reported diseases were acute upper respiratory infection, acute diarrhea, acute lower respiratory infection, skin diseases, and leishmaniasis. These diseases, which represented 41% of the total number of consultations, reflect common communicable diseases that affect refugees, internally displaced populations and hosting communities highlight the areas in need for prevention and control. While there are inherent limitations of the eWARN system, particularly reporting on selected diseases or symptoms, eWARN can prompt early detection of outbreaks, and, if used more long-term, the trends it provides can enhance preparedness. Taken these together, eWARN can contribute by rapidly detecting epidemic prone diseases with the aim of mitigating further transmission and ultimately reducing the burden of the disease.

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A GENERALIZABLE APPROACH TO INCORPORATE LOCATION INFORMATION IN NETWORK RECONSTRUCTION AND OUTBREAK ANALYSIS FOR IMPROVED UNDERSTANDING OF THE SPATIAL ASPECTS OF TRANSMISSION RISK

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Increasingly disease surveillance data, collected routinely and as part of outbreak response, capture a wealth of information which could inform the design of epidemiological interventions. However challenges exist in making use of these diverse data sources. Robust methods to make the most of these data to are required in order best support decision making. Geographic information, in the form of GPS coordinates or address of residence and/or health facility, is often collected but could be more effectively utilised. Furthermore, the relative importance of location in determining observed patterns of infection and transmission risk compared to other factors such as age or occupation remains poorly understood for many diseases. It is unclear whether simple models of distance are sufficient to explain the variation observed and design effective interventions or whether more complex information and data e.g. realistic

models of human movement are required. Here we introduce a new, weighting based method which incorporates spatial or similar distance-based information into our previously developed methods to quantify spatiotemporal variation in transmission. We use these methods to quantify the relative contribution of location on the risk of one individual infecting another. We apply this method to line lists from a variety of diseases including measles, Ebola, malaria outbreaks in near elimination settings and H7N9 influenza as well as simulated outbreaks. We carry out model selection to explore various assumptions about the relationship between locations of cases and likelihood of transmission occurring between them including binary near/far thresholds, euclididan distance, power law and gaussian kernels, gravity models, and population flow models. We demonstrate the applicability of our approach to a variety of contexts and discuss results in relation to public health intervention design. Our approach also provides the potential to incorporate other sources of information which can be converted into distance matrices such as age or molecular markers.

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TT SURGERY TRACKER - IMPROVING PATIENT CARE AS WE REACH ELIMINATION

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The global trachoma program has seen tremendous gains in the effort to eliminate blinding trachoma as a public health program, conducting over 230,000 trichiasis surgeries annually. With such gains comes the need to track not only the provision of the service, but the outcome of the surgeries provided. Few programs are conducting routine follow-up procedures in order to assess post-operative outcomes. Programs use templates from the International Coalition for Trachoma Control (ICTC) for consistent data collection, but the forms are paper-based, requiring lengthy processes to locate patient information and track patient outcomes. Due to these challenges, Sightsavers developed a mobile phone application to improve the systematic tracking of surgery provision in the global program. The android-based TT Surgery Tracker enhances timeliness and reporting of post-operative patient follow-up to improve quality of care. The tool documents surgeries conducted and the outcomes, by surgeon, to determine the need for additional training. It aims to increase follow-up completion through emailed reports to supervisors showing where and when follow-ups must take place. It also provides surgeons a monthly report, allowing them to assess their performance and providing encouragement for their efforts. Designated high-level workers can access up-to-date data online to view and download reports. Since November 2017, the application has been rolled out for testing in select districts in Nigeria and Tanzania. In total, 40 surgeons and recorders have been trained. To date, data have been collected on 896 and 517 surgeries and their subsequent follow-ups in Nigeria and Tanzania, respectively. Provisional results demonstrate that field teams are pleased with the Tracker's ease of use and benefits and Sightsavers is seeing positive results around improved efficiencies and program reporting - resulting in improved patient care. Once finalized in May 2018, the application will be provided to all country programs where enhanced trichiasis patient tracking is needed. It is anticipated that 10 country programs will be using the TT Tracker by the end of 2018.

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AN AGENT-BASED MODEL OF EFFECTIVE COVERAGE OF APPROPRIATE MANAGEMENT OF CHILD ILLNESS

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Effective coverage of correct management of child illness is dependent on seeking care from a healthcare provider and receiving appropriate care from that provider. Agent-based modeling (ABM) is a potential means of modeling effective coverage using both household and healthcare provider data. ABM uses simple rules to model complex behaviors. In ABM, heterogeneous autonomous micro-entities or agents, act and interact with each other via defined rules. Agents make decisions or execute behaviors based on a defined set of rules. These micro-actions result in macro-level dynamics. We created and ran an ABM of care-seeking and management of child illness using NetLogo 6.0 and the NetLogo R extension. We parameterized the model using data from a household survey on care-seeking for 385 child illnesses and 83 healthcare provider assessments we conducted in Southern Province, Zambia in 2016. We collected data on 1) the timing and location of care-seeking for child illness, 2) potential determinants of care-seeking including illness, child, caregiver, and household characteristics, and 3) healthcare provider accessibility, cost, and structural quality to manage child illness. Sick children and providers were included in the model as agents dispersed over a geospatial plane. We used multilevel models to assess the relative effect of various factors on mothers' decisions to seek care. These values were used to parameterize care-seeking actions in our ABM. Once a child accessed care from a provider, they were assigned to have received correct or incorrect care with a probability derived from the provider's structural quality score. We estimated effective coverage as the total proportion of sick children that were correctly managed. We refined the model over multiple iterations. We assessed model performance by comparing the ABM effective coverage estimates to our empirical estimates of coverage. We modified model parameters to assess how coverage would change with alterations in care-seeking behavior and provider structural quality. ABM can be an effective means of modeling complex behaviors where adequate data for parameterization exists.

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CLINEPIDB: THE CLINICAL EPIDEMIOLOGY DATABASE RESOURCE

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Population-based epidemiological studies provide new opportunities for innovation and collaboration among researchers addressing pressing global-health concerns, however open access to study data pose many challenges. ClinEpiDB (https://clinepidb.org) is an open-access online resource enabling investigators to maximize the utility and reach of their data and to make optimal use of data released by others. ClinEpiDB was developed using the existing infrastructure of EuPathDB (https://eupathdb.org), a collection of databases covering 170+ eukaryotic pathogens, along with relevant free-living and non-pathogenic species and select pathogen hosts, which provides a sophisticated search strategy system enabling complex interrogations of underlying data. Currently, data integration for ClinEpiDB has occurred or is in process for NIH-supported International Centers for Excellence in Malaria Research (ICEMR), the Gates Foundation-supported Malnutrition and Enteric Diseases Network (MAL-ED), and the Global Enteric Multicenter Study (GEMS) projects. In the process of data integration, a unified semantic web framework has been used to describe data generated from these studies. The ICEMR projects used comprehensive surveillance data to elucidate interactions between malaria parasites, their mosquito vectors, and human hosts. The MAL-ED and GEMS projects studied the etiology, incidence and impact of childhood enteric disease in low-income countries. Over 1500 different data variables about participants, their associated anthropometry, demographics, and disease episodes were collected in these clinical epidemiology studies. Query results can be statistically analyzed and graphically visualized via interactive web applications launched directly in the ClinEpiDB browser,

providing insight into distributions and exploratory associations with any observational covariates. The ClinEpiDB resource will continue to grow with integration of new datasets, enhanced tool development and significant user outreach and education.

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NOVEL METHODS FOR CONDUCTING A CENSUS TO DEFINE THE SAMPLING FRAME FOR A MALARIA HOUSEHOLD SURVEY IN ARTIBONITE, HAITI

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The Malaria Zero (MZ) consortium is working with the Ministry of Public Health and Population in Haiti to eliminate malaria by 2020. To inform the elimination strategy, a household survey was conducted in the communes of La Chapelle and Verrettes of Artibonite. In the absence of reliable census data to define an accurate sampling frame, we used novel methods to enumerate the population, monitor progress, and facilitate re-identification of the sampled households (HH) after selection. DigitalGlobe satellite images were imported into ESRI ArcGIS and overlayed with LandScan™ 1x1km grids that served as enumeration areas (EA). Images of all rooftops in the study area were digitized, and resulting maps used to develop the deployment plan for the enumerators. The maps were loaded onto tablets as geospatial PDFs with satellite and OpenStreetMap base layers to aid navigation. Enumerators captured GPS coordinates and details of each HH (e.g., inhabited, number living in HH, head of HH name), point of interest (POI; e.g., landmarks to assist in relocating a HH), and other digitized structures. Enumerators also talked with local guides and visited EA with no digitized structures to identify HH and POI that had been built since the date of the satellite images or to verify none existed. Data were collected on GSMA-enabled tablets for near real-time data synchronization using CommCare. Nightly, the coordinates of structures identified during the day were overlayed on those digitized using Quantum GIS to identify completed EA and areas of missed coverage. Each morning, these coverage maps were delivered to enumerators either on paper or via WhatsApp Messenger so they could return to the area if needed. Daily, coverage maps with cumulative numbers of HH, persons, and POI enumerated, and alerts on operational challenges (e.g., data connectivity, flooded roads) were sent to remote staff elsewhere in Haiti and the United States. In total, the census identified 33,060 households with an estimated population of 121,593 and 2,311 POI. The use of maps with digitized structures and daily coverage were novel methods that likely improved the quality of the population census.

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THE FEASIBILITY OF CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS): GENERATING CONSTRUCTS, VARIABLES AND THEMES TO PRODUCE CREDIBILITY IN QUALITATIVE RESEARCH

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CHAMPS aims to address childhood mortality by generating reliable data on the causes of death for children under 5. This process is complex as data are derived from various sources, including laboratory findings from minimally invasive tissue sampling (MITS) conducted on the body of a deceased child. The sensitive nature of this procedure inevitably evokes religious, cultural, and ethical questions that influence the feasibility of CHAMPS. Due to limited behavioral studies and theoretically sound paradigms related to child MITS, we developed an innovative qualitative methodology to determine the barriers, facilitators and other factors that affect the implementation and sustainability of CHAMPS surveillance across 7 diverse sites. We employed a multi-method grounded theory approach and analytical structure based on culturally specific frameworks comprised of focused constructs and variables. These frameworks provided direction for in-depth interviews, focus groups and observations needed to maximize the credibility of CHAMPS feasibility. The frameworks also guided data interpretation and collective analyses confirming how to articulate and define dimensions of CHAMPS feasibility within the cultural context of each site. Findings showed that the approach to MITS consent and procedure involve incorporating religious factors associated with timing of burial, use of certain consent terminology and methods of transporting the body. Community misperceptions and uncertainties of CHAMPS resulted in a need for rumor surveillance and consistency in information sharing. Other findings indicated that religious pronouncements, recognition of health priorities, attention to pregnancy and advancement of child health facilitates community acceptability. These findings helped form program priorities, site-specific adaptations in surveillance procedures, and verified the integrity of inferences drawn from diverse epidemiological and social data. Results informed appropriate community sensitization and engagement activities for introducing and sustaining mortality surveillance including MITS.

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SPATIAL PATTERNS OF WATER STORAGE PRACTICES AND UPTAKE OF VECTOR CONTROL BEHAVIORS FOR ZIKA TO IMPROVE PREDICTIVE MAPPING OF *AEDES AEGYPTI* IN CENTRAL AMERICA

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In 2015-2016 there was an outbreak of Zika virus throughout central and South America, a region where previously no cases had been recorded. This outbreak was associated with increased incidence of microcephaly and Guillain-Barre Syndrome; in one year it infected over 500,000 people (suspected and confirmed cases) in 40 countries and territories in the Americas. Zika virus is transmitted by *Aedes aegypti*, which is also the primary vector for Dengue, Chikungunya and Yellow Fever, diseases considered endemic to the region. *Ae. aegypti* has flourished in urban landscapes, where human-driven activities have created more suitable environments for larval development. While GIS data and maps have been used to create epidemiological maps to guide programmatic targeting of vector control activities, this has been challenging for Aedes-borne diseases, with entomological maps failing to align with human infection risk. We propose inclusion of human behavior to improve the precision of these predictive maps. A cross-sectional household survey will be implemented between April and June 2018 in the Dominican Republic, Honduras, Guatemala and El Salvador (n=600 per country). GPS points will be recorded for each household, to explore the spatial patterns of knowledge, attitudes and practices related to vector control behaviors. Using satellite imagery and remotely sensed data, environmental data previously reported to be associated with *Ae. aegypti* abundance will be overlaid on the survey data; bare soil coverage percentage, urbanization coverage percentage, water distribution, weekly rainfall and population density. These data will be used to explore spatial variation in vector abundance using available sentinel entomological data, and to explore linkages with household water storage practices and prevention behaviors. We hypothesize that incorporation of household adoption of recommended vector control behaviors will improve the fit of models, when compared to environmental factors alone.

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PREDICTING TICKBORNE INFECTIONS AND COINFECTIONS WITH DIAGNOSTIC DECISION TREES

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Tick-transmitted infections and coinfections with emerging pathogens are increasing in the United States (US) and complicating diagnosis and treatment. To identify the most common tickborne infections and coinfections and their vectors in the US and to design a decision tree approach to the diagnosis of tickborne infections, Internet search engines were queried with key words to select scientific articles on tickborne infections and coinfections over the reporting period, 2000-2018. Key words included ticks, emerging and reemerging tickborne infections and coinfections. Molecular studies of pathogen coinfection seroprevalence rates in ticks and animal reservoir hosts have confirmed that pathogen infections and coinfections with *Borrelia burgdorferi*, *Babesia microti*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, and *E. ewingii* do occur in regional zoonoses and that nymph and adult ticks that feed on multiple infected animal hosts can transmit these pathogens concurrently to humans during blood-feeding. Among the ten tick species capable of transmitting coinfections, the four tick species that have transmitted regional coinfections in the US include: (1) *Amblyomma americanum* in the East & Southeast; (2) *Dermacentor andersoni* in the Appalachians & Midwest; (3) *Ixodes pacificus* along the Pacific Coast; and (4) *Ixodes scapularis* in the Northeast and Upper Midwest. Mathematically solving diagnostic decision tree models that account for prior regionally confirmed human cases and current pathogen seroprevalence rates in ticks and their reservoirs will assist clinicians in predicting and diagnosing tickborne infections and coinfections and guiding appropriate antimicrobial therapy. Clinicians should suspect tickborne coinfections in returning travelers and vacationers with clinical and serological evidence of multiple infecting agents, especially in cases of unusual presentation or severity, prolonged duration, or non-response to single antibiotic therapy, typically with doxycycline.

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UNUSUAL PRESENTATIONS OF BABESIOSIS WITH DELAYED DIAGNOSIS IN THREE PATIENTS WITH INTACT SPLEENS AND MIXED INFECTIONS

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Babesiosis is an anthropo-zoonotic tick-borne disease of vertebrates. Of more than 100 known species, only *Babesia microti*, *B divergens, B duncani* and *B venatorum* have been reported in humans. The intra-erythrocytic parasite causes an infection that ranges from asymptomatic to life threatening . More severe and fatal cases have been in asplenic or immunocompromised individuals. some cases have been transmitted by blood transfusion. Here we report 3 unusual presentations, where 2 were initially misdiagnosed as pyelonephritis, and the other accidentally discovered during treatment of neuroborreliosis. One presented with fever for 2 weeks and left flank pain, treated at an urgent care center with macrobid for a urinary infection. She had leukopenia, thrombocytopenia and abnormal liver functions. On therapy with Ceftriaxone and doxycycline she developed a Jarisch-Herxheimer reaction (JH) with high fever and a new rash. *Babesia* smear and PCR was positive for *B microti*. The second patient admitted with left flank pain and fever for 1 week was treated with Levofloxacin. The admission diagnosis was pyelonephritis. Fever worsened and patient had multiple antibiotic changes, with no response. Finally a blood smear was positive with positive antibody test for *B microti*. She gave a history of Lyme Disease. Both responded to atovoquone and azithromycin (A&A). The third patient, while on therapy for neuroborreliosis with ceftriaxone developed fever, thrombocytopenia and anemia. She was empirically started on (A&A). The fever, thrombocytopenia and anemia resolved. Splenomegaly, Splenic rupture and splenic inflammation have been reported in babesiosis. But we are not aware of any misdiagnosed as pyelonephritis. Source of the left abdominal discomfort was the spleen. JH is known occur during therapy of Spirochetal infections such as *Borrelia*, *Treponema* and *Leptospira*. We believe JH was due to a mixed infection. *B burgdorferi* and *B miyamotoi* have been reported in *Ixodes scapularis* in the area. Lyme Western Blot was negative. In areas with heavy tick infestation, clinicians have to be vigilant to avoid misdiagnosis of tick-borne infections.

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PCR OF WHOLE BLOOD COMPLEMENTS SEROLOGY FOR CONFIRMATION OF EARLY LYME DISEASE AND ALLOWS FOR CONFIRMING A DIAGNOSIS IN 10% OF ACUTE PATIENTS WITHIN A NEGATIVE ANTIBODY WINDOW PERIOD

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Early Lyme disease is a clinical diagnosis when a patient presents with typical erythema migrans. However, atypical dermal lesions or febrile illness without erythema migrans requires laboratory testing to establish a diagnosis of Lyme disease. Such testing usually comprises serology, preferably of acute and convalescent samples. Directly detecting *Borrelia burgdorferi*, the agent of Lyme disease, has historically been impractical due to the need for invasive skin biopsies from which it may be cultured, and because of the low numbers of organisms in routine clinical specimens. *B. burgdorferi*, however, may be cultured from large volumes of whole blood sampled from early Lyme disease patients. It may be that more recent protocols for extracting larger volumes of blood for DNA and the use of sensitive real time PCR assays may also detect hematogenous *B. burgdorferi* from early Lyme disease patients. Accordingly, we analyzed 21,433 unique acute patient specimens submitted for Lyme diagnostic testing during 2016, focusing on those for which both serology and PCR testing had been requested. Real time PCR was performed using a pan-*Borrelia* primer set and any positive reanalyzed specifically for evidence of *B. burgdorferi* or *B. miyamotoi*. Of these, 1569 (7.3%) had laboratory results consistent with early Lyme disease. The majority (83.5%) of these 1569 samples were considered reactive in an IgM capture EIA with confirmation by immunoblot but were PCR negative. Another 5% were concurrently IgM positive and PCR positive. Interestingly, 11.5% were considered seronegative but PCR positive. We conclude that complementing serology with PCR incrementally improves the confirmation of a diagnosis of early Lyme disease, particularly for those patients within a window period when antibody may not yet be detectable.

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CLIMATE CHANGE INFLUENCES ON THE POTENTIAL GEOGRAPHIC DISTRIBUTION OF THE DISEASE VECTOR TICK *IXODES RICINUS*

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Ixodes ricinus is a species of hard tick that transmits several important diseases in Europe and North Africa, including Lyme borreliosis and tick-borne encephalitis. Here, we assessed the potential distribution of *I. ricinus* under current and future climate conditions to understand how

climate change will influence the geographic distribution of this important tick vector and associated tick-borne pathogens in coming decades. We used ecological niche modeling to estimate the geographic distribution of *I. ricinus* with respect to current climate, and then assessed its future potential distribution under different climate change scenarios. This approach integrates occurrence records of *I. ricinus* with six relevant environmental variables over a continental extent that includes Europe, North Africa, and the Middle East. Future projections were based on climate data from 17 general circulation models (GCMs) under 2 representative concentration pathway emissions scenarios (RCPs), for the years 2050 and 2070. The results show that the present and future potential distributions of *I. ricinus* showed broad overlap across most of western and central Europe, and in more narrow zones in eastern and northern Europe, and North Africa. Potential expansions were observed in northern and Eastern Europe. These results indicate that *I. ricinus* populations could emerge in areas in which they are currently lacking, posing increased risks to human health in those areas.

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MODELING ENVIRONMENTAL DRIVERS OF HOST-SEEKING BEHAVIORS THAT AFFECT BLACKLEGGED TICK HOST-FINDING SUCCESS

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The blacklegged tick (*Ixodes scapularis*) is the primary vector of *Borrelia burgdorferi*, the causative agent of Lyme disease in the US. The basic reproduction number (R_0) for pathogens transmitted by this tick is highly sensitive to the probability of larvae molting into nymphs and subsequently contacting a host. Incorporating regional variation in nymph host-finding success into models will allow for more accurate prediction of tick-borne pathogen persistence or decline. Here we present a novel approach to evaluating the relationship between host-finding success and host abundance that combines field data with a dynamic state variable model of tick questing behavior. Mark-recapture density estimates for the primary host, the white-footed mouse (*Peromyscus leucopus*) and on-host larval and nymphal densities from live-trapped mice, were obtained in 2015 and 2016 in mainland Connecticut and on Block Island, Rhode Island, with higher densities estimated at the island site. Tick overwinter survival - estimated from ticks enclosed in tubes - was >50% and did not differ significantly between sites. Ambient and leaf litter relative humidity (RH) were collected via HOBO weather stations and iButton data loggers during the questing season. Mean RH over this period varied significantly between sites (mainland air: 78.4%; mainland litter: 90.1%; island air: 90.2%; island litter: 91.6%). Using these data as dynamic state variable model inputs, we predicted host-finding success. Model predictions were then compared to host-finding success estimates for the two locations obtained by calculating the ratio of on-host nymph density to on-host larva density at the same site the preceding year, taking into account overwinter survival. Differences between the two sets of estimates were used to reparametrize the relationship between host population density and the probability of encountering a host while questing, which is a key function in our model. Informing the dynamic state variable model with field data served as an initial step towards disentangling the roles played by climate and host availability in determining tick host-finding success.

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HIGH SENSITIVITY DETECTION OF MULTIPLE TICK-BORNE INFECTIONS IN BLOOD AND URINE USING NANOPARTICLE ENTRAPMENT AND MASS SPECTROMETRY

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Tick-borne diseases (TBD) among humans are on the rise in North America. As ticks may be co-infected with several pathogens, there is a high likelihood of co-transmission of Lyme disease, babesiosis, and other TBDs to humans, thus affecting disease severity. To date, there are no FDA-approved assays for mass screening of TBDs. To address this need, our group uses a new class of biomarker harvesting nanocage technology, which, when combined with mass spectrometry (MS) can determine the presence of pathogen proteins shed in bodily fluids of mammalian hosts. Using the hamster model of babesiosis, our nanoparticle-MS approach identified several *B. microti* proteins in blood, serum, and urine. Surface and secreted antigens previously shown to elicit host immune responses against the parasite were particularly abundant compared to other proteins. Confirmation of expression of three of these antigens was shown in the cytoplasm and/or surface of infected hamster erythrocytes by immunological assays. Of note, urine samples from acutely infected animals harbored parasite proteins with housekeeping functions. Interestingly, the presence of urinary proteins was not exclusive to the experimental hamster model, as MS analysis of urine samples from human patients with TBD also revealed the presence of babesial proteins. Moreover, a subset of patients showed evidence of multiple co-infections, as peptides deriving from tick borne pathogens such as *Borrelia burgdorferi*, *Ehrlichia* sp., *Rickettsia* sp., *Anaplasma phagocytophilum*, and *Francisella tularensis* were also detected in urine. In summary, using a novel nanoparticle-MS approach, we have achieved a high level of analytical sensitivity necessary to detect multiple TBDs in blood and urine. This was previously difficult to achieve due to the low abundance of microbial markers concealed by the complex biomolecular matrix of bodily fluids from the host. Further adaptation of this technology into high throughput testing and/or immunochromatographic tests can provide a platform for a one-step, rapid analysis of multiple tick borne infections for use in screening, transmission control, and treatment.

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THE *BABESIA* OBSERVATIONAL ANTIBODY (BAOBAB) STUDY: SURVEILLANCE FOR *BABESIA MICROTI* IN KILOSA DISTRICT, TANZANIA

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Babesia is a genus of tick-borne intraerythrocytic protozoan parasites. Most human *Babesia* infections, ascribed to *B. microti*, are subclinical or mild, yet severe and fatal complications are well described. *Babesia* is globally ubiquitous yet human surveillance is lacking, particularly in Africa.

Findings from a pilot *B. microti* serosurvey in Kilosa district, Tanzania, suggested that *Babesia* may be present. In follow-up, a cross-sectional study was conducted July-August 2017: residents in a case hamlet that had clustering of subjects with high signal-to-cut off (S/CO) ratios for antibodies against *B. microti* in the pilot study, and a control hamlet that had lacked significant signal, were evaluated for *B. microti*. In addition to clinical evaluation and household inspections, subjects aged ≥15yrs (n=299) had 10ml whole blood drawn for evaluation by transcription mediated amplification (TMA) for *B. microti, B. divergens, B. venatorum* and *B. duncani*; *B. microti* indirect fluorescent antibody testing and rapid diagnostic testing (RDT) for *Plasmodium* spp. Those aged <15yrs (n=266) underwent RDT for *Plasmodium* and serological assessment with a *B. microti* ELISA. A total of 570 subjects participated (mean age 22 [<1 to 90yrs]) of whom 50.7% were female; 145 (25.5%) subjects were *Plasmodium* RDT positive (+). In those <15yrs, the median ELISA S/CO was 1.11 (IQR 0.80-1.50); the median S/CO in the case (n=121) and control (n=146) hamlets were 1.19 (IQR 0.81-1.48) and 1.06 (IQR 0.80-1.50) respectively (p=0.3). Children ≥5yrs old were more likely to have a higher S/CO ratio than those <5yrs old (p<0.001). Ninety-nine (37%) subjects <15yrs were *Plasmodium* RDT+. The median S/CO ratio (children <15yrs) did not differ by RDT status (p=0.18). In subjects ≥15yrs, no molecular positivity was detected for *Babesia*; four subjects were IFA reactive (two each at titers of 128 and 256). While an increase in *B. microti* seroreactivity with age is in keeping with true positivity, random distribution of *B. microti* seroreactivity in both the case and control hamlets raises concerns of cross-reactivity (e.g. *Plasmodium*, non-zoonotic *Babesia* spp.) or non-specificity.

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THE TICK APP: A SMARTPHONE APPLICATION TO ASSESS HUMAN BEHAVIORAL RISK FACTORS OF HUMAN-TICK CONTACTS

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Zoonotic diseases occur at the interface of human and ecosystem health. However, the lack of sufficient knowledge on the complex interactions between humans and the natural world has been a major barrier to the effective implementation of sustained disease control methods, with multiple examples across vector-borne disease systems. In the case of Lyme disease, individual human exposure will depend on the density of infected ticks (entomological risk) as well as on human activity and movement patterns affecting their exposure risk. As part of a broader project on the coupled natural and human dynamics of tick-borne diseases, here we report on the implementation of a smartphone application, The Tick App, as a survey tool to collect high spatial and temporal resolution data on human behaviors and movements associated with tick exposure. This app also aims to raise awareness among the general public by engaging users in tick identification and reporting. The ubiquity of smartphones allows for the potential to reach a large section of the population in a more cost-effective manner than most traditional epidemiological surveys. Traditional mechanisms of data collection (e.g., retrospective questionnaires) are subject to recall bias and do not capture specifics in intra-subject variability, particularly when participants are required to generalize an experience or behavior. The Tick App addresses this issue by allowing for real-time reporting. During the summer of 2017, 11 people piloted the app on Block Island, RI, with an average of 56 active sampling days. Based on this experience, a subsequent version of the app was made available to the public during the spring and summer of 2018 in the U.S. midwestern and northeastern regions. Its implementation was monitored closely in two areas within both regions across a gradient of endemicity, human demography and urbanization. Incorporating behavioral and movement data into empirically-informed tick-borne disease models will provide insights into the relative importance of factors driven by humans and nature, their interactions, and potential targets for intervention strategies.

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MODELING LYME DISEASE SYMPTOMATOLOGY IN ASSOCIATION WITH TICK-BORNE PATHOGEN CO-INFECTIONS IN HUNTING DOGS IN THE UNITED STATES

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In North America, infection with the spirochetal bacterium, Borrelia burgdorferi, transmitted by Ixodes scapularis and Ixodes pacificus ticks, is the primary cause of Lyme disease in humans and dogs. Moreover, Ixodes spp. are known to transmit other pathogens and there is a high potential for co-infection with multiple tick-borne diseases. However, the subsequent effects on symptom severity are not well described in the literature. Thus, by modeling both co-infection rates and Lyme disease severity, classified by the number of lyme positive symptoms, this project aims to shed light on the interplay between pathogens in a co-infected canine host. Blood samples collected from a cohort of approximately 650 hunting dogs around the United States were analyzed for B. burgdorferi, Anaplasma spp., Ehrlichia spp., and Babesia using qPCR and ELISA to detect presence or absence of tick-borne pathogen DNA or antibodies, respectively. Clinical signs of Lyme disease were also recorded and canine disease status was classified into 3 categories: Asymptomatic, classified by a positive Polymerase chain reaction (PCR) or serologic result (ELISA) and less than 2 lyme signs. Symptomatic, classified by a positive PCR or ELISA result and 2 or more lyme signs, or Negative, classified by a negative PCR and ELISA result. Co-infection and exposure rates were calculated using statistical analysis, following this, lyme symptomatic data was correlated with co-infecting tick-borne pathogens. Moreover, this data was then used to develop a regression model with lyme symptomatic as an outcome, that would assess relative risk associated with tick-borne disease co-infection By better identifying host-pathogen interactions in dogs, this study hopes to provide information regarding how Lyme disease may manifest in other co-infected hosts, such as humans. Therefore, providing information that may improve our ability to diagnose and treat Lyme disease.

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A DUO 4-PLEX REAL TIME PCR FOR DETECTION OF EIGHT TICK BORNE ZOONOSES IN KENYA

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Ticks can harbor multiple pathogens which they may transfer to humans. The ensuing zoonoses display non specific symptoms that make definitive diagnosis difficult. We report here the development and evaluation of multiplex qPCR assays for eight tick borne zoonoses (TBZ). The assays were organized in duo formats of 4-plex each. Format 1 was optimized for *Anaplasma phagocytophilum*, *Coxiella burnetii, Borrelia burgdoferi and Ehrlichia chaffeensis.* Format 2 was optimized for *Rickettsia spp*, *Bartonella spp*, *Borrelia spp* other than *B. burgdoferi* and *Babesia spp*. using synthetic plasmids; we show that the assays can specifically detect all the target sequences in the same reaction tube. Assay 1 had a limit of detection of 1gene copy for all targets. Assay 2 was less sensitive and on average had a limit of detection of 18 gene copies. In replicate tests, both assays had intra-assay variation of less than two cycles. Evaluation of 512 clinical samples collected from AFI patients attending hospitals in different counties revealed a 20 % prevalence of tick borne pathogens comprising *B. burgdoferi* (7 %), non *B. burgdoferi Borrelia spp* (4 %), *C. burnetii* (5 %), *A. phagocytophilum* (6 %), *Rickettsia* (2 %), *E. chaffeensis* (0.8

%), *Bartonella spp* (0.8%), and *Babesia spp* (0.4 %). The high analytical sensitivity suggests potential for the duo 4-plex qPCR for detection of common TBZ.

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APPLICATION OF WHOLE GENOME SEQUENCING TO STUDY THE GENETIC DIVERSITY OF FIELD COLLECTED *ORNITHODORUS* TICKS AND THE PATHOGENS THEY CARRY FROM NIGERIA

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Ornithodorus ticks are established vectors of relapsing fever in humans caused by *Borrelia* species and have also been reported to be potential vector for the recently emerging Alkhurma haemorrhagic fever virus. They have been reported from various parts of the world. However, there are not much genomic data utilizing sensitive techniques from Africa. Whole Genome Sequencing (WGS) using MinION (Oxford Nanopore), is a new and rapid third generation sequencing technology that has been successfully utilized to determine species of mosquito vectors and detect a variety of vector borne viruses. Using qPCR and WGS, we attempt to determine the species of *Ornithodorus* ticks and the pathogen they carry from a semi-arid Northeastern Nigeria. The sequence data generated was used to infer phylogenetic relationships among ticks as well as the pathogens detected. Genomic DNA was extracted from field collected ticks pooled by life stages. PCR analysis to identify tick species using primers against tick 16S gene and Sanger sequencing of resulting amplicons was initially carried out. The preliminary results by qPCR revealed the species of ticks to be *Ornithodoros savignyi.* Samples were further screened for the presence of *Borrelia* by sequencing of 16S, flagellin and IGS regions which revealed the presence of *Candidatus* Borrelia kalaharica. A recently proposed species that caused relapsing fever in a returning traveller from Southern Africa to Germany. The complete results from more extensive field sampling and analyses from sequence generated from the MinION will be presented at the meeting. This is the first report of relapsing fever *Borrelia* of public health importance from Nigeria. Furthermore, genomic data generated could help in better understanding of the diversity of tick species and for improved vector-borne disease diagnostics.

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SYNANTHROPIC RODENTS AS CARRIERS OF IMPORTANT ZOONOTIC PATHOGENS - FIRST REPORT FROM BHUTAN

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The number of acute undifferentiated febrile illness cases (AUFI) has been on the rise recently in Bhutan leading to misdiagnosis and inadequate treatment. Bhutan is a small country known for its rich biodiversity and strong conservation policies. It has approximately 80 rodent species. Rodents are well known reservoirs and vectors of many emerging and re-emerging infectious diseases, but little is known about their potential role in zoonotic disease transmission in Bhutan. A cross-sectional study of zoonotic disease pathogens in rodents was performed in Chukha district, Bhutan where many AUFI cases have been detected in people. Field collections were performed in 2016 and 2017. Rodents in and around houses and agricultural fields were trapped using live wire mesh traps. Following euthanasia, liver and kidney tissues were removed and tested by Polymerase Chain Reaction (PCR) for *O. tsutsugamushi* and other bacterial and rickettsial pathogens causing Bartonellosis, Borreliosis, human monocytic ehrlichiosis (HME), human granulocytic anaplasmosis (HGA), leptospirosis, and rickettsiosis. A phylogenetic analysis was performed on all rodent species captured and pathogens detected. Four out of the twelve rodents (33%) sampled in 2016 tested positive by PCR for a zoonotic pathogen. *Anaplasma phagocytophilum*, *Bartonella grahamii*, and *B. queenslandensis* were identified for the first time in Bhutan. *Leptospira interrogans* was also detected for the first time from rodents in Bhutan. Twenty-four additional rodents were collected in 2017, the results from these samples are still being analyzed and the final results will be presented during the meeting. Our study shows that sampling even a few rodents in Bhutan can provide important information about potential risks of rodent-borne zoonotic diseases. This is first of this kind of study conducted in Bhutan and more studies are warranted with a larger sample size and that cover more localities and diverse landscapes. We expect interesting findings from rodents sampled in 2017 and ongoing studies. This data will be used to better quantify the risk of rodent-borne zoonotic diseases in Bhutan.

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INCIDENCE OF HUMAN MONOCYTIC EHRLICHIOSIS IN SITES WHERE LONE STAR TICK INFESTATIONS HAVE RECENTLY EMERGED

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The Lone Star tick (*Amblyomma americanum*), has greatly expanded its distribution in the northeastern United States within the last decade. Once largely restricted to western Long Island and New Jersey south to Florida and westward to Texas, sporadic specimens have now been recorded from all northeastern states, and focal perpetuating infestations have been identified in Massachusetts. This aggressively human biting tick is a known vector for STARI/Masters Disease, human monocytic ehrlichiosis (HME), *Ehrlichia ewingii* ehrlichiosis, Rocky Mountain spotted fever, and tularemia; a common symbiont of Lone Star ticks, *Rickettsia amblyommatis*, is suspected of causing febrile illness in people. The public health burden of new Lone Star tick infestations has not been determined. We analyzed 37,133 blood samples submitted by clinical practices in CT, MA, RI, ME, NH, and VT during 2016-2017 for evidence of *Ehrlichia chaffeensis* (the agent of HME) DNA by polymerase chain reaction. By comparison, 21,082 blood samples were similarly analyzed from NJ, NY, PA, and MD, where Lone Star ticks have been endemic for at least two decades. Only two samples (0.005%) were positive from the New England sites whereas 0.63% of those from sites farther south were positive. We conclude that risk of acquiring HME and likely that of the other less common zoonotic infections transmitted by Lone Star ticks is very small in sites where they have recently invaded.

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RISK OF TICK-BORNE ENCEPHALITIS VIRUS INFECTION IN NORTHERN KAZAKHSTAN

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Tick-borne encephalitis (TBE) is becoming a growing public health problem in Kazakhstan. Recognized areas of spread of this infection are the eastern and south-eastern regions. At the same time, in recent years, there have been registered cases of TBE infection in the northern region of the republic, in particular in the Akmola region (24 cases in the period

of 2010 - 2017). The purpose of our research was to determine the tick species circulating in the northern region for the presence of TBEV. A total of 2,036 tick samples were collected in the territories of three oblasts of Northern Kazakhstan (Akmola, North Kazakhstan, Kostanay), divided into 257 pools according to species, gender, and collection location, and prepared for research. The species composition of ticks was represented mainly by *D. marginatus* and *D. pictus*, and to a lesser extent *Ix.persulcatus*. Homogenized tick pools were tested in parallel, using ELISA and PCR methods to compare and determine their viral load. All results were negative with ELISA. Using RT-PCR assays, the RNA of TBE virus was detected in 24 samples (9.3%). The highest rate of positive results was for the North Kazakhstan (15%) and Akmola (9.7%) oblasts. The tick species composition for positives was *D. marginatus* (62.5%), *D. pictus* (33.4%), and *Ix.persulcatus* (4.1%). The results of the tests show that the TBEV vectors are not only represented by *Ix. persulcatus* - the predominant vector in many parts of the world, but also with ticks of the *Dermacentor* genus (*D.marginatus* and *D.pictus*). This fact significantly increases the risk of infection for the population living in northern Kazakhstan and requires implementation of prevention and control. For the first time TBEV was isolated from ticks in the Kostanay oblast. In addition, it was confirmed that the sensitivity and specificity of the PCR method for TBEV determining is higher than that of antigen capture ELISA. In order to better understand the geographic distribution of infected ticks within Kazakhstan, it is necessary to have further long-term epidemiologic and entomologic TBEV studies.

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SUMMARY OF LABORATORY METHODS USED TO CLASSIFY CONFIRMED AND PROBABLE CASES OF SPOTTED FEVER RICKETTSIOSES—UNITED STATES, 2010-2015

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Spotted fever rickettsioses (SFR) are nationally notifiable diseases in the United States caused by several pathogens including *Rickettsia rickettsii* (the causative agent of Rocky Mountain spotted fever). Case classification is determined using clinical and laboratory criteria. Confirmed cases demonstrate a fourfold change in anti-SFR immunoglobulin G (IgG) serum antibody titers in properly timed paired samples using indirect immunofluorescence antibody (IFA) assay, polymerase chain reaction (PCR), immunohistochemistry (IHC), or culture. Cases are classified as probable when anti-SFR IgG or IgM antibodies can be detected using IFA, enzyme-linked immunosorbent assays (ELISA), dot-ELISA, or latex agglutination (LA). Qualitative assays, infrequently paired specimen collection, and concerns regarding an appropriate threshold of positivity suggest re-evaluation of laboratory criteria. We summarized surveillance data to characterize laboratory methods used to identify SFR cases with onset during 2010-2015 reported in the United States. Using the current SFR national case definition, we determined the proportions of cases identified by each laboratory method. CDC received 14,478 SFR supplemental case reports during 2010-2015. SFR was confirmed in 112 (0.8%) reports, of which 87 demonstrated a fourfold change in IgG titers by IFA, and 25 by PCR, IHC, or culture. The remaining 14,366 (99%) met criteria for probable SFR. Elevated IgG titer was the most commonly reported finding, and was reported in at least one specimen by IFA exclusively (n=9314; 65%) or in combination with other methods (n=2416; 17%); 5522 (47%) recorded one or more titer value <1:128. IFA IgM (3%), ELISA (9%), LA (<.1%), and dot-ELISA (0%) were utilized infrequently independent of other methods. SFR reports rarely show sufficient laboratory evidence to reach confirmed classification. A large proportion of probable cases are supported by a low IgG titer <1:128. Qualitative laboratory criteria and IgM IFAs are used infrequently, can be difficult to interpret, and do not significantly contribute to our understanding of SFR burden in the United States.

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HOST ASSOCIATION AND PREVALENCE OF RICKETTSIAE IN FLEAS COLLECTED FROM DOMESTIC ANIMALS IN NORTHEAST CAMBODIA

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Flea-borne rickettsiae are a source of acute undifferentiated febrile illnesses worldwide. These pathogens are present, but are not well understood in Cambodia where poor resources and infrastructure limit the early detection in both humans and vectors. The aim of this study was to measure the prevalence of flea-borne rickettsiae in vector populations from host animals in Rattanakiri and Stung Treng Provinces, Cambodia. From November 2016 to March 2017, fleas were collected from hosts using direct inspection methods, preserved in 70% alcohol, and transferred to the laboratory. A total of 13,068 fleas were collected from dogs, cats, and from murid rodents. Specimens were identified morphologically, pooled by species and host, and stored at -20°C until DNA extraction. Extracted nucleic acids of pooled flea specimens were tested by species-specific qPCR assays. Flea pools containing unidentified *Rickettsia* spp. were subjected to multilocus sequence typing. Four species of fleas were collected from dogs: *Ctenocephalides felis orientis*, *Ctenocephalides felis felis*, *Pulex irritans*, and *Echidnophaga gallinacea*, three species from cats: *C. felis orientis*, *C. felis felis*, and *P. irritans*, and a single flea species, *Xenopsylla cheopis,* was collected from murid rodents. Rickettsiae were identified in all flea species and associated with all host animals, and included detections of *Rickettsia felis, Rickettsia asembonensis,* and Candidatus *Rickettsia senegalensis. Ctenocephalides felis orientis* collected from dogs and *X. cheopis* collected from rodents were found to harbor both *R. asembonensis* and *R. felis* on individual hosts. These findings confirm the presence of flea-borne rickettsiae, including the pathogenic *R. felis,* circulating in flea populations in Northeast Cambodia. More research is needed to understand the burden of rickettsial diseases in Cambodia, which will better inform healthcare professionals, and lead to more accurate diagnoses.

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GENETIC STRUCTURE AND PHYLOGEOGRAPHY OF *XENOPSYLLA CHEOPIS*, THE FLEA VECTOR OF PLAGUE IN MADAGASCAR

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Plague is a zoonotic disease that reaches humans via the bites of infected fleas. Hundreds of plague cases are reported every year in Madagascar where the flea, *Xenopsylla cheopis,* is the transmission vector. Evolutionary analyses of the genetic diversity in fleas can provide information on their population structure and dispersal patterns in relation to disease spread. The aims of this study were both to determine whether *X. cheopis* populations are genetically structured, and to infer the evolutionary history and dispersal patterns of this species at the country level. Nine microsatellite markers were used to genotype 325 individuals from 21 geographic locations. Genetic structure within populations was analysed using Pritchard's clustering method which revealed the existence of two major geographically structured genetic clusters. Whereas fleas from the southeastern and western regions belonged to cluster one, those from

the southwestern regions belonged to cluster two. Flea populations in the northern and eastern regions displayed a high degree of admixture and those in the Central Highlands, which were comprised primarily of cluster two, displayed a moderate degree of admixture. Complete mtCOII sequences were determined and were used together with sequences sampled from elsewhere (ten from La Réunion, three from Mayotte, one from Cambodia, nine from China and seven from Iran) to perform discrete phylogeographic analyses. This analysis suggested that *X. cheopis* may have originated in Madagascar (probability = 0.92). However, the paucity of samples from outside Madagascar means that this result should be interpreted with caution. Pathways of geographical spread were inferred between Madagascar and China and, between locations within Madagascar. This study highlights the occurrence of genetic structure and geographical spread of *X. cheopis* in Madagascar. Genetic admixture at certain locations within Madagascar provides strong evidence that despite the Madagascan *X. cheopis* populations being genetically structured, there is some gene flow between these populations in certain parts of the country.

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REGIONAL DIFFERENCES IN MORTALITY RISK IN PATIENTS HOSPITALIZED WITH TICK-BORNE DISEASES IN THE UNITED STATES

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Tick-borne diseases are increasing in incidence in the United States, however data on regional differences in clinical outcomes are limited. Using a national database of hospital records, we aimed to identify regional differences in hospitalizations and mortality from tick-borne disease. Data were examined at 156 US hospitals from 2009-2015 in the *Cerner Healthfacts* database to identify hospitalized tick-borne disease cases via administrative codes. Multivariable regression was used to evaluate mortality risk by patient and facility-level factors; adjusted odds ratios (aOR) and 95% confidence intervals (CI) were estimated. Overall, 3,125 hospitalized tick-borne disease patients were identified (mean annual incidence=28 cases/100,000 hospitalized persons): 66% had Lyme disease, 18% tick-borne fever, 14% ehrlichiosis, 6% babesiosis, 3% rickettsiosis, and 5% multiple diagnoses. Cases averaged 49±23 yrs, were 53% male, and had a mean Elixhauser comorbidity index of 2.1±2.0. Among cases, 41 (1.3%) died (mean age=68±16 yrs; range=33-90 yrs). Mortality did not vary by tick-borne disease (p=0.6) but did by region, with rates ≥2-fold greater in the South (2.6%) and West (3.2%) and lower in the Midwest (0.3%) and Northeast (0.6%) (p<0.0001). After controlling for key factors, death was associated with older age (10-unit increase: aOR=1.5, 95% CI=1.2-1.8), higher comorbidity index (1-unit increase: aOR=1.4, 1.2-1.6), and being in the South (aOR=5.9, 95% CI=2.6-13.7; p<0.0001) or West (aOR=10.7, 95% CI=2.4-48.5; p=0.002) versus the Northeast. When evaluated by sub-region, patients in West South-Central states (AR, LA, OK, TX) were 8-times more likely to die (aOR=8.5, 2.6-28.2) compared to those in New England (CT, ME, MA, NH, RI, VT); this area remained at higher risk even when limited to only Lyme disease patients (aOR=8.4, 95% CI=2.0-36.3). Significant differences in mortality exist by US region in hospitalized tick-borne disease patients. High-risk areas may reflect more virulent disease. Greater awareness may also exist in hyperendemic areas, resulting in more rapid diagnosis and treatment to limit severe outcomes.

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MURINE MODELS OF ROBUST AND WANING SCRUB TYPHUS IMMUNITY UTILIZING HOMOLOGOUS AND HETEROLOGOUS STRAINS OF *ORIENTIA TSUTSUGAMUSHI*

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The febrile illness scrub typhus is caused by the obligately intracellular bacterium *Orientia tsutsugamushi*. Unique characteristics of the pathogen and human infection have impeded progress towards a vaccine for protection against this potentially fatal disease. The abundance of *O. tsutsugamushi* strains in the endemic Asia-Pacific region with geographical overlap and poor cross-protective immunity between strains as well as short-lived immunity against the homologous strain in human infection is one such hurdle. To understand this transient immunity, animal models which demonstrate protection and the deterioration of this protection are necessary. We set out to build upon current understanding of this ineffective protection by utilizing our recently developed murine models, sublethal intradermal infection followed by challenge via lethal hematogenous dissemination. Preliminary data from our lab have shown that mice surviving an initial infection with *O. tsutsugamushi* Karp strain are resistant to subsequent lethal homologous challenge after 430 days. After acute infection, passive antibody transfer or immune splenocyte transfer conferred homologous protection from death, whereas more than a year later immune splenocytes alone did not afford complete protection. A different phenotype was observed in mice that were initially infected sublethally with Gilliam strain. Mice which received heterologous challenge after acute infection did not show signs of illness for up to 30 days after Gilliam strain infection. Waning protection indicated by weight loss mirroring the severe course in naïve mice after lethal challenge was observed after challenge with Karp strain at 270 days post initial heterologous infection while protection from death was maintained. At 425 days, 20 percent of mice succumbed to heterologous Karp strain challenge. Elucidation of the antibody and T cell contributions to homologous and heterologous challenge is currently underway and will contribute to understanding the protective immune response, laying the groundwork for development of an effective vaccine.

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TUNGIASIS IN MADAGASCAR: A NEGLECTED TROPICAL PARASITE EXACERBATED BY CLIMATE CHANGE?

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Itchy, swollen feet are a common occurrence in rural Madagascar thanks to the female sand-flea (*Tunga penetrans* or *T. trimamillata*), an ectoparasite that burrows into skin to drink blood, lay eggs, and die after four to six weeks. Advanced cases of sand-flea disease, or tungiasis, can lead to altered gait, inability to work, secondary infection and death. Previous research in Brazil showed that tungiasis peaks during the dry season and decreases sharply with onset of rains, possibly due to washing-away of the larvae, pupae, and nymphs. In this study, human surveys and climate data were combined to investigate the relationship between climate and tungiasis. Eighty-six residents were interviewed in three rural villages of eastern Madagascar, and data were collected on weather patterns associated with tungiasis. Present-day and model climate data based on four IPCC Representative Concentration Scenarios (RCPs) were used to predict climate and tungiasis prevalence in the years 2050 and 2070. The interviews revealed that rural Malagasy experience a high sand-flea burden, especially children and the elderly. All interviewees had experienced tungiasis, and 10% were actively infected. Active infections were present in 25% of children (<18 years) and 21% of elders (>40 years) but 0% of adults. In total, 91% remove fleas with unsterilized

needles, and 40% reported secondary infections. As prevention, 35% of respondents rub feet with toxic kerosene or insecticide. Of the interviewees, 94% reported that sand-fleas are more common on hot days in the dry-season months of September to December. The results from all four RCPs predict a shortening wet season (precipitation >250 mm), while the dry (<80 mm) and very-dry (<50 mm) seasons are predicted to lengthen. The impact of tungiasis in rural Madagascar is likely to increase with climate change due to a lengthening dry season. This study provides evidence that tungiasis is a neglected tropical disease deserving of higher priority for planetary-health research and control. The presented method of pairing disease seasonality with climate projections can be expanded to endemic regions globally.

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MOLECULAR CHARACTERISTICS OF *RICKETTSIA* IN TICKS COLLECTED ALONG THE SOUTHERN BORDER OF MONGOLIA

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Ticks are second only to mosquitoes in their ability to act as vectors of disease. Consequently, workers or populations that frequently enter tick habitats are at an increased risk of contracting a tick-borne disease. This holds true in Mongolia, where pastoral-herders make up 26% of the country's population (of 3 million) and are at high risk of tick bite exposure. A total of 4028 *Dermacentor* and *Hyalomma* ticks were collected from southern Mongolia across five aimags (provinces) including Bayankhongor, Dornogobi, Gobi-Altai, Khovd, and Umnogobi. Ticks were separated into 786 pools by tick genus and location (pool size 2-6 ticks). Extracted genomic material was tested using qPCR methods and primers targeting *rickettsia* 23S-5S ribosomal RNA intergenic spacer. Both Maximum likelihood estimations (MLE) and minimum infection rates were calculated. A total of 505/786 tick pools tested positive for rickettsia bacteria. Based on Mt values, we suspect that at least four, possibly six, different species of *rickettsia* are circulating, based on qPCR melting curves, with major variations in detection rates by sampled region and tick genus. The highest MLE rate of 48.4% (95% CI 41.7-56.5%) was observed in *Dermacentor* ticks from the Gobi-Altai region. Additionally a MLE rate of 7.6% (95% CI 6.2-9.2%) was observed in *Hyalomma* ticks collected in Umnugobi, warranting further testing. Sequence results pending. *Rickettsia* bacteria are circulating at a high rate within native tick species in Mongolia. Further steps are required to identify the exact species of rickettsia circulating in these regions. Given the severity of clinical symptoms of rickettsia in neighboring China and Russia, it is possible these same pathogens are circulating in Mongolia, but the extent remains unclear at this time.

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THE ROLE OF TWO FEMALE ATRIAL PROTEASES IN THE REFRACTORINESS OF *ANOPHELES GAMBIAE* MOSQUITOES TO FURTHER MATINGS

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Although the reproductive rate of *Anopheles gambiae* mosquitoes is a key aspect of their capacity to transmit malaria, the fertility of these vectors depends on a single copulation event as females become refractory to mating after a first copulation. Interfering with mating is, therefore, a promising alternative to control mosquito populations in endemic areas, potentially reducing malaria morbidity and mortality. In this study, we show the involvement of two female atrial serine proteases in shaping the mating receptivity of *An. gambiae* females. These two proteolytic enzymes ensure correct processing of the mating plug, a gelatinous structure composed of male seminal secretions that is transferred to the female reproductive tract during mating. We show that correct processing of the plug by these proteases is necessary to trigger a series of events that lead to the reduction of mating receptivity experienced by females after a first copulation. Moreover, these proteases are associated with and possibly contribute to the formation of a peritrophic matrix-like structure secreted by female atrial cells, which surrounds the plug after mating and may be critical for its function. These results shed new light on the mechanisms regulating female receptivity to mating in *An. gambiae*, with possible implications for reducing the reproductive output of these mosquitoes.

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CRISPR/CAS9 GENE EDITING TO BLOCK MALARIA TRANSMISSION

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The development of genetically modified (GM) mosquitoes for malaria control has gained momentum through the recent advances in gene-drive technology and an increased understanding of vector-pathogen interactions. While most work aiming at the development of GM malaria resistant mosquitoes, suitable for a population replacement strategy, has focused on the over-expression of anti-parasitic genes, here we have explored a strategy relying on CRISPR/Cas9-based inactivation of mosquito encoded *Plasmodium* agonists. During its sporogonic cycle inside the mosquito vector, *Plasmodium* engages in intimate interactions and relies in numerous *Anopheles*-derived host factors, which act as facilitators of infection. The C-type lectins CTL4 and CTLMA2 have been identified as *Plasmodium* agonists (Osta *et al*., 2004). In our recent work we showed that the C-type lectin-mediated protection against parasite melanization in the African vector *A. gambiae* is dependent on infection intensity, rather than the mosquito-parasite combination (Simões *et al*., 2017). RNA interference (RNAi)–based silencing of both lectins resulted in melanization and reduction of live oocysts, albeit RNAi results in only partial gene silencing. We hypothesized that the knockout (KO) of CTL4 would result in complete melanization of the parasites, and consequently a complete halt of *P. falciparum*'s reproductive cycle inside the mosquito. We are currently using CRISPR/Cas9 technology for germline targeted CTL4 KO in *A. gambiae*. We have selected three guide RNA (gRNA) target sequences and generated gRNA-overexpressing *A. gambiae* transgenic lines that were crossed with the Vasa::Cas9 strain and outcrossed with the wildtype docking line, to generate CTL4-KO *A. gambiae* mutants. These CTL4-KO mosquitoes are being studied for their potential parasite blocking and fitness impact of total CTL4 disruption.

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ASSOCIATION OF THE FREQUENCY OF *KDR* ALLELES V1016L AND F1534C WITH PYRETHROID RESISTANCE IN TWO *AEDES AEGYPTI* POPULATIONS IN PERU

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In Peru, over 20 million people are at risk of *Aedes aegypti*-borne diseases including dengue, Zika and chikungunya. As reports of insecticide resistance increase, vector control efforts may become compromised. Of particular concern is resistance to the pyrethroid class of insecticides. Mutations on the voltage gated sodium channel can contribute to pyrethroid resistance. In Latin America, the principal mutations are V1016I and F1534C, collectively referred to as *kdr*. Wild populations of *Ae. aegypti* were sampled transversally using ovitraps in 2 urban centers, Calleria (jungle) and El Porvenir (coastal). WHO bioassays were conducted to determine susceptibility to permethrin (0.75%) and alpha-cypermethrin (0.05%) in F1 female progeny between 3-5 days old. Following the biosassays, individuals were phenotyped as resistant or susceptible and DNA was extracted. The V10116I and F1534C mutations were detected by real-time PCR. Permethrin and alpha-cypermethrin resistance was detected with bioassay mortalities of 45.4% and 21.8%, respectively, in Calleria, and 58.3% and 95%, respectively, in El Porvenir. The frequencies of the *kdr* alleles differed between the populations. In Calleria, F1534C was fixed (n=93 mosquitoes tested), whereas the frequency was 0.68 El Porvenir (n=71 mosquitoes tested). The frequency of V1016I in Calleria was 0.45, whereas it was only 0.06 in El Porvenir. We detected an association between the V1016I allele and phenotypic resistance to both pyrethroids ($p < 0.05$), suggesting that this mutation is contributing to the resistance observed. However, the F1534C allele was only associated with phenotypic resistance to alpha-cypermethrin ($p<0.01$) but not permethrin ($p>0.05$). All individuals positive for the 1V016I allele also had the F1534C allele.

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KINETIC AND STRUCTURAL CHARACTERIZATION OF THE GLYCOLYTIC ENZYME PYRUVATE KINASE 1 FROM *AEDES AEGYPTI* MOSQUITOES

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A recent study from our laboratory has shown that glucose oxidation is required to support ammonia detoxification in blood-fed *Aedes aegypti* mosquitoes. Pyruvate kinase (PK) catalyzes the last step of the glycolytic pathway. In most organisms, one or more allosteric effectors control PK activity; however, the kinetic properties and conformational structure of PK in mosquitoes have not been previously reported. In this study, two genes (AaPK1 and AaPK2) encoding PK were identified in the *A. aegypti* genome. The gene AaPK1, which encodes a 529 amino acid protein with an estimated molecular weight of ~57 kDa, was cloned and the recombinant protein was expressed and purified in bacteria. AaPK1 kinetic parameters were determined. Multiple allosteric effectors of AaPK1 were identified and the binding of those molecules was analyzed by a thermal shift assay. The recombinant protein was also crystallized and its 3D structure determined at 4Å resolution. We found that Ala, Gln, Pro, Ser or fructose-1-phosphate displayed a classic allosteric activation on AaPK1. We also found that ribulose-5-phosphate acted as an allosteric inhibitor of AaPK1, but its inhibitory effect was reversed in the presence of some amino acids (AAs). In addition, the allosteric activation of AaPK1 by AAs was abolished in the presence of fructose-1,6-bisphosphate (F16BP). Moreover, the allosteric activation of AaPK1 by Ala and Ser was diminished by the addition of glucose-6-phosphate. Interestingly, the addition of F16BP or the mixture of F16BP and specific AAs increased the melting temperature of the enzyme, confirming the association between the sugar, the AAs and the enzyme. The AaPK1 structure shows the presence of two independent allosteric sites. All together, our data reveal how specific AAs and phosphorylated sugars tightly regulate conformational dynamics and catalytic changes of AaPK1. Mechanistic insights from our data can aid in the understanding of the role of AaPK in sugar- and blood-fed mosquitoes.

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A ROLE FOR THE JNK PATHWAY IN 20E-DEPENDENT OVIPOSITION IN *ANOPHELES GAMBIAE*

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The reproductive success of the *Anopheles gambiae* mosquito is a major determinant of its capacity to transmit the malaria-causing parasite, *Plasmodium falciparum*. We recently demonstrated that 20 hydroxyecdysone (20E), an ecdysteroid hormone transferred from male to female during copulation, licences the female to oviposit developed eggs. However, the molecular mechanisms and signalling pathways driving this lifelong behavioural switch remain incompletely understood. Microarray analysis of females before and after mating revealed a head-specific, mating-associated gene signature enriched in transcripts associated with wounding and melanisation, two responses linked to the activation of the Jun N-terminal Kinase (JNK) pathway. This link between mating and JNK signalling was confirmed by western blotting documenting an increase in phosphorylated JNK (pJNK, the active form of the kinase) in the heads of mated females. Notably, RNAi-mediated depletion of JNK, or the transcription factors Jun and Fos that act downstream of JNK, partially inhibited mating-induced oviposition, demonstrating a requirement for the JNK pathway in this mating outcome. Conversely, activation of the JNK pathway in virgin females by RNAi-mediated depletion of the JNK phosphatase, Puckered, a negative regulator of the JNK pathway, was able to mimic both the biochemical (activation of JNK in the head), and biological (induction of oviposition) consequences of mating, suggesting that activation of the JNK pathway was sufficient *per se* to induce oviposition. A second oviposition stimulus in the form of exogenous 20E, injected in to virgin females, induced a similar activation of JNK in the head, whereas the oviposition normally observed blood-fed virgins after 20E injection was significantly reduced in females depleted of JNK pathway components. Together, these data demonstrate an unexpected requirement for the JNK pathway, more traditionally associated with immunity and stress resistance, in 20E-dependent post-mating responses in the most important vector of malaria.

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STEROID HORMONE SIGNALING REGULATES *PLASMODIUM FALCIPARUM* DEVELOPMENT IN THE *ANOPHELES GAMBIAE* FEMALE

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Anopheles gambiae are the major African vectors of the malaria parasite *Plasmodium falciparum*. The vectorial capacity of these mosquitoes is partly due to their high reproductive rate as well as their ability to support *Plasmodium* development—these two processes are intimately linked since when a female mosquito takes an infected blood meal, oogenesis and parasite development proceed concurrently. Here we examined whether sporogonic development of *Plasmodium* parasites is influenced by processes driving oogenesis. After infecting *An. gambiae* females

with a *P. falciparum* culture, we found an unexpected positive correlation between the number of eggs a female mosquito develops and the number of oocysts found in her midgut. Furthermore, transgenic ablation of ovarian germ cells prior to infection caused a significant decrease in parasite numbers, indicating that infection depends on the occurrence of oogenesis. We next enzymatically impaired the function of the steroid hormone 20-hydroxyecdysone (20E), a key factor in the regulation of oogenesis, and found that reducing hormone titers also reduces parasite numbers. Moreover, disrupting 20E signaling via RNAi silencing of the 20E nuclear Ecdysone Receptor (EcR) breaks the positive egg-parasite interaction, implicating 20E in regulating reproduction-immunity trade-offs. Strikingly, however, disrupting 20E signaling induces faster parasite development, leading to more salivary gland sporozoites at earlier time points post blood meal. Lipidomic analyses of blood meal-induced changes show that the effects of hormone signaling on the parasite extrinsic incubation period (EIP) are likely due to 20E-mediated accumulation of neutral lipids in the midgut post blood meal. Consistently, interfering with lipolytic pathways by silencing a mosquito TAG Lipase phenocopies the effects of *EcR*-silencing by inducing faster parasite development—although the mechanism by which parasites utilize lipids is still being determined. Overall, these data demonstrate a remarkable influence of steroid hormone signaling on vector-parasite interactions critical for malaria transmission.

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THE ROLE OF THE *ANOPHELES* LIPOLYTIC MACHINERY IN MOSQUITO REPRODUCTION AND *PLASMODIUM FALCIPARUM* DEVELOPMENT

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Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin;} Once mobilized, lipids are loaded onto lipophorin, in the hemolymph, for delivery to various tissues such as ovaries, fat body, and flight muscles. Here, we elucidated the specific role of lipid mobilization in shaping both *Anopheles gambiae* reproduction and *Plasmodium falciparum* parasite development in the female mosquito. We initially performed targeted lipidomic analyses of various mosquito tissues after a blood meal to assess dynamic changes in major lipids, especially triacylglycerols (TAGs) and other neutral lipids. These analyses revealed a coordinated accumulation and depletion of TAGs, DAGs, MAGs, and CE across key mosquito tissues, suggesting the occurrence of lipolytic and lipogenic pathways. Bioinformatics analysis of the *Anopheles* lipolytic machinery involved in the mobilization of TAGs, revealed the presence of a putative TAG lipase that appears to be specific to insects. RNA interference (RNAi) against this TAG lipase and an associated protein that is generally involved in the breakdown of TAGs identified lipid mobilization as central in determining reproductive success of the main malaria vector. Specifically, TAG lipase silencing significantly impaired lipid mobilization from the midgut, reduced egg development, and abolished fertility. Interestingly, while silencing of this lipase did not affect *P. falciparum* oocyst numbers, it induced faster parasite development. This study identifies the regulation of TAG/DAG equilibrium as critical for the reproductive success of *Anopheles* mosquitoes.

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A CASCADE OF TYROSINE KINASE PHOSPHORYLATION REGULATES EGG DEVELOPMENT IN *ANOPHELES GAMBIAE* MOSQUITOES

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Anopheles gambiae mosquitoes are the principal vectors of the malaria-causing parasite, *Plasmodium falciparum.* The high reproductive rate of these females is strictly related to egg production, an intricate process which requires vertebrate blood in order to accumulate nutrients for later development of the embryo. The steroid hormone 20-hydroxy-ecdysone (20E), found throughout life in both females and male, is responsible for most of the changes that occur in female during this process. Modification of the function or the way in which acts this hormone could generate new opportunities to reduce the number of females transmitting malaria. To this aim, we focused on decoding the complex signaling cascades triggered by blood feeding that lead to egg development in the female *A. gambiae* mosquitoes. Our studies show that the 20E partly regulates oogenesis via a pathway that involves activation of tyrosine kinases to enhance protein tyrosine phosphorylation of an array of different proteins, which in turn might contribute to the 20E-induced physiological changes leading to oogenesis. Injection of a tyrosine kinase inhibitor reduces the number of eggs developed after blood feeding, and results in additional egg development defects, suggesting that tyrosine phosphorylation might play a pivotal role in the correct outcome of the reproductive cycle. Moreover, enzymatically blocking the function of 20E reduces tyrosine phosphorylation levels after blood feeding, suggesting the latter process is regulated by 20E signaling cascades. More detailed analysis revealed that the released 20E after a blood meal induces phosphorylation of phospholipase C gamma (PLCγ), an important mediator of growth. Silencing of this PLCγ lead to a significant decrease in the number of eggs, phenocopying the results obtained with the tyrosine kinase inhibitor and the effects of 20E on PLC appear mediated by the 20E nuclear hormone receptors, Ecdysone Receptor (EcR). Our results reveal novel signaling pathways that are involved in translating the 20E signal into egg development and may help designing novel methods to reduce the reproductive fitness of *A. gambiae* females.

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THE 20 TOOLS OF VECTORBASE: A BIOINFORMATICS AND POPULATION BIOLOGY RESOURCE FOR INVERTEBRATE VECTORS OF HUMAN PATHOGENS

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VectorBase.org is a free online resource that provides 20 different tools, including four new ones made available in the last year. Visit us in the exhibit hall for a demonstration of any of these tools with your species or data set of interest. If you never used VectorBase or you are a novice user, we recommend you start with the Genome Browser or with 'Find a Data Display (new!)', with this tool you can choose a gene, region or variant, and obtain an automatic generation of the relevant visualizations that include many different types of figures and tables. Some of the other 20 VectorBase tools are briefly described as follows. Apollo is an instantaneous, collaborative, genome annotation editor, designed to support geographically dispersed researchers. Search, Advanced Search and BioMart, are used for simple and complex, big and small-scale data mining queries. BLAST finds regions of local similarity between sequences. HMMER looks for homologous genes, but unlike BLAST it aims to be more accurate and better to detect remote homologs. A background pipeline

generates Comparative Genomics data, browse for precomputed displays of protein (e.g., gene trees) and DNA comparisons. The Expression Browser and Map has microarray and RNAseq data from different experiments showing differential expression, all data is processed through the same pipeline so that results can be compared side-by-side. Use the Genotype Explorer (new!) to browse variation such as SNPs and INDELs in field samples, and the Variant Effect Predictor (VEP) to determine the effect of variants on genomes. Galaxy is an open, web-based platform for data intensive biomedical research. The Population Biology (PopBio) resource is used for visualization, search and analysis of population data, including genotypes and phenotypes, mostly from field collections. Use a map or the Sample Explorer (new!) to query the metadata from the Genome Browser and PopBio samples. The REST API (new!) provides direct programmatic access to VectorBase species data. For Help or feedback email info@vectorbase.

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ADOPTION OF HOUSEHOLD VECTOR CONTROL BEHAVIORS FOR *AEDES AEGYPTI* AMONG AT-RISK POPULATIONS IN FOUR COUNTRIES

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Zika is a public health concern that, if acquired during pregnancy, has particularly adverse impacts on neonatal and child health. Since the onset of the epidemic in the Americas in 2015, there have been 3,720 confirmed cases of congenital Zika syndrome, named for a cluster of birth defects associated with Zika virus infection, including severe microcephaly. However, 80% of adults infected with Zika are asymptomatic, leading to low perceived severity of the disease. Dengue and Chikungunya are considered endemic in the region and, compared with Zika, have higher mortality rates and confer much more debilitating symptoms in both adults and children. With the recent Zika epidemic, the U.S.G. Department of State dedicated funding for the Zika response in April 2016, prioritizing efforts and programming to minimize negative pregnancy outcomes in South and Central America and the Caribbean. The Zika response is being implemented in a setting where other *Aedes aegypti*-borne diseases have long been public health concerns. In this context, a cross-sectional household survey will be implemented between April and June 2018 in targeted USAID programming implementation areas in the Dominican Republic, Guatemala, El Salvador and Honduras (N=2400). The survey will collect information on individuals' knowledge, risk perceptions, individual and household vector control practices, self-efficacy, perceived ease of practice of prevention behaviors, and injunctive social norms pertaining to prevention practices. This research will assess the comparative perceived risk and perceived severity of Zika, Dengue and Chikungunya, and how these perceptions modulate vector control and prevention practices within households. The objective is to inform prevention strategies aimed at using social and behavior change campaigns to increase adoption of household vector control activities that will reduce transmission of diseases by the *Aedes aegypti* vector.

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RESISTANCE OF *AEDES AEGYPTI* POPULATIONS TO DELTAMETHRIN, PERMETHRIN, AND TEMEPHOS IN CAMBODIA

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Dengue fever is a major public health concern in Cambodia with over 185,000 cases reported annually. *Aedes aegypti* is the primary vector for dengue transmission and is currently targeted with insecticide treatments during outbreaks. This study characterized the insecticide resistance status of 8 populations of *Ae. aegypti* from four different geographical areas. Urban and rural villages were selected as collection points within each area. Villages were selected by the National Dengue Control Program according to geographical representation, dengue incidence, and recent use of temephos (within the previous 2 years). The susceptibility of *Ae. aegypti* to temephos, permethrin, and deltamethrin was evaluated in accordance with World Health Organization protocols and test kits. All the field populations showed resistance to temephos compared with the sensitive strain with resistance ratio 50 (RR_{50}) varying from 3.3 to 33.78 and RR_{90} from 4.2 to 47 compared with the sensitive strain, demonstrating an installed and generalized resistance of larvae to the temephos in Cambodia. *Ae aegypti* adult populations were highly resistant to permethrin regardless of province or rural/urban classification with an average mortality of 0.02%. Seven of the 8 field populations showed resistance to deltamethrin. These results and those of neighboring countries are alarming. From a regional point of view, it seems essential to rapidly change control methods and replace temephos with another intervention. These results are alarming for dengue vector control, as widespread resistance may compromise the entomological impact of larval control operations. Unfortunately, loss of susceptibility to these insecticide classes limits the efficacy of interventions in the event of an epidemic and impact on emergency suppression of Aedes mosquitoes. Regular continuous monitoring of resistance is necessary in order to select the most effective adulticides for dengue control in Cambodia.

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AN EVALUATION OF THE EFFICACY OF AIRCRAFT DISINSECTION PROCEDURES AT AUSTRALIAN AIRPORTS

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The inadvertent transport of live *Aedes aegypti* on aircraft originating from disease-endemic countries is a problem for Australia's biosecurity measures. Recently, our international airports have intercepted an unprecedented number of exotic mosquitoes during routine surveillance, which suggests 1) existing aircraft disinsection procedures are inadequate, and 2) airports are a likely source of exotic invasions. Mosquitoes captured at 8 Australian ports revealed that 996P and 1023G knock-down resistant (*kdr*) mutations were present in 53 out of 79 mosquitoes. Susceptible mosquitoes caught in northern Queensland airports were deemed local as *A. aegypti* in the area lack *kdr* mutations. The World Health Organization (WHO) recommends 0.2 g/m^2 of permethrin as the target dose for residual aircraft treatments, and is designed to kill landing

mosquitoes over 8 week intervals despite the gradual decay of residues (the lower tolerable threshold is 2 mg/m^2). A resistant *A. aegypti* colony at 100% 996P/1023G *kdr* mutation frequencies was used as proxy for mosquitoes intercepted at Australian airports to determine the impacts of WHO recommended permethrin doses on various aircraft surfaces. Results revealed the operational impact of residual treatments to be low, even for susceptible mosquitoes on some surfaces. Bioassays performed on permeable surfaces (i.e. carpets) with 0.2 g/m^2 permethrin showed mortality of <50% in susceptible mosquitoes exposed for 30 min. High-performance liquid chromatography (HPLC) analysis of swabs taken from permeable surfaces revealed that permethrin recovery was considerably less than the applied amount. Using free flight rooms and mosquitoes displaying natural resting and flying behaviours, we also showed that patchily treated environments typical of treated aircraft cabins and holds do not result in the universal exposure of mosquitoes. This study provides evidence of the ineffectiveness of aircraft disinsection procedures, at least in relation to residual treatments. New chemistries and more effective application methods are desperately required to protect our airports and most vulnerable territories.

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AEDES POPULATIONS AND THEIR SUSCEPTIBILITY TO INSECTICIDES IN KINSHASA, DR CONGO

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Aedes aegypti and *Ae. albopictus* mosquitoes transmit several important viruses to humans such as yellow fever, dengue, chikungunya and zika. The health threat posed by Aedes borne viruses is rising especially in urban environments. In the absence of curative treatments against arboviruses, vector control remains essential, and many rely on the use of insecticides. With Aedes increasingly resistant to insecticides, updated data on susceptibility is needed to guide control measures. The Democratic Republic of Congo (DRC) is one of the countries highly threatened by arbovirus outbreaks. Despite the long-standing risk, data about Aedes are lacking. This study aimed to determine population and susceptibility to insecticides of Aedes in Kinshasa. Aedes larvae were collected in Kinshasa from January to June 2017. They were reared to adults and identified to species following Huang and Highton morphological keys. WHO tube assays were carried out to assess susceptibility of female *Ae. aegypti* and *Ae*. *Albopictus*. 8,798 adult mosquitoes were obtained from aquatic stages of which 62% were Aedes, which comprised of three species: *Ae. aegypti* (29%), *Ae. albopictus* (70%), and *Ae. vittatus* (0.5%). All sampled *Ae. aegypti* belonged to the *formosus* subspecies. Mortalities recorded from WHO tube assays indicated high prevalence of resistance to DDT (25 and 36% for *Ae. aegypti* and *Ae. albopictus*, respectively), confirmed resistance to deltamethrin (73 and 92%), marginal suspected resistance to permethrin (97 and 100%), and propoxur (98 and 100%), but full susceptibility to bendiocarb and malathion (all 100%). This study is the first to provide distribution and data on susceptibility of *Aedes aegypti* and *Aedes albopictus* to insecticides in Kinshasa. With the exception of DDT, most tests indicate limited resistance and provide valuable baseline data for future studies and to guide control measures. More studies on Aedes populations and their susceptibility to insecticides, and mechanisms involved in resistance are needed across DRC.

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A WHOLE TRANSCRIPTOMIC APPROACH TO CHARACTERIZE INSECTICIDE RESISTANCE MECHANISMS IN *ANOPHELES ARABIENSIS* (DIPTERA: CULICIDAE) FROM ETHIOPIA

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The development of widespread insecticide resistance is of increasing concern in Ethiopia because of its implications for malaria vector control failure. To improve our understanding of the specificity of resistance mechanisms and to facilitate the design of management strategies that minimize the likelihood of selecting for cross-resistance, we undertook an exploratory transcriptomic approach to identify markers of metabolic resistance in *Anopheles arabiensis*. A total of 237 *An. arabiensis* were collected as blood-fed adults from house walls in Asendabo, Oromia Region in July-September 2017. F_1 progeny, generated by forced-oviposition, were phenotyped as resistant or susceptible to malathion and permethrin using standard CDC bottle bioassays. Target site resistance markers (L1014F *kdr* and G119S *Ace-1*) were screened using PCR. RNA was extracted from bioassay survivors, unexposed individuals, and susceptible reference populations and submitted for Illumina RNA-sequencing. The average mosquito mortality was 72% (range of 61-83%) and 77% (50-100%) when exposed to the diagnostic doses of malathion and permethrin, respectively. This population demonstrated intense deltamethrin resistance (average mortality of 84% at 10X the diagnostic dose) but was fully susceptible to carbamates (bendiocarb and propoxur). *Ace-1* mutations were not present in any mosquitoes tested in malathion bioassays (n=173). The L1014F *kdr* mutation was detected in 52% (30/58) of permethrin-exposed *An. arabiensis*, with allele frequencies of 0.65 and 0.26 in surviving and dead mosquitoes, respectively. Analysis of RNA-seq data is ongoing to identify candidate genes associated with resistance. The absence of *Ace-1* target site polymorphisms and the moderate *kdr* allele frequency suggest that metabolic mechanisms are likely driving organophosphate resistance and contributing to pyrethroid resistance in this population. As little is known about the molecular basis of metabolic resistance in *An. arabiensis*, these results will provide the most comprehensive analysis in this species to date.

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VARIABLE RESISTANCE TO INSECTICIDE IN *AEDES AEGYPTI* IS EXPLAINED BY COMBINED *KDR* MUTATIONS AND METABOLIC GENE OVEREXPRESSION IN BURKINA FASO

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Aedes aegypti control in Burkina Faso is an urgent concern owing to recurrent and expanding dengue outbreaks. The situation of *Ae. aegypti* resistance to insecticides in Africa suffers from lack of data and, for the available data, heterogeneity of resistance, which may have a temporal, methodological or biological explanation. We report here the insecticide resistance situation for *Aedes aegypti* in Burkina Faso. Larvae were collected from the most representative breeding sites in urban, semi-urban and rural localities of Burkina Faso. WHO and CDC protocols bioassays were performed on larvae and adults using insecticides from

the four major classes and in somed cases included the synergist PBO. Taqman RT-PCR was used to check for knockdown resistance (kdr) mutations. Quantitative Real-time PCR was used to analyze the expression of candidate P450 genes potentially involved in insecticide resistance. Larval stages displayed susceptibility to organophosphates with highest lethal concentrations (LC50 and LC99) generally recorded in the urban localities. Adult resistance to insecticides varied according to locality and breeding site. As elsewhere in Africa (where recorded) Ae. aegypti were susceptible to organophosphates but suspected to be resistant to carbamates in the urban area. The urban and semi-urban populations were resistant to permethrin and deltamethrin with at least partial restoration of susceptibility by PBO, whereas in contrast, the rural area population was susceptible to the insecticides tested. Molecular analysis reveal the presence of the V1016I and F1534C knockdown resistance (kdr) mutations and the absence of S989P and V1016G. The frequency of each kdr mutation was higher in the urban and semi-urban areas than in the rural area. Quantitative PCR analysis of expression of metabolic resistance candidate genes detected an aggregate overexpression of P450 genes, which combined with kdr mutations is likely to explain the phenotypic resistance differences observed.

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FINE-SCALE SPATIAL AND TEMPORAL HETEROGENEITIES IN INSECTICIDE RESISTANCE PROFILES OF *ANOPHELES ARABIENSIS* IN RURAL SOUTHEASTERN TANZANIA

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Programmatic monitoring of insecticide resistance in disease vectors is mostly done on a large scale, often focusing on differences between districts, regions, or countries. However, local heterogeneities in residual malaria transmission imply the need for finer-scale data. This study reports small-scale variations of insecticide susceptibility in *Anopheles arabiensis* between three neighbouring villages across two seasons in Tanzania, where insecticidal bed nets are extensively used, but malaria transmission persists. WHO insecticide susceptibility assays were conducted on female and male *An. arabiensis* from three proximal villages, Minepa, Lupiro, and Mavimba, during dry (June-December 2015) and wet (January-May 2016) seasons. Adults emerging from wild-collected larvae were exposed to lambda-cyhalothrin, deltamethrin, permethrin, DDT, dieldrin, bendiocarb, propoxur, pirimiphos-methyl and malathion. A hydrolysis probe assay was used to screen for L1014F (*kdr-w*) and L1014S (*kdr-e*) mutations in specimens resistant to DDT or pyrethroids. Synergist assays using piperonly butoxide (PBO) and triphenol phosphate (TPP) were done to assess pyrethroid and bendiocarb resistance phenotypes. There were clear seasonal and spatial fluctuations in phenotypic resistance status in *An. arabiensis* to pyrethroids, DDT and bendiocarb. Pre-exposure to PBO and TPP, resulted in lower knockdown rates and higher mortalities against pyrethroids and bendiocarb, compared to tests without the synergists. Neither L1014F nor L1014S mutations were detected. This study confirmed the presence of pyrethroid resistance in *An. arabiensis* and showed small-scale differences in resistance levels between the villages, and between seasons. Substantial, though incomplete, reversal of pyrethroid and bendiocarb resistance following pre-exposure to PBO and TPP, and absence of *kdr* alleles suggest involvement of P450 monooxygenases and esterases in the resistant phenotypes. Further bioassays are recommended to quantify the strength of resistance, both biochemical and molecular analysis to elucidate specific enzymes responsible in resistance.

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EFFICACY OF TWO TYPES OF PYRETHROID AND PIPERONYL BUTOXIDE (PBO) COMBINATION NETS (OLYSET PLUS AND PERMANET 3.0) AGAINST PYRETHROID RESISTANT MALARIA VECTORS IN SOUTHERN BENIN

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The efficacy of Long-lasting insecticidal nets is today threatened by widespread resistance to pyrethroids; the insecticide of choice for treating bed-nets. PBO, a synergist that inhibits specific metabolic enzymes within mosquitoes has been combined with pyrethroids to enhance the potency of bed-nets against pyrethroid resistant vector populations. Five PBO and pyrethroid combination nets with varying characteristics and technical specifications have to-date received interim WHO recommendation for field use. In this study, we compared the efficacy and wash resistance of two types of PBO plus pyrethroid combination nets (Olyset Plus and Permanet 3.0) against pyrethroid resistant malaria vectors in experimental huts in Cove, Southern Benin.Laboratory assays were performed to help explain the findings in the experimental huts. Mortality rates of malaria vectors from the experimental hut station exposed to pyrethroids in CDC bottle bioassays increased significantly after pre-exposure to PBO (from 30% to 70%) demonstrating the presence of mixed-function oxidases in the vector population. Mortality of wild free-flying pyrethroid resistant An gambiae entering the experimental huts was only 22% with Olyset Net (a pyrethroid-only net). The PBO+pyrethroid LLINs killed significantly higher proportions of mosquitoes than Olyset Net. Mortality was significantly higher with Permanet 3.0 (44%) than Olyset Plus (32%) when both LLINs were unwashed ($P<0.05$). With nets washed 20 times, mortality decreased significantly with Permanet 3.0 (from 44% unwashed to 17% after 20 washes, $P<0.05$) but did not change significantly with Olyset Plus (32% unwashed vs. 28% washed 20 times, $P>0.05$). In conclusion, both types of PBO nets demonstrated improved efficacy against pyrethroid resistant malaria vectors compared to a standard pyrethroid-only LLIN. Permanet 3.0 showed a greater effect when unwashed while Olyset Plus was substantially more wash resistant.

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ASSOCIATION BETWEEN *VGSC*1014 ALLELES AND FEEDING PATTERNS OF *PHLEBOTOMUS ARGENTIPES*

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Control of the sandfly vector *Phlebotomus argentipes* is an essential tool for visceral leishmaniasis elimination in the Indian subcontinent disease hotspot. Currently, the main control measures are based on pyrethroid insecticides that share cross-resistant mechanisms with DDT, notably knockdown resistance *(kdr)* mutations in the para voltage-gated sodium channel (*Vgsc*). We detected three *kdr* mutations linked strongly with DDT resistance and predictive of pyrethroid tolerance in *P. argentipes.* Here we present results on *kdr* distributions in relation to sandfly control activities to better understand selection and operational impacts of insecticide resistance. We screened a temporal sample of *P. argentipes* females from Mymensingh (Bangladesh), collected during a pyrethroid-based indoor residual spraying (IRS) programme, for *Vgsc*-1014 alleles using TaqMan assays and blood meal origin using two PCR assays. In Mymensingh, all *kdr* mutations were less common than in Bihar (India) potentially suggesting a lower current or past selective pressure. Data from Bihar showed *kdr* frequencies covaried spatially with intensity of DDT (and now pyrethroids) IRS programmes, but in Mymensingh temporal trends in *kdr* did not link

with IRS implementation. However, most *P. argentipes* with human blood exhibited *kdr* genotypes (81%), including a large fraction possessing the phenylalanine alleles (52%) associated with the strongest resistance phenotypes. In contrast, sand flies with animal blood meals possessed primarily wild type leucine allele ($\chi^2_3 = 96.09$; $P << 0.001$). This strong association between human feeding and *kdr* mutations in a region with pyrethroid spraying suggests an operational impact of *P. argentipes kdr* and provides further evidence to support screening of *kdr* mutations in surveillance and control programs.

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IDENTIFICATION OF SEX SPECIFIC ACTIN GENES IN *ANOPHELES ALBIMANUS*, A MAIN MALARIA VECTOR IN CENTRAL AMERICA AND THE CARIBBEAN

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Malaria is a life threatening disease that affects millions of people worldwide. There are four *Plasmodium* species that can cause malaria when transmitted to humans by female *Anopheles* mosquitoes. *Anopheles albimanus* is one of the two main vectors of malaria in the Mesoamerican region where the transmission of the disease is still active. In Guatemala, despite the distribution of insecticide treated nets malaria persists, focalized transmission in several departments is persistent. The Sterile Insect Technique (SIT) is a biological control method that can reduce insect pests by releasing a large number of sterile males, but the mosquito rearing process and fitness cost to the males still need to be improved. Sex sorting is one of the key steps of the production that need new technologies to be more efficient in a large scale production. The SIT was successful in the 1970´s at controlling *An. albimanus* in a lake ecosystem in El Salvador. In *Aedes sp*. mosquitoes, there are sex specific muscle actins and the indirect flight muscle actin promoter has been used to produce flightless females by the Release of Insects Carrying Dominant Lethal (RIDL) system. With the aim of improving SIT and to develop a method to target females, we extracted RNA and performed 3´-RACE to differentiate the actins expressed throughout the *An. albimanus* life cycle. Interestingly, we found four sex specific actins, two female-specific and two male-specific. Our current studies will evaluate the effects of knocking down these sex-specific genes. Our research could potentially result on a genetic methodology to selectively eliminate the females from the production of sterile male mosquitoes for release, making this process cheaper and more feasible to implement in the region.

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ATTRITION, BIOLOGICAL EFFICACY, INSECTICIDE RESIDUE AND PHYSICAL DEGRADATION OF LONG LASTING INSECTICIDAL NETS USED FOR MALARIA CONTROL AND ELIMINATION IN THE PHILIPPINES

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In the Philippines, long lasting insecticidal nets (LLINs) played a major role in reducing malaria burden over the past decade. Out of 83 provinces 74 of them have been declared malaria free with many provinces showing zero malaria cases in the past two to three years. Only eight provinces (Cagayan, Davao del Norte, Maguindanao, Mindoro Occidental, Palawan, Sultan Kudarat, Sulu, Tawi-Tawi) have indigenous malaria cases. With these recent improvements, the program direction shifted from control to sub national elimination with the vision malaria-free country by 2030. Despite the huge investments in the use of LLIN, the information on prolonging its use and efficacy is lacking. Thus, this study addressed issues of attrition, biological efficacy, insecticide residue and physical degradation under villagers conditions. Surveys of 462 households using restrospective questionnaire was conducted to determine attrition. Samples of distributed LLINS at varying age were collected to further determine biological efficacy using field collected *Anopheles flavirostris* following WHO Standard Cone Bioassay. Proportional hole indexes (pHI) based on WHO guidelines were also estimated to assess physical degradation. High pressure liquid chromatography analysis of coded LLIN samples measured insecticide residues. Four LLIN brands (*Dawa Plus 2, Olyset, Permanet 2, Interceptor)* distributed from 2012 to 2015 previously distributed by the National Malaria Control and Elimination Program were collected from study sites (Cagayan and Palawan). Based on the survey, an overall 12% attrition rate was obtained. Around 88% of the current nets are still in good condition and can be used any time. Most (91.08%) LLIN are being used with 93.75% of these bed nets were used the night before the interview. 95.02% of the respondents said that the bed nets are used all year round. Further analysis is being done to understand better association of attrition, community practices, biological efficacy, insecticide residue and physical degradation of LLIN to provide evidence for public health policy makers guidance in achieving maximum benefits on LLIN use.

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DYNAMICS OF INSECTICIDE RESISTANCE INTENSITY, KNOCKDOWN RESISTANCE (KDR) GENE FREQUENCY AND MECHANISM OF INSECTICIDE RESISTANCE IN ANOPHELES GAMBIAE S.L. ACROSS FIVE ECOZONES OF NIGERIA

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Effective malaria control depends heavily on continued pyrethroid susceptibility in *Anopheles gambiae* s.l. in Nigeria. Resistance intensity and mechanisms were determined at multiple collection stations at six sentinel sites within five ecozones of Nigeria. Deltamethrin and DDT susceptibility tests were done on *An. gambiae* s.l. mosquitoes using CDC bioassay method. Polymerase chain reaction (PCR) assays were used for species identification. Resistance intensity and PBO synergist assays were also done using standard methods. The *kdr* genotypes were determined in *An. gambiae* s.l. using allele-specific PCR assays. *An. gambiae* s.l. showed resistance to deltamethrin in four of the five ecozones- rainforest, Guinea, Sudan and the Sahel-savannas. High frequency of deltamethrin resistance was observed in the rainforest (Oyo) with mortality ranging from 16 (95%CI 8.8-23.4) to 36 (95%CI 26.6.-45.4) percent, while in the Sahel savanna (Sokoto) mortality ranged from 69 (95%CI 59.9-78.1) to 77 (95%CI 68.8-85.2) percent. Deltamethrin resistance at 5x and 10x diagnostic concentrations was recorded at three of four collection stations

in the rainforest part of Oyo and at all four stations in the rainforest (Ebonyi). In five sites, piperonyl butoxide (PBO) synergist assays indicated that elevated oxidases are the only contributors to pyrethroid resistance. In the rainforest, additional resistance mechanisms are likely as PBO did not increase mortality to 100%. *Kdr* gene frequencies for deltamethrin-exposed mosquitoes varied slightly across the ecozones with a range of 0.13 in the Guinea savanna to 0.29 in the Sahel savanna; those for DDT-exposed mosquitoes varied from 0.04 in the Sudan-savannah to 0.23 in the Sahel savanna. Higher *kdr* gene frequencies were observed in deltamethrin-exposed than in DDT-exposed mosquitoes in five sentinel sites. This study documents deltamethrin resistance in multiple ecozones of Nigeria with higher intensity resistance in rainforest (Ebonyi and Oyo) sites. Resistance mitigation strategies should be considered for Ebonyi and Oyo and may include distribution of non-deltamethrin, PBO, or next generation LLINs.

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INSECTICIDAL AND REPELLENT ACTIVITIES OF NATURAL PRODUCT DERIVATIVES FROM THE MADAGASCAN MEDICINAL PLANT *CINNAMOSMA FRAGRANS* AGAINST THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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New insecticides and repellents are needed to improve control the *Aedes aegypti* mosquito, which is the primary vector of several emerging arboviruses, including Zika, yellow fever, chikungunya, and dengue fever. Recent work by our group has identified a drimane sesquiterpene dialdehyde (cinnamodial: CDIAL) from the Madagascan medicinal plant *Cinnamosma fragrans* that is an agonist of mosquito transient receptor potential A1 (TRPA1) channels and exhibits promising activities as an insecticide and repellent against *Ae. aegypti*. The goal of the current study was to generate insights into CDIAL's insecticidal and repellent activities by screening the bioactivities of several chemical derivatives of CDIAL containing modifications to one or both of its electrophilic aldehyde functions. All of the chemical derivatives exhibited reduced or nominal antifeedant activity (a proxy for TRPA1 modulation and repellency) compared to CDIAL. While most of the derivatives exhibited dramatically weaker insecticidal activity against larvae and adult females of *Ae. aegypti* compared to CDIAL, two compounds retained similar or improved insecticidal activity against larvae compared to CDIAL. The results indicate that the electrophilic aldehyde functions of CDIAL are critical to its activation of TRPA1 and repellent activity, but not its toxicity, which confirms our earlier work suggesting that the toxicity of CDIAL is independent of TRPA1 modulation. Current efforts are developing additional analogs of CDIAL to better understand its structure-activity relationships and mechanism of toxic action on mosquitoes.

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THE THUMB TEST: A BENCHTOP TEST FOR QUANTIFYING THE EFFECTS OF INSECTICIDE-TREATED NETTING ON MOSQUITO BEHAVIOR

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Long lasting insecticidal nets (LLINs) are essential for malaria control in Africa, but their future is threatened by resistance to pyrethroid insecticides, the primary insecticide class used. To accelerate the screening of potential new chemicals or net types, we developed the 'Thumb Test', a simple benchtop assay to quantify the effects of novel net treatments on mosquito behavior during host seeking, and on survival and a range of sub-lethal effects post-exposure. Bloodfeeding (on the operator's thumb) through the test net can be permitted or prevented, and measurement of detailed behavioral events at the net interface are video recorded (20min) for subsequent analysis to measure events ranging from pre-contact repellency, contact-irritancy and bloodfeeding interference. Individual mosquitoes are used, allowing precise determination of correlations between duration of LLIN contact and the effects of exposure. Testing 4 mosquito strains (2 susceptible, 2 resistant) on untreated net, PermaNet 2 and Olyset LLINs, response rates varied from 13 to 57%; all strains responded, and all would land and bloodfeed through LLINs. Bloodfeeding duration was reduced in all strains on LLINs compared to untreated netting ($P<0.05$): *e.g.* mean bloodmeal duration by Banfora (IR strain) was 8.40 min on untreated net, but 5.03 and 3.55 min on PermaNets and Olysets respectively ($p<0.05$). Post-exposure, survival of resistant mosquitoes was reduced from an average of 14 days after bloodfeeding through untreated nets to less than 9 days on LLINs; when bloodfeeding was prevented, longevity post-exposure of resistant mosquitoes fell from 15 to 8 days on untreated net and LLINs respectively. Further validation is nearing completion and a comprehensive evaluation and standard operating procedure are being developed. The test is simple to operate, inexpensive to install in a basic laboratory setting and generates considerable data per mosquito tested. We present it as a method with significant potential to contribute to the search for novel LLIN treatments and as an addition to the existing WHO test repertoire for evaluating resistance in vector populations.

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A BASELINE ENTOMOLOGICAL REPORT OF MOSQUITO VECTORS AND INSECTICIDE RESISTANCE IN UGANDA PRIOR TO A LLIN NATIONAL UNIVERSAL COVERAGE CAMPAIGN

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Pyrethroid resistance, due to both target site mutations (kdr) and metabolic mechanisms, has been reported across Africa and may threaten malaria control. One approach to overcoming this threat is to combine a pyrethroid insecticide on LLINs with a synergist, piperonyl butoxide (PBO), which is capable of inhibiting cytochrome P450s, and potentially overcoming pyrethroid resistance in anopheline vectors. In Uganda, insecticide resistance mutations were first observed in Anopheles gambiae in 2001, and the problem of resistance has been increasing. Recent data suggest that pyrethroid resistance is now widespread across Uganda and that the causal mechanisms include kdr, cytochrome P450s, carboxylesterases, and glutathione-s-transferases. At least one specific P450 has been reproducibly associated with pyrethroid resistance in Uganda and data suggest that Ugandan-specific resistance markers may explain 50% of the variance seen in pyrethroid resistance. Previous work has demonstrated that insecticide resistance can be very heterogeneous on a fine spatial scale which may not be detected by traditional phenotypic monitoring methods. A cluster-randomised trial designed to evaluate the impact of long-lasting insecticidal bednets (LLINs), with and without PBO, distributed via a national universal coverage campaign in 2017/2018 is underway in 104 clusters (health sub-districts) across 48 districts in Uganda. Since variations in insecticide resistance between clusters could be a major confounder of our primary objective, we undertook a baseline entomological survey across the study site before the universal LLIN distribution campaign. Mosquitoes from 10 randomly selected households in all 104 clusters were collected using prokopack aspirators. Up to 50

Anopheles gambiae s.l. per cluster were investigated for Ugandan-specific resistance markers to obtain cluster specific estimates of resistance. We present the results of these baseline entomological and genotype resistance surveys and discuss their implications.

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INTENSITY OF PYRETHROID RESISTANCE IN ANOPHELES GAMBIAE S.L. IN THE DEMOCRATIC REPUBLIC OF CONGO

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Pyrethroid resistance is an important concern of the National Malaria Control Program in the Democratic Republic of Congo (DRC), as pyrethroid-treated bed nets are the primary malaria vector control intervention used in the country. Resistance monitoring has revealed widespread resistance, but it is unknown to what extent this resistance is compromising control efforts. Measuring the intensity of resistance may improve our understanding of operationally significant resistance. The intensity of resistance of *Anopheles gambiae* s.l. was monitored in Kinshasa in 2016 and 2017, and in 6 sites throughout the country in 2017. CDC bottle bioassays using one, two, five, and ten times the diagnostic dose of permethrin and deltamethrin were conducted using *An. gambiae* s.l. collected as larvae. One round of these assays were conducted at 5 sites in and around Kinshasa province in 2016 six months prior to a distribution of deltamethrin treated bednets in Kinshasa province in December 2016. Three subsequent rounds of intensity assays were then conducted 2, 6, and 10 months after the distribution to monitor changes in the intensity of resistance. In the sites outside of Kinshasa, a single round of intensity assays was conducted in 2017. Resistance to deltamethrin and permethrin was noted in all sites at all time points. In Kinshasa, resistance to permethrin was intense: survival was 16-100% and 0-95% when mosquitoes were exposed to 5 and 10x the diagnostic dose, respectively. Resistance to deltamethrin was less intense: survival was 0-74% and 0-23% when mosquitoes were exposed to 5 and 10x the diagnostic dose, respectively. The results from the six sites tested outside of Kinshasa showed similar patterns, with 64-90% and 20-84% surviving 5 and 10x permethrin exposure, and 0-39% and 0-20% surviving 5 and 10x deltamethrin exposure, respectively. Pyrethroid resistance is a pressing issue for malaria control which threatens the gains made in the past ten years of scaling up vector control in DRC. Monitoring of the intensity of insecticide resistance should continue; there may be a need to consider distribution of next generation nets (PBO nets, bi-treated nets).

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APPLICATION OF A NOVEL MULTIPLEX ASSAY TO ASSESS ANOPHELES MALARIA TRANSMISSION ECOLOGY IN THE CENTRAL HIGHLANDS OF MADAGASCAR

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Due to variability in mosquito habitat preference, feeding patterns, and susceptibility to various measures of insect control; it is vital that we efficiently characterize the vector's bloodmeal. Using field-collected *Anopheles* mosquitoes from two villages in the highlands of Madagascar in December 2017, we designed and utilized a novel Ligase Detection Reaction and Fluorescent Microsphere Assay to efficiently identify the mosquito, query the mammalian host, and test for the presence of malaria parasites. We collected the following species using a combination of pyrethroid spray catch and barrier screens: *A. arabiensis* (8), *A. coustani* (164), *A. funestus* (11), *A. gambiae* (14), *A. maculipalpis* (96), *A. rufipes* (85), *A. squamosus* (87), and six specimens of an unknown *Anopheles* species. There was an overall concordance of 82% between LDR-FMA and morphologically identified specimens. This assay successfully detected multiple hosts in the blood, revealing a high zoophilic trend in the sample, even amongst traditionally anthropophagic species. Furthermore, numerous mosquitoes were *Plasmodium* positive: 2.6% *P. falciparum*, 4.8% *P. vivax*, 0.8% *P. malariae*, and 1.2% *P. ovale*; 31.4% of which constituted mixed-species infections. Finally, we briefly discuss a potentially novel *Anopheles* mosquito capable of harboring *Plasmodium* parasites. This multiplex assay enables efficient characterization of large mosquito samples and simultaneous pathogen detection to monitor and incriminate disease vectors in Madagascar. Furthermore, it provides valuable insight into mosquito transmission ecology in a region dominated by submicroscopic infection, a potential barrier to the ultimate goal of total malaria eradication.

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BEHAVIORS AND MOLECULAR IDENTIFICATION OF *ANOPHELES* SPECIES IN KARAMA, WEST SULAWESI, INDONESIA

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Information regarding local malaria vector compositions in association with their bionomic traits is vital for targeting susceptibilities in mosquito behaviors and implementing effective malaria interventions. Many studies rely on morphological identification of mosquitoes, limiting identification to visually distinct species. Sulawesi, Indonesia is a region with high species diversity, with previous documentation of eight malaria vectors and species complexes. However, vector molecular identification, distribution, ecology, and vector behaviors have not been well characterized in West Sulawesi, Indonesia. Mosquitoes were collected in Karama, Indonesia a village located in the northwestern regency Mamuju, West Sulawesi using human-landing collections, barrier screens, barrier screens with eaves, and kelambu traps. Mosquitoes were morphologically typed and then molecularly distinguished based on ribosomal DNA ITS2 and mitochondrial DNA CO1 sequences. Collection methods were evaluated and compared based on morphological and molecular identities. The presence of *An. barbirostris subgroup clade V* in West Sulawesi, Indonesia in confirmed here for the first time. While morphological identifications recognized 19 species, 16 distinct species groups were identified through molecular analyses. Of the 16, six could not be identified through comparison to published sequences. Peak activity times for molecularly identified *An. barbirostris* and *An. vagus* indicated activity in first half of the night. Kelambu traps collected the most Anopheles species (n=14) as well as the highest frequency of Anopheles (n=2,924). Morphological identification of secondary vectors in this study was generally inaccurate, underlining the importance of using molecular analysis in conjunction with morphological investigations. Collection methods indicated certain biases that can be tailored to future entomological studies. Detailed knowledge about primary and secondary malaria vectors and bionomic characteristics that effect malaria transmission and intervention effectiveness are central in the pursuit of malaria elimination.

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CHARACTERIZATION OF RIBOSOMAL DNA OF ANOPHELES FLUVIATILIS SPECIES T AND U FROM A SYMPATRIC POPULATION

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Anopheles fluviatilis sensu lato is a vector of primary importance responsible for malaria transmission in forest and foothills of India. Provisionally this species was recognized as a member of four sibling species—species S, T, U and V, identifiable based on the chromosomal inversions present on chromosome 2 and 3. However recently based on the genetic barcode of three members of species complex, i.e., S, T and U, these nominal species were made conspecific. To resolve this issue, we characterized a stretch of partial ribosomal DNA (rDNA) of two sympatric species, species T and U, encompassing from 5.8S rDNA to D3 domain of 28S rDNA. PCR products were amplified from individual mosquitoes using a single PCR (1.75 kb) and sequenced through primer-walking. Analysis of sequences revealed four unique nucleotide locus where fixed differences exist between species T and U. Since these differences are species-specific and fixed in a sympatric population, our data disapprove the synonymy status of species T and U.

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SIBSHIP RECONSTRUCTION REVEALS RELATIONSHIPS BETWEEN LARVAL ABUNDANCE AND OVIPOSITION BEHAVIOR WITHIN AND AMONG ANOPHELES ARABIENSIS HABITATS

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Strategies to control malaria vectors at the larval stage appear increasingly important with acknowledgement of the limitations of primary interventions which target only adults biting and resting indoors. Larviciding strategies would benefit from knowledge on habitat productivity and the proportions of female mosquitoes that oviposit at multiple breeding sites to allow improved targeting. To investigate these questions, we applied genetic markers to *Anopheles arabiensis* larval samples from artificial and natural ponds to investigate relatedness within and among habitats. In a controlled experiment, 18 artificial ponds were left uncovered for 4 days to allow oviposition by wild females, then covered for a further 6 days to prevent further egg laying; larvae were sampled daily from day 5. Additionally, *Anopheles* natural habitat categories, 'puddles' and 'drainage ditches', were identified and larvae sampled. The larvae were identified to species and *An. arabiensis* samples genotyped using microsatellites. We used BAPS and COLONY softwares, to provide methodologically-independent reconstructions of sibling groups. In the ponds, COLONY (which is widely used but known to artificially partition large families) identified more families than BAPS but results were well correlated (r=0.7). In both cases the number of families predicted the total number of larvae ($r^2 \approx 0.5$), supporting our hypotheses that more larvae result from more females laying rather than simply better survival of an equivalent number of families. From BAPS results, 64% of females had deposited larvae in multiple ponds and from COLONY, 45%, suggesting a high frequency of skip oviposition behaviour. In the natural habitats, the number of families strongly predicted larval abundance ($r^2 \approx 0.8$) and this relationship did not differ between habitat types (P=0.19), with an overall average of 2.4±0.84 larvae per family. The sharing of habitat by multiple females and high skip-oviposition rate shown here are favorable for the success of an autodissemination approach for larval control and may be a factor in maintenance of high genetic diversity in *An. arabiensis* populations.

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CONSTRUCTION OF THE EFFICIENT GENOMIC EDITING SYSTEM WITH CRISPR/CAS9 IN VECTOR MOSQUITO, *AEDES ALBOPICTUS*

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Aedes(Stegomyia)albopictus , also known as Asian tiger mosquito, is a mosquito originally inhabiting in the countries of Asia but becoming more and more emerging throughout the world recent years. It can transmit several arboviruses including Dengue, Zika and Chikungunya virus, which already becomes a public health threat. Nowadays, genetic control is one of the most promising methods for mosquito population suppression. However, there is little research in gene editing of *Ae. albopictus*. In the present study, we first employed two genes, kynurenine hydroxylase and dopachrome conversion enzyme, to generate germ-line mutations by CRISPR/Cas9 system. Following Cas9-sgRNA RNPs injection into pre-blastoderm embryos, 30–50% of fertile survivors produced alleles that failed to complement existing *kh* and *yellow* mutation. We conclude that CRISPR/Cas9-mediated gene editing system is accomplishable in *Ae. albopictus*, offering an efficient tool for gene editing in mosquito population management. Moreover, our study represents a step toward

the application of large scale, species-specific population control technologies targeting *Ae. albopictus* that rely on Cas9-based gene drive mechanisms.

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PHYLOGEOGRAPHIC RELATIONSHIPS AND GENE FLOW PATTERNS OF GLOBAL COLLECTIONS OF AEDES AEGYPTI (DIPTERA: CULICIDAE) BASED UPON THE MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT 1 GENE

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The phylogeographic relationships among global collections of the mosquito *Aedes aegypti* was evaluated using 143 sequences from the mitochondrial Cytochrome C Oxidase 1 gene (CO1) including fourteen new sequences from Sri Lanka. The BEAST2 analysis implementing a CO1 arthropod molecular clock estimated that *Ae. aegypti* arose as a species ~614 thousand years ago (kya) in the late Pleistocene, which gave rise to two lineages. At 545 kya an "early" East African clade and at 372 kya an East African "late" clade arose. The "early" East African clade gave rise to three lineages, one of which is distributed throughout all tropical and subtropical regions, a second that contains Southeast Asian/Sri Lankan mosquitoes and a third that contains mostly New World mosquitoes. West African collections were not represented in this early clade. The "late" East African clade gave rise to East and West lineages. This late clade continues to differentiate throughout Africa which eventually gave rise to branches that spread globally. A Bayesian analysis of migration rates suggested abundant gene flow between India/Pakistan and the rest of the world and high rates of gene flow between East and West Africa and between Sri Lanka and Southeast Asia. This may reflect commercial movement of mosquitoes given the location of Sri Lanka along the key shipping route between the Malacca Straits and the Suez Canal and the fact that Sri Lanka contains some of the busiest harbors and ports in the region.

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A TRANSCRIPTOMIC APPROACH TO IDENTIFY MOSQUITO TARGETS FOR BLOCKING PLASMODIUM VIVAX AND PLASMODIUM FALCIPARUM TRANSMISSION

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Plasmodium falciparum and *Plasmodium vivax* are responsible for the majority of malaria cases worldwide. Whereas *P. falciparum* interactions with its mosquito vectors has been the focus of many studies, little is known on the interactions of *P. vivax* with its vectors. We capitalized on the peculiar situation of Madagascar where both parasite species are prevalent along with the most studied mosquito vectors: *Anopheles gambiae* and its sister taxa *Anopheles arabiensis*. After establishing a field laboratory, we infected local *An. arabiensis* mosquitoes with *P. falciparum* and *P. vivax*. Midguts from infected mosquitoes were collected and processed for a comparative RNAseq analysis to identify common or unique pathways to both/each parasite species and to better understanding the specificity of the interaction between each parasite species and their common vector, *An. arabiensis*. Preliminary results of this RNAseq analysis will be presented and discussed.

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GENOME-WIDE ASSOCIATION STUDY IDENTIFIES GENES UNDERLYING DENGUE VIRUS SUSCEPTIBILITY IN WILD-TYPE *AEDES AEGYPTI* FED ON VIREMIC HUMAN BLOOD

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Natural populations of *Aedes aegypti* mosquitoes vary in their susceptibility to dengue virus (DENV) infection but the underlying genetic basis has not been elucidated. Although single nucleotide polymorphisms (SNPs) in genetic markers as well as expression of certain genes have been linked to variation in DENV susceptibility, the *Ae. aegypti* genes containing causative polymorphisms are unknown. In this study, we performed a genome-wide association study (GWAS) followed by gene-silencing assays to identify *Ae. aegypti* genes functionally involved in natural DENV susceptibility. Wild mosquitoes collected in Ho Chi Minh City, Vietnam were tested for DENV susceptibility within three generations of colonization in the laboratory. They were scored for DENV infection after experimental exposure to DENV viremic blood by feeding directly on naturally infected dengue patients. Genomic polymorphisms associated with DENV infection status were identified using a recently developed *Ae. aegypti* SNP chip with genome-wide distribution. A shortlist of candidate genes located near SNPs significantly associated with DENV infection status is currently being tested by RNAi-mediated gene knockdown *in vivo*. Knockdown of one candidate gene resulted in a significant increase of DENV infection prevalence, indicating an antiviral function. Further characterization of this gene and other shortlisted candidates is ongoing. Our study is the first GWAS in wild-type *Ae. aegypti* that identifies candidate genes associated with DENV susceptibility that are subsequently validated at the functional level. Our results contribute to elucidate the genetic basis of natural DENV susceptibility in *Ae. aegypti* and will provide target genes that could be manipulated in future *Ae. aegypti* genetic control strategies.

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INSIGHTS INTO GENOMIC, CHROMOSOMAL AND ECOLOGICAL DIFFERENTIATION OF THE MALARIA MOSQUITO ANOPHELES MESSEAE SENSU LATO

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A dominant malaria vector *Anopheles messeae sensu lato* (*s.l.*) is a highly polymorphic species with a wide geographic distribution throughout Eurasia. Five highly polymorphic inversions associated with geographical distribution of the species have been reported. A sister species, *An. daciae,* was discriminated from *An. messeae sensu stricto* (*s.s.*), further referred as *An. messeae,* based on five fixed nucleotide substitutions in the Internal Transcribed Spacer 2 (ITS2) of ribosomal DNA. However, genome-wide genetic divergence and chromosomal differentiation between two species remained unexplored. In this study, we analyzed ITS2 sequences and karyotypes in 289 larvae specimens of *Anopheles* from three locations in Moscow region. Five individual genomes for each species were sequenced. Our study determined five previously described fixed substitutions in ITS2 of *An. messeae* whereas the ITS2 sequence in *An. daciae* had both *An. messeae* and *An. daciae* variants present simultaneously in the first three positions of each individual. Fixed differences between *An. messeae* and

An. daciae were found only in the last two positions. Only one mosquito was identified as a hybrid between *An. messeae* and *An. daciae* based on heterogeneous substitutions in all five positions. Our genome sequence comparison has demonstrated the genome-wide divergence between the two species. The divergence was extremely high on the X chromosome. A cytogenetic comparison of *An. messeae* and *An. daciae* samples demonstrated that two species significantly differ from each other by the frequencies of the polymorphic inversions. Inversion X1 was fixed in *An. messeae* but was polymorphic in all *An. daciae* populations. Frequencies of 3 polymorphic autosomal inversions were higher in *An. messeae* than in *An. daciae.* Each of the populations had distinct species composition suggesting different ecological preferences of the two species. Thus, our study revealed the high level of genetic differentiation between *An. messeae* and *An. daciae* in Moscow region. The research was supported by the Russian Science Foundation grant No 15-14-20011 to I.V.S. and RFBR grant No 18-04-01117A to M.I.G.

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GENETIC VARIATION IN CULEX TARSALIS ASSOCIATED WITH BLOODMEAL HOST CHOICE

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Culex tarsalis is the principal vector of several human pathogens in the western United States, including West Nile (WNV), western equine encephalomyelitis, and St. Louis encephalitis viruses. Of these, WNV causes the greatest annual disease burden, and incidence of WNV disease is highest in areas where *Cx. tarsalis* is the predominant vector, such as the northern Great Plains and California's Sacramento Valley. Because of its status as an important bridge vector of zoonotic arboviruses, the ecology of *Cx. tarsalis*, including feeding behavior, has received significant research attention, but genetic studies are limited, perhaps due in part to the lack of a reference genome. Genetically distinct populations have been identified in the US using microsatellite markers, but they have not been characterized regarding phenotypic differences such as vector competence, insecticide resistance, or host-seeking behavior. In this study, we hypothesized that ornithophilic and opportunistic genotypes of *Cx. tarsalis* occur in sympatry and the relative abundance of these genotypes influences the frequency of overall mammalian blood meals. To test this, we sequenced 40 blood-fed adult *Cx. tarsalis* females, including 13 that fed on mammals, 2 that fed on reptiles, and 24 that fed on birds. In addition, we deep-sequenced one female mosquito from an inbred colony (Kern County, CA) and assembled a draft reference genome using SPAdes (v3.11) and MEGAHIT (v1.1.2). Due to inadequate reference assembly quality (N50=1789), we performed a preliminary analysis on the mitochondrial genome (15,566bp). A principal components analysis based on 1,044 mitochondrial SNPs revealed evidence for population structure among *Cx. tarsalis* collected from the same site in Davis, CA. We also observed a lower nucleotide diversity among mammal-fed (pi=0.001) versus bird-fed (pi=0.002) *Cx. tarsalis*. This pattern is consistent with the hypothesis that particular genotypes are more likely to feed on mammalian hosts.

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EFFECTS OF FOREST LANDSCAPE ANTHROPIZATION AND LAND-USE AND LAND-COVER CHANGE ON THE ECOLOGY OF *AEDES* ARBOVIRUS VECTORS IN LARGE INDUSTRIAL PALM AREAS, CÔTE D'IVOIRE

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In Cote d'Ivoire, forest landscape anthropization (FLA) and land-use and land-cover (LULC) change have been associated with a tangential transmission of zoonotic *Aedes* mosquito-borne arboviruses to humans. We assessed the effects of FLA and LULC change on *Aedes* ecology in large industrial palm areas in yellow fever (YF) and dengue (DEN) foci. From January 2013 to December 2014, *Aedes* eggs, larvae and adults were sampled across a gradient of FLA (sylvatic, peridomestic and domestic ecozones), and four land-covers (Forest, polyculture, palm monoculture and housing area) using ovitraps, larval surveys and human-baited double-net traps. In total, 28,276 *Aedes* specimens belonging to 11 species (*Ae. aegypti*, *Ae. africanus*, *Ae. dendrophilus*, *Ae. fraseri*, *Ae. furcifer*, *Ae. lilii*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. palpalis* and *Ae. vittatus*) were found. *Aedes* species richness (7 species) and abundance (1.36 ± 0.14 *Aedes*/ovitrap/week) were higher in peridomestic ecozone. *Ae. aegypti* proportion was higher in domestic (81.0%), followed by peridomestic (62.6%) and sylvatic (37.3%) ecozones. Only four *Ae. aegypti* specimens were found in palm monoculture. *Aedes* showed higher species richness in forest (11 species), and higher abundance in polyculture (n = 28,276; 60.9%). *Ae. aegypti*, *Ae. dendrophilus* and *Ae. vittatus* biting rate was 34.6 and 7.2-fold higher in polyculture (21.5 bites/person/day (b/p/d)) and housing area (4.5 b/p/d), respectively, compared to forest (0.6 b/p/d). *Ae. aegypti* displayed strong anthropophagy inflicting 93.0% of bites to humans. *Aedes* biting activity showed bimodal daily feeding cycles, with stronger magnitude in polyculture. Conclusion, FLA and LULC change strongly drive *Aedes* ecology leading to the migration and proliferation of forest-dwelling zoophagic and anthropophagic vectors primarily biting humans in peridomestic ecozone, housing area and polyculture. This may link enzootic to epizootic and epidemic cycles of arboviruses thus raising the risk of transmission of YF and DEN viruses from wild animals to humans around their homes and farms. There is a need for an integrated vector management.

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INSECTICIDE SUSCEPTIBILITY AND ROLE OF *AN MELAS* IN AN AREA OF MALARIA RESIDUAL TRANSMISSION IN SENEGAL

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Background: The global decrease of malaria incidence in Senegal has changed the disease epidemiology; with malaria occurring mainly in hot spot of residual transmission. *An. melas* is one of the member of the *An. gambiae* s.l. complex found in brackish environment of the country, where its display high density. Despite its known role as secondary/focal malaria vector, little is known about the bionomic of *An. melas,* including as its susceptibility to insecticides. In the context of malaria elimination, it is therefore vital to evaluate the role of *An. melas* in the transmission as well as and its susceptibility to insecticides currently in use in malaria hot-spot areas. **Methods**: The study villages were chosen in the coastal zone in the Saloum River Delta. The malaria vector populations dynamics was monitored using the human landing catches (HLC) and pyrethrum spray catches (PSC) methods. **Results**: During the first study year, a total of 1933 Anopheles specimens (HLC = 700 and SPC =1293) were collected. *An gambiae* s.l. (95% ; n= 1892) was the dominant anopheline species . The molecular identification of members of the Gambiae complex and the search for the infection of the vectors collected are being carried out for the determination of the involvement of the different species in the residual transmission. Susceptibility tests to the WHO-approved insecticides, carried out in adults of *Anopheles gambiae* s.l. collected from brackish water showed that resistant to permethrin (91.3%), but fully susceptibility to organophosphate (100%) and carbamate (100%). **Conclusion and perspective:** This study ongoing shoud provide an up-to-dated information of the level of involvement of *An. melas* in malaria

transmission and its status of insecticide suceptibility in an area of malarial transmission hot-spot. These data are essential for targeted vector control interventions to achieve elimination goal in Senegal. Mots clés: Malaria, Transmission résiduelle, *An melas*, insecticide susceptibility, Sénégal.

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WIDE DISTRIBUTION OF THE MAYARO VIRUS VECTOR HAEMAGOGUS SPECIES IN TRINIDAD, WEST INDIES

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Emerging mosquito borne diseases have posed a major challenge to public health systems in many regions of the world within recent time. The Mayaro Virus (MAYV) is an emerging mosquito borne disease that was first reported in the island of Trinidad in 1954, but is now known to be endemic to Central and South America. This virus is only known to cause sporadic cases and limited outbreaks, but may have the potential to develop into a major epidemic in the future. The primary vector for MAYV is Haemagogus which is also the vector for the yellow fever virus, is also endemic to the region. Managing any possible future MAYV epidemic will require knowledge on the distribution of the vector. The last survey for *Haemagogus* in the island of Trinidad was in 1995, when the vector was reported to be confined to the Northwestern peninsula. The current study aims to update the distribution of Haemagogus in this island. A survey for the vector was conducted over the period March 2017 to March 2018. Adult mosquitoes were collected off human bait during 60 trips to major forested areas in the island . Larvae were also collected after breeding in tyre traps from all sites sampled. The results show that Haemagogus is now widely distributed in the island, with a total of 155 pools of the mosquito collected from all five zones surveyed. The average density of adult mosquitoes collected during the wet season (June-November) was six times the amount collected in the dry season (December-May). The species collected included Hg. janthinomys , Hg. equinus , Hg Leucocelaneus and Hg. celeste. Hg. janthinomys was the most adaptive species, which was found in all zones and accounted for eighty-eight percent of mosquitoes collected in the survey. Hg. janthinomys was predominantly found in the Ramsar site Caroni swamp and surrounding villages. This mangrove wetland serves as a habitat for a number of birds and wild mammals, but not primates which are known to be the main reservoir in sylvatic cycle of the MAYV. These new findings have significant epidemiological implications for the sylvatic cycle of this medically important species.

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CATTLE ODOR IS A POWERFUL ATTRACTANT FOR EXOPHAGIC MALARIA VECTORS

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Background: As currently implemented, malaria vector surveillance in sub Saharan Africa uses tools that are mostly able to target endophagic and endophilic mosquitoes leaving exophagic blood seeking and feeding behaviour largely undescribed. We evaluated the recently developed Host Decoy Trap (HDT) and compared it to the gold standard, Human Landing Catch (HLC), in western Kenya. HLCs are favoured because they elicit a more natural range of *Anopheles* biting-behaviour compared to other sampling tools, and therefore, in principle, provide the most reliable profile of the biting population. The HDT incorporates the main host stimuli that attract blood meal seeking mosquitoes and can be baited with the odours of live hosts. **Results:** Mosquito numbers and species diversity varied significantly between HLCs and HDTs baited with human (HDT-H) or cattle (HDT-C) odour, revealing important differences in behaviour of *Anopheles* species. Significantly higher proportions of *An. arabiensis* were caught in HDT-Cs, 0.94 ± 0.01 and HDT-Hs, 0.76 ± 0.09 than in HLCs, 0.45 ± 0.05 per trapping night. The proportion of *An. gambiae s.s.* was highest in HLC (0.55 ±0.05) followed by HDT-C (0.20 ± 0.09) and least in HDT-C (0.06 ± 0.01). An unbaited HDT placed beside corralled cattle overnight caught mostly *An. arabiensis* with proportions of 0.97 ± 0.02 and 0.8 ± 0.2 in presence and absence of cattle respectively and a mean of 10.4 (2.0-55.0) *Anopheles*/night near cattle, compared to 0.4 (0.1-1.7) *Anopheles*/night in an unbaited HDT indicating that the HDT can be effective without the need for directed odour. **Conclusions**: The capability of HDTs to combine host odours, heat and visual stimuli to simulate a host provide the basis of a system to sample human- and cattle-biting mosquitoes. HDT-C is particularly effective for collecting *An. arabiensis* outdoors. The HDT offers the prospect of a system to monitor and potentially control *An. arabiensis* and other outdoor-biting mosquitoes more effectively.

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ENVIRONMENTAL CHARACTERISTICS OF NYSSORHYNCHUS DARLINGI BREEDING SITES AND SPATIOTEMPORAL RELATIONSHIP WITH MALARIA CASES IN 8 VILLAGES IN PERI-IQUITOS, AMAZONIAN PERU

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Due to its propensity to bite and rest outdoors, *Nyssorhynchus darlingi*, the major malaria vector in Latin America, is unlikely to be completely controlled by traditional vector interventions such as long-lasting insecticidal nets and indoor residual spraying. Therefore, alternative control measures, such as larval source management (LSM), should be considered. A previous study in the peri-Iquitos region of Amazonian Peru found *Ny. darlingi* larvae presence to be associated with lower forest cover level, proximity to humans, and algae. In Brazil, *Ny. darlingi* breeding sites have been associated with the edges of deforested areas and with man-made water bodies, such as fish ponds and microdams. No recent published studies have characterized the larval habitat of *Ny. darlingi* in peri-Iquitos. From 2016-2017, we sampled 88 water bodies in 8 villages in peri-Iquitos for *Nyssorhynchus* larvae and recorded environmental and chemical characteristics, conducting 5-6 collections per water body. We collected 750 *Ny. darlingi* from both natural and artificial breeding sites. Similar to previous studies, the presence of *Ny. darlingi* was associated with lower enhanced vegetation index (EVI) and more people living within 100m of the water body. Additionally, *Ny. darlingi* presence was associated with the presence of grass and amphibians, lower light intensity (lux), increased cloud cover, and the presence of non-*Ny. darlingi Nyssorhynchus* and *Anopheles* larvae. Houses in the study villages in which inhabitants were diagnosed with malaria during the study period were located closer to *Ny. darlingi*-positive water bodies than houses in which no one was diagnosed with malaria, suggesting that LSM targeting *Ny. darlingi* breeding sites could be an effective control method in this region. This was a pilot study intended to provide preliminary data about the characteristics of *Ny. darlingi* breeding sites in the study villages. Future studies involving more

intensive sampling in these villages will provide a more comprehensive determination of the signature of water bodies that would be the most appropriate targets for LSM.

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USING SWARM INTELLIGENCE TO SIMULATE MOSQUITO BEHAVIOR AGAINST SPATIAL REPELLENTS

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Swarm intelligence is a method that deals with collective behavior of individuals that coordinate using decentralized control and self-organization. In natural system, examples of swarm behaviors include ants colonization, fish schooling, etc.. Male mosquitoes are known to exhibit swarm behavior, i.e., males aggregate over specific and conspicuous swarm markers to attract females and repeatedly at the same places over time. Similarly, female mosquitoes are attracted to humans or certain attractant odors, or are repelled by repellent. The aim of this study is to determine if pyrethroid resistant *Anopheles gambiae* mosquitoes behavior differentially from susceptible mosquitoes when against repellents. We used particle swarm optimization (PSO) approaches under the laboratory and MalariaSphere settings. In the laboratory setting, we used 3D tracking system to track the mosquitoes' movement under three conditions: nothing, attractant, and repellent. In the greenhouse setting, we used mark-release-recapture experiments. Both laboratory and greenhouse experiments are ongoing. In the simulation, we assumed a constant release and both attractant and repellent chemicals diffused randomly and omnidirectional in the cubic cage. The velocity component in the PSO was modified by an attraction/repulsion rate and concentration. Simulation result indicated that without attractant/repellent, mosquitoes might fly anywhere clueless. In the presence of attractant odor, all mosquitoes were attracted to the source of the odor. For the repellent, mosquito behavior depends on repellency property of the repellent and mosquito insecticide resistance status. For insecticide susceptible mosquitoes, simulation found that all mosquitoes were eventually repelled to the farthest side of the wall from the release spot. While resistant mosquitoes could stay close to the repellent release spot. When cumulative repellent concentration became high, mosquitoes were pushed away from the repellent release spot. Simulation results are being validated using the experimental data in the MalariaSpheresand laboratory.

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ASSESSING THE RELATIVE ABUNDANCE OF MALARIA VECTORS TRAPPED INDOORS AND OUTDOORS IN CHIKHWAWA, SOUTHERN MALAWI

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Monitoring the abundance of malaria vectors is important for assessing potential changes in malaria transmission. Most of the current sampling methods for malaria vectors are designed to work indoors, but the importance of sampling outdoors is increasingly being recognized together with the potential for outdoor malaria transmission. The Suna trap is a recently developed odour-baited trap, which can be set either indoors or outdoors. Because the trap is designed to attract host-seeking mosquitoes, two Suna traps simultaneously set indoors and outdoors at the same house may potentially compete with each other, biasing any comparison of indoor and outdoor sampling. Our objective was to assess the potential for this competition between traps. The study was conducted for 24 nights in March-May 2017 using a 12 × 12 Latin Square design in 12 houses in a rural area of southern Malawi. Suna traps baited with an attractant blend and carbon dioxide were set at each house in one of three ways: one trap indoors, one trap outdoors or one trap indoors with an additional trap outdoors. Differences in the number of mosquitoes collected were tested using t-tests. We collected 134 female *Anopheles* mosquitoes, 90% of which were identified molecularly as *Anopheles arabiensis.* The number of mosquitoes collected in indoor traps did not differ between houses where a trap was simultaneously used outdoors or not (P = 0.928). Likewise, the indoor trap did not affect outdoor sampling: catch sizes for outdoor traps did not differ between houses where a trap was simultaneously used indoors or not (P = 0.745). Simultaneous indoor and outdoor use of Suna traps at the same house does not reduce the number of mosquitoes collected in either trap, and therefore indoor and outdoor sampling do not need to be done on separate nights. Researchers and malaria control programmes interested in sampling mosquitoes indoors and outdoors with Suna traps can set traps in both locations on the same night, saving time and effort.

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FINE SCALE BIOTIC AND ABIOTIC EFFECTS OF WEST NILE VIRUS ILLNESS IN HUMANS

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Since 1999, West Nile virus (WNV) has moved rapidly across the United States, resulting in thousands of human cases. The number of cases and rate of mosquito infection (MIR) varies across years, with an uneven geographic distribution at regional and local scales. Besides differences in the type and amount of mosquito control, fine-scale differences in WNV risk among geographic locations are related to variable microclimates, vegetation and drainage systems, socio-political factors, property tax base of mosquito abatement districts (MADs), and population-level difference in vector biology. Because of the interactions among these multiple factors that affect the locally variable risk of WNV illness, it has been especially difficult to determine the effect of mosquito control on human risk potential. Cook County, comprising the city of Chicago and surrounding suburbs, is among the areas hardest hit by WNV in the United States. After the 2002-2003 WNV outbreak, the four independently operating MADs in the area revised and improved their preparedness and treatment protocols to better address the specific threats posed by WNV. However, despite these changes and active mosquito control efforts, there are consistent, WNV positive pools of adult mosquitoes annually and greater than 350 confirmed WNV human cases and 12 deaths reported in the past 5 years in Cook County alone. Using multivariate statistical approaches to investigate the biotic and abiotic factors that drive the spatially variable risk of WNV in Cook County, our prior study found that hot and dry weather conditions and higher MIR in earlier weeks and higher percentages of the white population increased the probability of an area of having a WNV human case. The higher proportion of high and medium intensity urban areas and open water sources in an area decreased the probability of observing a WNV human case. We aim to add catch basin density and historical mosquito abundance to our existing model at finer spatial scales to create the most robust human WNV risk model to date.

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COMPETITIVE INTERACTIONS IN AEDES MOSQUITOES AT CONSTANT AND FLUCTUATING TEMPERATURES

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Mosquitoes have evolved specific responses to variation in environmental conditions and these responses have implications on the outcome of species interactions such as intraspecific and interspecific competition. Investigations on competitive interactions in container mosquitoes so far have mostly been done at constant temperatures despite the importance of fluctuating daily temperatures as a better indicator of field conditions. This study investigated the effects of constant and fluctuating daily temperatures estimated as diurnal temperature range (DTR), on

inter- and intraspecific larval competition between *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.). Larvae of laboratory established colonies were reared under controlled conditions at three constant (20°C, 27°C and 32 °C) and three fluctuating (20°C DTR 10, 27°C DTR 10, and 32 °C DTR 10) low, medium and high-temperature treatments. Overall, fluctuating temperatures led to lower larval survival, and smaller adults than constant temperatures, for both species. At low and medium temperatures, *Ae. albopictus* larval survival and adult mass were minimally influenced by interspecific competition with *Ae. aegypti*. However, at high temperatures, both interspecific and intraspecific competition decreased larval survival and adult mass for both species. These findings improve our understanding of the relationship between temperature variation and species interactions and provide valuable information for modeling of container mosquito population dynamics and intrinsic growth rate of vector populations.

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UPGRADE OF A PHYSICAL GENOME MAP FOR AEDES AEGYPTI

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Nearly 4 billion of the world's population is currently at risk to contract mosquito borne diseases, with almost 2500 million people prone to dengue infection. *Aedes aegypti* is the principal vector of Dengue, Chikungunya and Zika viruses. Efforts are ongoing to facilitate a better understanding of the *Aedes aegypti* genome to pave ways for vector control. Among mosquitoes, high-coverage genome maps for the African malaria vector *An. gambiae* and *An. albimanus* were developed, covering 84.5% and 98.2% of the *An. gambiae* and *An. albimanus* genomes, respectively. The recent Hi-C-based scaffolding approach assigned 93.6% of the original L3 *Ae. aegypti* draft genome assembly to three chromosomes thus creating the L4 assembly. However, the genomic coordinates of L4 assembly do not have any correspondence with chromosomal bands. Further, it lacks data from male sex locus as the L4 assembly was based on females only. To develop a fine-scale physical genome map anchoring genomic contigs to their chromosomal positions, we compared the *Ae. aegypti* assembly coordinates of ~500 previously and newly mapped BAC clones from the NDL library with their positions on the chromosomes using multicolor fluorescence *in-situ* hybridization (FISH). We performed repeat masking of the BAC-end sequences and aligned them to the recent L5 assembly which includes the male sex locus (M locus). We were able to match 403 BAC-end sequences with the L5 genome assembly. We identified contradictions of the genome coordinates with chromosomal positions of 10 BAC clones, which represent 2.6% of the mapped BAC clones. Overall, the BAC-clone-based map validated the high quality of the L5 assembly and presents a final resolution of 5-10 MB, the highest density map for *Ae. aegypti* until now. The genome coverage of the map equals 93.4% representing the second highest genome coverage among mosquitoes. We used this map to validate the sex locus in different strains of *Ae. aegypti*. These efforts will help to identify, target and manipulate epidemiologically important traits and to develop potential new strategies for vector control.

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ENTOMOLOGICAL INVESTIGATIONS INTO RIFT VALLEY FEVER VIRUS TRANSMISSION POTENTIAL ON FEEDLOTS IN COLORADO

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Rift Valley Fever Virus (RVFV) is an emerging arbovirus that infects ruminants and humans. The 20th century saw notable range expansions of the virus to new geographies outside of continental Africa. Many gaps remain in our knowledge of potential transmission cycles of RVFV in the United States. Several species of mosquito native to the U.S. are competent vectors in the laboratory, with especially high vector competence observed of Cx. tarsalis. We conducted field studies to determine the extent to which native mosquitoes could bridge transmission of RVFV from potential wildlife hosts to domestic ungulates at feedlots in Colorado. We used a paired-site design, sampling mosquitoes at feed lots and nearby control sites in Colorado from early May to late August using CDC light traps. We characterized mosquito diversities at each site using Hill numbers, and investigated differences between mosquito community assemblages by habitat type (feedlot vs control) using ordination and model-based analysis of multivariate abundance data. Blood meal analysis was performed on engorged specimens to determine differences in feeding behaviors at feedlots versus control sites. We also compared West Nile Virus (WNV) infection rates in Cx. tarsalis collected at feedlots and nearby control sites to investigate whether dispersal is limited across the feedlot/wildlife interface. Mosquito diversities were generally higher on control sites than on paired feedlot sites. Mosquito community assemblages differed significantly by site type (feedlot vs. control). Cx. tarsalis fed on a high diversity of vertebrates hosts at control sites, but predominantly cattle on feedlots, indicating potential to bridge potential sylvatic transmission to domestic ungulates. Infection rates of Cx. tarsalis with WNV did not differ between paired sites, offering evidence that Cx. tarsalis mosquitoes disperse readily across the feedlot/wildlife interface. Overall, these results show that while feedlots impact the mosquito community measures, Cx. tarsalis may bridge hypothetical sylvatic transmission cycles to domestic ungulates.

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INCREASED ADULT *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) ABUNDANCE IN KAOHSIUNG CITY, TAIWAN

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The assumption that vector abundance differences might drive spatial and temporal heterogeneities in vector-borne disease transmission is common, though data supporting it is scarce. Here, we present data from two common mosquito species *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* Say, biweekly sampled as adults, from March 2016 through December 2017, with BG-sentinel traps in two neighboring districts of Kaohsiung City (KC), Taiwan. One district has historically been a dengue transmission hotspot (Sanmin) and the other a coldspot (Nanzih). We collected a total 41,027 mosquitoes, and we found that average mosquito abundance (mean ± S.E.) was higher in Sanmin (*Ae. aegypti*: 9.03 ± 1.46 ; *Cx. quinquefasciatus*:142.57 ± 14.38) than Nanzih (*Ae. aegypti*: 6.21 ± 0.47 ; *Cx. quinquefasciatus*: 63.37 ± 8.71) during the study period. In both districts *Ae. aegypti* and *Cx. quinquefasciatus* population dynamics were sensitive to changes in temperature, the most platykurtic environmental variable at KC during the study period, a pattern predicted by Schmalhausen`s law, the principle stating that organisms are more sensitive to small changes in environmental variables whose average value is more uncertain than its extremes. Our results also suggest that differences in *Ae. aegypti* abundance might be responsible for spatial differences in dengue transmission at KC, and our comparative approach, where we also observed a significant increment in the abundance of *Cx. quinquefasciatus* in the dengue transmission hotspot, suggests this area might be more likely to experience outbreaks of other vector borne diseases, and should become a primary focus for vector surveillance and control.

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XENODIAGNOSIS TO DETECT *L.DONOVANI* INFECTION IN VISCERAL LEISHMANIASIS, POST KALA-AZAR DERMAL LEISHMANIASIS AND ASYMPTOMATIC SUBJECTS

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Visceral leishmaniasis (VL) is a chronic protozoan parasitic disease causing substantial mortality and disability. VL Elimination campaign has been running on the Indian Subcontinent since 2005. However, to achieve and sustain elimination of VL, knowledge gaps on infection reservoirs and transmission need to be addressed. In the present study, we investigated the role of *L. donovani* exposed individuals from across the infection spectrum in driving the transmission of endemic VL. Direct xenodiagnosis was performed using 10 male and 30 female *P. argentipes* sand flies on active VL patients (n=74), Endemic healthy aymptomatics (EHC; n=56) and PKDL patients (n=08). After 60 hrs of post blood meal feeding, only blood fed flies were dissected and infections of sand flies were evaluated by microscopy. We found varying sand fly feeding response (3-94%, Mean: 66.7%) with VL patients, 56.2-89.6% (Mean: 65.4) with Post kala-azar (PKDL) patients and (3-94%, Mean: 66.7%) with EHCs. Modeling analysis for the proportion of flies that get infected with VL within each class of severity of disease showed that infectivity of flies correlates with severity of disease. We found 24.02% (ODDs ratio: 69.5) of flies infected in the highly severe disease patients (splenic score=5) compared to 0.45% infection in flies in subjects with splenic score-1. In PKDL, we found infection in flies was higher when exposed on skin lesions; and nodular forms are more infectious compared to macular forms. Importantly, none of the asymptomatic subjects was able to transmit parasite to flies. The study on human shows that VL and PKDL patients harbored parasites in sufficient numbers to promote infections in vector sand flies. <!--EndFragment-->

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DENSITY AND INDEX OF MOSQUITOES BEFORE AND AFTER HURRICANE MARIA IN SOUTHERN PUERTO RICO

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In 2017 and 2018 the distribution of mosquitoes has varied due to environmental and climatic factors, such as Hurricane Maria. Our objectives: 1) Evaluate the environmental and climatic factors related to the density and distribution of mosquitoes during the dry period of 2017 and the dry period of 2018; 2) Determine entomological index for 2018. 3) Determine hotspots and coldspots in the southern region of Puerto Rico. An ecoepidemiology substudy in the southern region of Puerto Rico was performed to evaluate the environmental factors, climatic factors and density of mosquito. It was observed that when the wind speed increases the frequency of mosquitoes decreases ($r = -0.8263$) ($p<0.05$). In extreme temperature and pressures the distribution of mosquitoes was lower, however, precipitations led to an increase in mosquitoes. In the dry period during 2017 it was observed that the mosquito density was 20 mosquitoes, while in the dry period of 2018 the mosquito density was 128 mosquitoes. During the rainy season of 2018 (after Hurricane Maria) the larvae entomological index was 59%, the pupal entomological index was 36% and the oviposition entomological index was 72%. During the dry season of 2018 were 53%, 29% and 65% respectively. In the southern region of Puerto Rico, the hotpots were statistically different compared to the coldspots (z score > 2.58) ($p<0.001$). The hotspots and coldspots maintained a pattern of grouped values ($p<0.05$). Adjusting for collected samples the hotpots were not statistically different compared to the coldspots (z score $= -1.65 - 1.65$) ($p>0.001$). Therefore, understanding the environmental factors, climatic factors, and the entomological index leads to knowing the distribution of mosquitoes and being able to carry out interventions and vector control.

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LEVERAGING ARBOVIRAL SURVEILLANCE DATA TO INFORM PUBLIC HEALTH RESPONSES IN BARBADOS

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The island nation of Barbados (pop. ~285,000), located in the Caribbean, has long contended with public health threats posed by *Aedes*-transmitted diseases, in particular, dengue fever. More recently, both Chikungunya and Zika joined dengue as challenges to public health. Management of arboviral diseases in Barbados is necessary not only for protecting the health of the resident population, but also ensuring the continued success of the island's thriving tourism industry. The establishment of *Aedes* mosquitoes in Barbados and throughout most of the Caribbean has rendered islands vulnerable to emerging pathogens and outbreak events. In 2015, Zika spread rapidly throughout the Americas, and its proliferation through the Caribbean followed suit. Barbados reported its first confirmed autochthonous Zika transmission to the Pan American Health Organization (PAHO) in January 2016, a month before the global public health emergency was declared. Following detection of suspected Zika cases on Barbados in 2015, 926 individuals were described as suspected cases, and 147 lab confirmed cases were reported through December 2016. Our work on dengue incidence in Barbados from 2013-2016, in which georeferenced cases of dengue fever reported to the Ministry of Health (n=1,117) were aggregated to health districts, demonstrated that cases were not randomly distributed in space. Utilizing local Moran's I and spatial scan statistics, we detected areas of significantly high and low disease activity that shifted between years, an indication of spatially and temporally variable disease transmission risk. The ability to detect critical hotspots of transmission activity enables public health agencies to target mosquito control efforts, strengthening the capacity to curtail outbreaks and respond to, and reduce, the threat of emerging mosquito-borne pathogens in the Caribbean. Moving forward, we recommend further incorporation of spatially explicit methods of outbreak detection into existing surveillance frameworks.

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GEOSPATIAL ANALYSIS OF HIGH-RISK AREAS FOR VECTOR-BORNE TRANSMISSION USING REMOTE SENSING TECHNOLOGIES

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Mosquitoes kill more humans than any other creature on earth. As a consequence of a wide range of mosquito breeding habitats globally, the high abundance of competent arboviral vector species in existence, and lacking international mosquito control infrastructure, arboviral diseases cause an estimated 300 million infections and 500,000 deaths annually. While effective insecticides exist, modest vector control department budgets prohibit their broad distribution, warranting cost-effective measures for enhanced and targeted insecticide applications. Our current project was a collaborative effort to develop a predictive

model of mosquito breeding habitats utilizing advanced remote sensing technologies. To ensure maximum public health benefit, we also evaluated the association of the model's detected mosquito breeding habitats to real-world mosquito abundance and mosquito pool arboviral disease status within our selected study areas. Our current project in Harris County, Texas focuses on *Culex sp*. and *Aedes spp*. mosquitoes in the downtown-ship channel corridor and the suburban communities of Spring and Katy. We employed a variety of remoting sensing techniques including object-oriented image classification, vegetation and water indices, and spectral readings for enhanced classification. This unique project brought together a diverse group of experts in an innovative way to tackle one of the most important public health concerns of our time.

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IDENTIFICATION OF *PLASMODIUM FALCIPARUM*-INFECTIOUS *ANOPHELES COLUZZII* MOSQUITOES USING NEAR-INFRARED SPECTROSCOPY

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The prevalence of infectious mosquitoes is used to assess the effectiveness of vector control against malaria. Mosquitoes with salivary gland sporozoites can be identified by microscopy following manual dissection, ELISA technique or using expensive molecular methods. These low throughput techniques are impeding routine entomological surveillance especially in areas with high mosquito densities. This study evaluates the potential of near-infrared spectroscopy (NIRS) technique to differentiate between *Plasmodium* infected and uninfected mosquitoes. Colony of *Anopheles coluzzii* mosquitoes were fed on blood collected from gametocyte positive volunteers naturally infected with *Plasmodium falciparum* using direct membrane feeding assay in Burkina Faso. Two hundred and ten mosquitoes (111 in exposed group and 99 in control group) were killed 14-18 days-post blood-feeding and scanned using NIRS. Infection status was confirmed in individual mosquitoes using qPCR as the reference method. The overall predictive accuracy for differentiating infectious from uninfectious mosquitoes was 72%, and the false negative and false positive rates were 37% and 20% respectively. The area under the receiver operating characteristic curve was 0.80. The moderate diagnostic accuracy observed here might be improved by modifying the machine learning methods used to convert spectral data into estimates of infectious status or by improvements in the lights source, fibre optics, or NIR sensors. NIRS is a rapid and non-destructive scanning technique that has the potential to be used to measure sporozoite prevalence though further work is needed to increase its precision and validate the technique in wild caught mosquitoes.

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HOST DECOY TRAPS ARE HIGHLY ATTRACTIVE TO OUTDOOR BITING MALARIA VECTORS

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Sampling outdoor biting malaria vectors is vital to understanding peri-domestic transmission risk and evaluating outdoor control interventions and is particularly important in pre-elimination settings or where residual vectors may maintain transmission. However, current methods for measuring outdoor biting suffer from serious ethical and technical limitations. To address this, we developed a host decoy trap (HDT), which exploits known behaviors of *Anopheles* mosquitoes. By incorporating olfactory, visual and thermal stimuli, the HDT mimics a human host and attracts *Anopheles*, inducing them to land and become trapped. We tested the HDT against human landing catches (HLC) and CDC light traps indoors and outdoors in four different ecological and epidemiological settings in west Africa: 1) an area of intensive rice irrigation and 2) traditional Sudanian savanna subsistence farming in Burkina Faso; 3) an area of urban coastal malaria transmission in Benin; and 4) a low vector density setting with seasonal transmission in highland Cameroon. Latin square replicates were completed across a range of climatic periods and vector densities. We conducted a simultaneous participatory technology assessment to determine the user acceptability of each method, focusing on ease of use, perceptions of risk and confidence. In total, 19,289 *Anopheles* were collected. Outdoors, HDTs matched or outperformed HLC on vector abundance across all settings, except for one village in Burkina Faso where the HLC caught more *Anopheles*. HDT catch was significantly higher than HLC in higher density periods. The CDC light trap caught fewest *Anopheles*. Indoors, the HLC caught consistently more *Anopheles* than the other methods. Field collectors cited high confidence in the HDT relative to the other methods, finding it easy to use, although more effort was required to set up the trap. They generally disliked HLCs as their sleep was disturbed, impacting on the following day's activity, and they perceived risks associated with exposure to vector-borne diseases and the night air. The HDT has potential as an acceptable standardized outdoor sampling method for malaria vectors.

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PLASTICITY OF HOST SELECTION, NON-RANDOM HUMAN FEEDING AND SPATIAL HETEROGENEITY OF EXPOSURE TO MALARIA VECTORS IN PAPUA NEW GUINEA

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In Papua New Guinea (PNG), malaria transmission persists despite decade-long implementation of long-lasting insecticidal nets (LLINs) nationwide. The resilience to LLINs is attributed to ecological and behavioral factors rather than insecticide resistance. We investigated host selection and spatial distribution of human-vector contact of four *Anopheles* species in four villages of PNG, two with high (>70 %) and two with low (<55 %)

LLIN use. Anophelines were sampled using barrier screen and landing catch methods. Mosquito and blood meal host species were identified by PCR methods. Human blood meals were individualized using microsatellite DNA profiling. The ratio of human to nonhuman blood meals for *Anopheles punctulatus*, *Anopheles farauti* and *Anopheles longirostris* conformed to the census ratio in villages with low LLIN usage but was lower in villages with high LLIN usage. In contrast, the ratio of human blood meals for *Anopheles koliensis* was high in all villages regardless of LLIN usage. These results show that *Anopheles farauti*, *Anopheles punctulatus* and *Anopheles longirostris* are opportunistic in their host selection and utilize non-human hosts when access to humans is reduced, whereas *Anopheles koliensis* is highly anthropophilic. Of 79 individual people identified in 214 human-fed *Anopheles farauti* blood meals from one of the villages, only a few people (13%) contributed to most (64%) blood meals, indicating non-random human host selection. *Anopheles* landings per person-night (an estimate of bites per person-night) and *Anopheles* density per barrier screen-night were both spatially heterogeneous and overdispersed for all species, indicating aggregation of vectors in certain microenvironments of the villages. The number of landings on humans was greater outdoors than indoors. The spatial heterogeneity of mosquito abundance and exposure may result in spatial heterogeneity in risk of infection within a village. Alternative vector control methods such as treatment of domestic animals with endectocides can be implemented alongside the LLINs to target the outdoor-biting opportunistic vectors.

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EFFECT OF LARVAL DIET, AGING, AND ZIKV INFECTION ON IMMUNE GENE REGULATION IN AEDES AEGYPTI

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Arboviruses transmitted by mosquitoes are a significant cause of mortality throughout the world. Transmission of these disease-causing agents is dependent upon the existence of competent arthropod vectors that have the capability to maintain the pathogen, then transmit it to a subsequent host. Multiple studies of vector competence have suggested that the interaction between pathogens and the mosquito immune system plays a role in determining competence of the mosquito as a disease vector. In this study, we analyzed relative expression of a suite of immune genes from ZIKV infected *Aedes aegypti*. Mosquitoes were reared as part of a larger study investigating the effects of larval diet on vector competence. Standard extraction techniques and cDNA synthesis, followed by qRT-PCR, using previously published primer sets, was used to analyze relative expression at the transcriptomic level. Significant differential expression was observed between mosquitoes from different larval diets and between ZIKV-infected and control mosquitoes in all immune genes analyzed, the general trend being down-regulation of genes following viral infection and under nitrogen-poor rearing conditions. These results represent a step towards illuminating the influence of diet and viral infection on the immune response in mosquitoes, which may have subsequent consequences for the transmission of arboviruses. Results will also be presented from ongoing RNAseq analyses of the effect of larval diet on global transcript expression and a study using the same suite of *Aedes* immune genes to measure gene expression as mosquitoes age under laboratory conditions.

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SIMULATING THE EFFECTS OF CLUMPED EGG LAYING ON MOSQUITO POPULATION DYNAMICS IN RELATION TO GENE-DRIVE INTERVENTIONS

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Gene-drive based vector control methods are a rapidly developing tool in the fight against malaria. They utilise highly targeted insertions of genes to express specific traits, such as biases in offspring sex ratio or inhibited vector competence, which are preferentially inherited by copying themselves between chromosomes. Although theoretically self-sustaining, most gene-drive methods are sensitive to numerous aspects of local mosquito population dynamics. Often however, due to gaps in our knowledge, mathematical modelling of gene drive systems makes highly simplifying assumptions about key aspects of mosquito ecology. There is an urgent need to better understand fine scale population processes to improve predictions of the likely impact of gene-drive releases and refine development of target product profiles. The principal drivers of local mosquitoes dynamics can be traced to the larval stages, where density-dependent mortality in larval habitats is a key regulator of local adult density. In this study, we explored these drivers by simulating non-homogenous egg-laying over time and analysing its relationship with differing functional forms of density dependence. We developed a discrete-time stochastic model of mosquito population dynamics, which incorporated the random, temporal clumping of egg laying. The model was fitted to historical longitudinal mosquito trapping data using advanced particle MCMC methods. From this we were able to better quantify the relationship between clumping of egg laying and density-dependent regulation of larval populations, and thus refine estimates of the mosquito reproduction number, Rm - a key determinant of the predicted impact of gene-drive interventions. We find incorporation of

clumping of egg-laying improves model fit, and reduces estimates of Rm, thus making establishment and spread of gene-drive constructs more likely than predicted by models assuming higher Rm values.

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PARITY, PRESENCE, AND PREVALENCE: USING MOSQUITO DATA AND GONOTROPHIC CYCLE DYNAMICS TO MONITOR DISEASE IN ELIMINATION-TARGETED SETTINGS

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Mosquito-borne diseases represent a substantial portion of the total global disease burden. In the case of malaria, onchocerciasis, and lymphatic filariasis there are global elimination campaigns underway. Sustaining public and political support near the end of elimination campaigns can be difficult due to the difficulties posed by accurate prevalence and incidence estimation due to low case numbers. Xenomonitoring, the use of vector data to detect pathogen presence in human populations, has been cited as a potential tool for improving surveillance at low prevalences. However, there is an expectation that effective xenomonitoring requires financially infeasible sample sizes and yields intractable data. Despite these issues, the potential benefits of xenomonitoring are obvious; data collection would be non-invasive and allow greater access to hard-to-reach populations, giving a clearer snapshot of transmission. Using a novel feeding cycle model we estimate the probability of a parous vector being infectious given different human prevalence levels of a particular disease, to provide a simple tool for relating infection in mosquitoes to that in humans. The more cycles a vector has completed, the higher the probability it will be infectious, leading to a relationship between parity and prevalence that will vary depending on mosquito species and both infection and mosquito longevity. We calculate explicit expressions for the entomological inoculation rate, the basic reproductive number, and vectorial capacity. These important measures of vector-borne pathogen transmission are widely used but can be hard to estimate, our results describe a method of direct calculation from mosquito data and allow investigation of the potential impact of vector control measures. We use these tools to present techniques for calculating sufficient sample sizes when confirming presence or absence of disease, as well as demonstrating the relationship between human and vector prevalence across a range of diseases.

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TOWARDS TARGETED IRS: MAPPING AND TRACKING THE INDOOR RESTING BEHAVIORS AND PREFERENCES OF AEDES AEGYPTI IN SEMI-FIELD EXPERIMENTS

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The peridomestic behavior of *Aedes aegypti*, the primary mosquito vector of arboviruses to humans, is often speculated or unknown despite the considerable body of literature available. Its endophilic propensity is an attractive target for control by indoor residual spraying (IRS). However, IRS needs large insecticide quantities, is potentially harmful to householders and is often poorly accepted. A deeper understanding of *Ae. aegypti* indoor resting habits could identify key indoor sites that are often/always used for resting, greatly reducing the area requiring IRS. In the literature *Ae. aegypti* is often reported to rest on surfaces below 1.5 m height, but reports provide minimal information on resting activity over time. We investigated the basic resting behavior of *Ae. aegypti* in Recife, Brazil using an unfurnished room, with white walls and ceiling coated in a non-setting adhesive (the Sticky Room). In a first experiment, the entire room was coated, and adult mosquitoes (both sexes) and different physiological stages were released. Forty minutes later, the mean percentages and standard deviations (SD) collected within 1 m from the floor were 76.1 (7.2), 66.9 (7.2), and 60.6 (6.9) for males, unfed and bloodfed females respectively. In the second experiment, only two 20 cm strips of adhesive were applied to the walls, as horizontal bands covering 0-20 cm and 160-180 cm from the floor. Twenty-four hours later, the percentages and SD of males, unfed and fed females captured on the 20-cm sticky strip closest to the floor were 85 (11), 90 (4), and 79 (17), respectively. These preliminary results indicate that in the absence of visual or other stimuli, the majority of *Ae. aegypti* adults have a strong tendency to visit a restricted area of the walls adjacent to the floor. The current series of experiments employs a video system to track mosquito movement and resting preferences in a more complex environment, incorporating modular 3D shapes (simulated furniture) and cryptic resting sites. We present these experimental results as evidence for the potential of householder-delivered targeted IRS as an effective means of *Aedes* control in urban communities.

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PHYLOGENETIC ANALYSES OF ALL FOUR DENGUE VIRUS SEROTYPES DETECTED IN WESTERN KENYA, 2014-2015

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Reports of dengue virus (DENV) cases and outbreaks provide evidence of ongoing DENV transmission in Kenya. Yet, little is known about the circulating virus strains, limiting our understanding of viral transmission dynamics. To address this gap in knowledge, we used next-generation sequencing to characterize the phylogeny of all four DENV serotypes (DENV-1 to -4) identified in acutely febrile children, living in western Kenya between 2014-2015. The subjects were participants in our study of pediatric arboviral disease burden, who presented with fever to either of two regional health centers that served the communities of Chulaimbo and Kisumu. RNA was extracted from blood samples and reverse transcribed to cDNA in Kenya. cDNA was then shipped to the US for further analysis. We used the MiSeq platform (Illumina, San Diego, CA), to sequence cDNA from children who were positive for DENV by conventional PCR. To date, seven genomes have been fully sequenced: four DENV-1, and one each of DENV-2, -3, and -4. Phylogenetic analysis reveals four different genotypes as well among the four serotypes, with possibility of exhibiting distinct levels of genetic variation. Further whole-genome molecular evolutionary analysis may reveal varying trends in selection pressures and phylodynamics among the four co-circulating DENV serotypes. These data provide valuable insight into the evolution of DENV in Kenya and provide an important reference for future investigation of arboviral transmission dynamics.

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EVALUATION OF NEXT GENERATION SEQUENCING AND BIOINFORMATICS PIPELINE FOR PATHOGEN IDENTIFICATION USED AT THE DEPARTMENT OF VIROLOGY, ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES (AFRIMS), BANGKOK, THAILAND

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Next Generation Sequencing (NGS) technologies and bioinformatics tools provide broad detection of various pathogens in clinical specimens and are widely used in public health laboratories. Different protocols/pipelines have been developed for pathogen identification and discovery. To obtain optimal pipelines, evaluation and standardization is necessary. The Department of Virology, AFRIMS, participated the Next Generation Sequencing and Bioinformatics Consortium Pathogen Discovery Pilot supported by Global Emerging Infections Surveillance (GEIS) and conducted by Walter Rees Army Institute of Research (WRAIR) in collaboration with NMRC-BDRD and USAMRIID-CGS. A panel of 20 unknown extracted nucleic acid samples was created, validated, and shipped to AFRIMS with clinical case descriptions. To identify pathogens in the panel, four NGS runs were performed using the Illumina MiSeq with ten samples per run. Sample preparation procedures included 1) RNA sequencing using TruSeq Stranded Total RNA with Ribo-Zero Globin kit, 2) DNA sequencing using QIAseq FX DNA sample preparation kit. The data were processed and analyzed using four bioinformatics pipelines included EDGE, CLARK, Kraken and Trinity2. Adenovirus, influenza, Zika, dengue, chikungunya, and Japanese encephalitis viruses, *Acinetobacter baumannii*, and *Klebsiella Pneumoniae* were identified. All detected pathogens were confirmed by reference mapping and conventional laboratory assays included PCR, RT-PCR, and 16s sequencing. Phylogenetic analysis was performed with some pathogens. Results were sent to WRAIR for evaluation. AFRIMS was able to identify 95% of the pathogens in the panel (95% CI: 75-100), 87.5% sensitivity (95% CI: 47-99), 100% specificity (95% CI: 74-100), 12.5% false negative rate (95% CI: 1-53), and 0 false positive rate (95% CI: 0-24). Our data demonstrated that RNA sequencing and bioinformatics pipelines were optimal for identification of both DNA and RNA pathogens. The pilot helped to monitor the quality and accuracy of our NGS/bioinformatics pipeline and provided useful suggestions for improvement.

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HOUSEHOLD-BASED CLUSTER INVESTIGATIONS DURING AN OUTBREAK OF DENV-2 IN AMERICAN SAMOA

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The U.S. territory of American Samoa (AS) experienced outbreaks of chikungunya virus (CHIKV) in 2014, dengue virus type-3 (DENV-3) in 2015, and Zika virus (ZIKV) in 2016. The AS Department of Health reported 594 laboratory-positive cases (10.8 per 1,000 pop.) from a dengue virus type-2 (DENV-2) outbreak during November 1, 2016–January 12, 2018. As this outbreak followed an outbreak of ZIKV, it provided an opportunity to evaluate DENV infection after ZIKV exposure. Household cluster investigations were conducted during September–October 2017 to identify individual and behavioral risk factors for DENV infection. We hypothesized that antibodies from previous infection with heterologous flaviviruses may confer protection against symptomatic dengue disease, as opposed to the antibody-dependent infection enhancement seen in some cases of heterotypic sequential DENV infection. PCR- or NS1-positive dengue patients were visited within 30 days of illness onset, and households within 50 meters of index case-patients' households were invited to participate. Questionnaires were administered to collect data on individual- and household-level characteristics, behaviors, and recent symptoms of acute febrile illness. Serum specimens were collected and tested by RT-PCR and ELISA for nucleic acid and IgM/IgG antibodies against DENVs, ZIKV, and CHIKV. Among 228 participants, 9 (3.9%) were dengue positive (DENV+), 2 by RT-PCR and 7 by IgM. Seven DENV+ participants (77.8%) were febrile, as compared to 23.3% of DENV-negative participants (Chi2 p-value 0.0002). In unadjusted logistic regressions, age <18 years, income over $25,000/year, recent febrile illness, and having a septic tank (Chi^2 p-values = 0.013, 0.010, 0.004, and 0.042, respectively) were associated with DENV infection. Next steps include measurement of anti-ZIKV IgG antibodies by microimmunoassay and analyses of associations between DENV disease and previous ZIKV infection. As recurrent DENV outbreaks have affected AS, patients may be susceptible to severe dengue. Mosquito avoidance behaviors should be stressed, and those with acute febrile illness should seek medical care.

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EFFECT OF THE SALIVA OF DENGUE TRANSMITTER MOSQUITO *AEDES AEGYPTI* ON DENGUE VIRUS INFECTION OF PRIMARY CULTURES OF FIBROBLASTS

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Dengue virus (DENV) is transmitted to humans by female *Aedes aegypti* and *Aedes albopictus* mosquitoes, which inoculate virus into the dermis and epidermis while feeding. Previous studies have demonstrated that skin cells are permissive to dengue virus including hematopoietic cells and non-hematopoietic cells, such as fibroblasts, keratinocytes and epithelial cells. These findings suggest that dengue viral replication begins in the skin cells, and these cells will have an important role in the disease process. Fibroblasts are abundant cells in the skin capable of responding to infection in very early stages of infection. Although little information has been generated regarding the additional effects of the saliva of the vector on the infection with Dengue virus. The objective of this work was focused on evaluating the effect of the different components of saliva and DENV-2 on primary skin fibroblasts. Further, we observed a higher viral titer in the supernatants of fibroblasts from different donors, which were infected and

treated with the salivary gland extracts. These data corroborate the fact that some of the components of the saliva might contribute to increasing the viral infection . Furthermore, we evaluated different protein fractions obtained through HPLC, to elucidate the proteins that may be involved in the enhancement of the viral load. Our results strongly suggest that the protein responsible for this effect was a 34 kDa protein. We observed an increase in the infection of fibroblasts in the presence of this protein. This data is consistent with the observed in human keratinocytes, where it has been reported that this protein is capable of increasing the infection of dengue virus in these cells. Since we observed that the 34 kDa protein was partially responsible for this effect and that pre-incubation of the whole extracts was able to further increase in the viral load, we sought to evaluate if the 34 kDa protein is capable of interacting with the Envelope (E) protein of dengue virus through an ELISA and immunofluorescence assays.

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WHOLE GENOME ANALYSIS REVEALS HIGHLY LOCALIZED TRANSMISSION CHAINS AND A GRAVITY DYNAMIC OF DENGUE 3 SPREAD WITHIN A HYPERENDEMIC CITY IN THE PERUVIAN AMAZON

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Dispersal hotspots are thought to play a critical role in transmission control during dengue virus (DENV) epidemics. However, data addressing fine-scale spatial dynamics of virus spread are rarely available. From 2002 to 2008 there was a DENV-3 outbreak in Iquitos, a hyper-endemic city and transport hub in the Peruvian Amazon. This provided a unique opportunity to examine the fine-scale spatial dynamics of DENV-3 dispersal using phylodynamic analysis of a large collection of DENV-3 whole genomes. To do so we sequenced 240 DENV-3 positive human sera collected in Peru between 2002 and 2015. The resulting genomes were combined with publicly-available DENV-3 sequences into a dataset of 1055 genomes (174 from Iquitos, 66 from other regions of Peru, 231 from nearby countries and 584 from non-South American countries). Routes of DENV-3 dispersal into Iquitos, and subsequent dispersal through the city's four subdistricts (Belen, Iquitos, San Juan and Punchana), were estimated using time-scaled trees with ancestral geo-state construction. Our results show the 2002-2008 Iquitos epidemic was seeded via Ecuador (prob = 0.99), with an estimated introduction time of about 6 months prior to the first reports of herald cases (TMRCA Jan 2001, 95% HPD Dec 2000 - July 2001). The central subdistrict of Iquitos, which is the most populous, occupied the most ancestral tree space, suggesting it was the origin of epidemic strains sampled in the peripheral, less populated districts of Belen, San Juan and Punchana. Following introduction into the periphery, localized transmission was estimated to last a maximum of 2.54 yrs, with a median strain persistence time of 0.53, 0.57 and 0.60 yrs, respectively. We conclude that DENV-3 epidemic dynamics in Iquitos are characterized by highly localized transmission chains, fine-scale persistence and a gravity dynamic of virus spread, supporting a hypothesis of substantial peri-domestic transmission that has major relevance for transmission control.

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PREVALENCE OF CHIKUNGUNYA AND DENGUE VIRUS EXPOSURE AMONG CHILDREN IN WESTERN KENYA

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Chikungunya virus (CHIKV) and dengue virus (DENV) are among the several neglected arboviral diseases that have caused increased epidemics in the recent past globally. In Kenya, however, their occurrence is often underreported due to the low awareness by public healthcare providers and systematic surveillance and also the limited resources available. The objective of this study was to measure the seroprevalence of CHIKV and DENV among asymptomatic children in western Kenya and establish the population at risk of exposure. In 2015, a longitudinal prospective cohort study of 1378 non-clinical subjects aged 2≤17 years was initiated in western Kenya (Chulaimbo and Kisumu villages). Blood samples were obtained and sera tested for CHIKV and DENV exposure using standardized CHIKV IgG and DENV IgG ELISA protocols. During the one year of surveillance, the total prevalence of asymptomatic CHIKV and DENV exposure in the cohort was approximately 6.89% (95/1378) and 0.73% (10/1378) respectively. The total asymptomatic prevalence in the ≤5 year old age group was 0.51% (7/1378) and 0.15% (2/1378); 6-10 years was 2.83% (39/1378) and 0.07% (1/1378); 11-17 years was 3.55% (49/1378) and 0.51% (7/1378) respectively. Our results demonstrate that exposure to CHIKV is more common across all age groups as compared to DENV in western Kenya, with a greater proportion of those exposed ranging between the ages of 6-17 years. This could be due to the fact that this sub-population belongs to the school-going age bracket thus increased duration of exposure to the biting by the vector hence high transmission. The ≤ 5 years of age were less likely to be exposed owing to the fact that most of them had not attained the school going age thus spend their time indoor. Our findings have important implications for assessment of CHIKV and DENV transmission, infection and disease.

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INFLUENCE OF TEMPERATURE AND RAINFALL ON THE EPIDEMIOLOGY OF ARBOVIRAL DISEASES IN ENVIRONMENTALLY DISTINCTIVE REGIONS OF BRAZIL

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Arboviral diseases, such as dengue, chikungunya, and zika, continue to present a substantial and increasing threat to global public health. The timing and severity of epidemic outbreaks are strongly associated with vector suitability, driven primarily by seasonal oscillations in temperature and rainfall. Local variations in climate thus naturally induce significant heterogeneities in arboviral incidence across space and time. However, the relative roles of temperature and rainfall in the emergence and spread of arboviruses in environmentally distinctive settings has only been partially explored. To this end, we constructed a climate-dependent individual-based model to compare the effects of precipitation and temperature on the timing and magnitude of several epidemic outbreaks of dengue, chikungunya and zika, across climatically different regions of Brazil from 2014 to 2017. Our results confirm the important influence of temperature and rainfall on the temporal variation in vectorial capacity, which crucially shapes arboviral disease epidemiology in these regions. Importantly, we also find that the relative effects of different climate variables is strongly dependent on the specific environmental setting, with temperature or rainfall being the main driver for seasonal epidemics in one region but not in another. This further implies that estimation of the latency period between peak annual rainfall, for example, and the timing of an arboviral disease outbreak must be determined at a local scale. Our findings thus contribute to a better understanding of the influence of climate on arboviral disease dynamics and highlight the importance of considering local climatic dependencies when generalizing disease suitability or intervention strategies to wider spatial contexts.

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DENGUE VIRUS IS AN EMERGING CAUSATIVE AGENT OF ACUTE ENCEPHALITIS SYNDROME (AES) IN INDIA: RESULTS FROM A FOUR-YEAR ENHANCED AES SURVEILLANCE STUDY IN INDIA

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In India, acute encephalitis syndrome (AES) outbreaks are reported annually. While Japanese encephalitis virus (JEV) accounts for 5-35% of reported cases, the etiology of most AES cases in India remains unknown as testing for other pathogens is not routinely performed. As part of the Global Health Security Agenda in India, we established a tiered network of 20 district laboratories and five referral laboratories in three high AES burden states and implemented a testing algorithm to evaluate etiology of AES. All patients fulfilled the WHO AES case definition - "*Acute onset of fever in a person of any age, with change in mental status and/or new onset of seizures (excluding simple febrile seizures)*". Serum and/or cerebrospinal fluid (CSF) samples were collected from AES in-patients from January 2014- December 2017. All samples were evaluated for JEV IgM antibodies. JEV IgM-negative sera were tested for IgM to scrub typhus, dengue virus, and West Nile virus; JEV IgM-negative CSF was tested by PCR for *S.pneumoniae, N.meningitidis, H.influenzae*, herpes simplex virus type 1(HSV), and enteroviruses. Of 10,207 AES patients, 58% were male and the majority (63%) were <15 years. CSF and sera were obtained from 58% of patients; the rest had either CSF (17%) or serum (24%). Overall, an etiological agent was identified in 46.3% patients. JEV IgM was detected in CSF/serum of 16.2% (1658/10,207), while in JE-negative cases, where sera was available, scrub typhus accounted for 16.6% of cases(1165/7011), followed by dengue (5.7%; 340/5992). Dengue was detected in all three states of Assam, Uttar Pradesh and West Bengal. In 6/708 patients where sufficient sample was available, we were able amplify dengue virus RNA in CSF. Through a tiered network of labs and an algorithmic approach, etiologies other than JE were identified in 30% of AES patients; identification would have been limited only to JE (16%) detection in prior routine surveillance. Furthermore, JEV, scrub typhus, and dengue accounted for 94% of all cases where an etiological agent could be identified. These results clearly suggest that dengue virus is emerging as an important etiological agent of AES in India.

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DYNAMICS OF T CELL RESPONSES PRIOR TO AND AFTER SECONDARY DENGUE VIRUS INFECTION

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Cytokines secreted by T cells contribute to both protective immunity against dengue virus (DENV) and the pathogenesis of dengue disease. The aim of this study was to define the dynamics and the magnitude of T cell responses over time after DENV infection. We measured responses to DENV peptides using an IL-2/IFN-γ dual color enzyme-linked immunospot (ELISPOT) assay. Peripheral blood mononuclear cells (PBMC) from Thai children prior to and after secondary DENV-1 and DENV-2 infection from a 5-year prospective cohort study were stimulated with overlapping peptide pools of structural and non-structural proteins from the four DENV types. Overall, responses to DENV peptides demonstrated a cytokine phenotype hierarchy of IFN-γ > IL-2 > IFN-γ/IL-2. Responses to DENV peptides were low or absent prior to secondary infections. Subjects who experienced a secondary DENV-1 infection showed higher cytokine responses post-infection compared to secondary DENV-2 or subjects with subclinical infections. The trends in responses to DENV peptides over 3 y post-infection were highly variable. These data demonstrate a lasting but variable impact of secondary DENV infection on DENV-specific T cell responses.

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HIGH THROUGHPUT LIVE-IMAGE CYTOMETRY-BASED NEUTRALIZATION ASSAYS FOR DENGUE AND ZIKA VIRUSES

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Virus neutralization tests such as plaque and micro-focus reduction neutralization tests (PRNT and mFRNT) are critical tests in flavivirus diagnosis, vaccine study, and sero-surveillance. Since Zika virus (ZIKV) and dengue viruses (DENVs) co-circulate in areas with *Aedes* mosquitoes, simultaneous NT for ZIKV and DENV is usually required. Wild-type DENV and ZIKV replicate slowly, making PRNT time-consuming. Previously, we developed West Nile virus (WNV)-based chimeric DENV 1-4 and ZIKV, which reduced the duration of traditional PRNT and mFRNT by 50%. To further improve and streamline the assay, we developed live chimeric reporter DENVs and ZIKV (R-WN/DENVs and R-WN/ZIKV) expressing green fluorescent protein in infected cells that can be automatically analyzed by cytometer or fluoro-spot reader. The chimeric reporter virus seed lots used for NT assay were evaluated by RT-PCR and flow cytometry to confirm 100% reporter gene integrity of the reporter viruses. Using these reporter viruses, we developed a reporter-mFRNT (R-mFRNT) which can be measured within 24-30 hours after cell infection. Using human clinical sera, we verified the neutralization Ab titers obtained from the R-mFRNT were identical or similar to titers obtained by traditional PRNT or mFRNT. Due to the use of same WNV-based chimeric platform, all the reporter viruses have similar replication efficiency and provide a fast and practical means to obtain NT results to ZIKV and DENVs simultaneously. In addition, the live-image based R-mFRNT allows for a true high-throughput detection directly from cell culture without any cell fixation or other additional procedures.

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PUBLIC HEALTH IMPACT OF VACCINATION WITH DENGVAXIA IN THE PRESENCE OF INTRA-URBAN VARIATION IN DENGUE VIRUS TRANSMISSION

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Dengvaxia from Sanofi Pasteur is currently the only licensed dengue vaccine. Results from phase-III trials indicate that people with no history of natural dengue virus (DENV) infection who receive Dengvaxia experience an elevated risk for severe disease upon subsequent natural infection. This finding has shed light on the importance of spatial variation in

DENV transmission history, with this vaccine only recommended for use in areas with a high probability of prior DENV infection among potential vaccinnees. This recommendation has prompted efforts to measure DENV transmission intensity through the analysis of serological survey data. However, DENV transmission is known to be highly spatially heterogeneous, even at intra-urban scales. We performed a modeling analysis to understand whether spatial aggregation of serological data at the scale of a city could lead to unintended harm in the presence of spatial heterogeneity in DENV transmission below the scale of the city as a whole. We used a modified SIR model that allows for up to four distinct DENV infections over a person's lifetime to examine the differential impacts of vaccination on two sub-city communities with different transmission intensities but connected by human mobility. Vaccination coverage of 80% led to a decrease in the number of cases on a city-wide scale and had varied effects on a sub-city scale. It was found that under uniform vaccination across two districts of the city with differential transmission intensity, the district characterized by low transmission had an increase in the incidence of severe disease while the district characterized by high transmission had a decrease in the number of infections. This work has implications for vaccination campaigns in cities in which there is spatial heterogeneity in transmission that is unaccounted for in the aggregation of serological data at a city-wide scale.

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SPATIOTEMPORAL VARIATION IN DENGUE TRANSMISSION INTENSITY IN JAKARTA, INDONESIA

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The characterisation of both spatial and temporal variation in dengue transmission intensity is of key importance for optimal implementation of new control and prevention strategies, including vaccination programmes and the release of *Wolbachia*-infected mosquitoes. Large seroprevalence surveys are often unfeasible due to the associated costs, placing reliance on disease surveillance data for assessment of local dengue epidemiology. Though the quality of dengue disease surveillance data is often uncertain due to lack of laboratory-confirmation and unspecific clinical manifestations, analysis of the relationship between age and the observed disease incidence can provide valuable insights into the underlying nature of dengue transmission. Jakarta, the capital of Indonesia, a dengue hyperendemic country, has a population of over 10 million people. In Jakarta, clinically-confirmed, hospitalized cases of dengue haemorrhagic fever (DHF) are reported to the epidemiological surveillance system and reported online. We used a mathematical model in a Bayesian inferential framework to estimate dengue force of infection, basic reproduction number, and reporting probabilities from age-stratified dengue case-notification data for 44 sub-districts (administrative level 3) in Jakarta over a 10-year period, 2008-2017. We assumed all 4 dengue serotypes to be in circulation and equally transmissible. The model was used to explore varying assumptions about the contribution of primary, secondary and post-secondary dengue infections to the observed disease incidence. Under the simplest assumption that only secondary infections contribute to the observed disease incidence, the average annual force of infection in Jakarta was estimated at 9.7% (97.5%CrI: 9.6-9.8%) for the 10-year period. This ranged from an average of 6.3% to 13.2% per year between sub-districts. Further model extensions allowing for all (primary-quaternary) dengue infections to contribute to the observed disease incidence will allow further characterisation of the spatial and temporal distribution of dengue transmission in Jakarta.

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INCREASED SIALIC ACID LEVELS IN SERUM ARE ASSOCIATED WITH VASCULAR LEAK AND SEVERE DENGUE DISEASE

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With an estimated 400 million infections and approximately 100 million cases each year, dengue is the most common arboviral infection. Infection with any of the four dengue virus (DENV1-4) serotypes results in a spectrum of outcomes, from asymptomatic infection to classic dengue fever (DF) to dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), characterized by vascular leakage and shock. Previous research has established that DENV non-structural protein 1 (NS1) directly induces endothelial hyperpermeability *in vitro* and vascular leak *in vivo.* Further, our group demonstrated that DENV NS1 disrupts the endothelial glycocalyx layer (EGL) *in vitro,* triggering the degradation and shedding of key structural components, including sialic acid and heparan sulfate proteoglycans. In addition, studies in human populations have shown that increased levels in serum of EGL components such as hyaluronic acid, heparan sulfate and syndecan-I are associated with plasma leakage and severe dengue disease. Here, using a murine model of DENV-induced vascular leak, we demonstrate that sialic acid and NS1 levels in circulation correlate with increasing viral inoculum and DENV-induced morbidity and are significantly higher ($p=0.0026$ for sialic acid and $p<0.0001$ for NS1) in mice that succumb to DENV infection. Additionally, preliminary experiments using liquid chromatography-mass spectrometry (LC-MS) indicate that free sialic acid levels in human serum samples from dengue studies in Nicaragua are significantly elevated in DSS cases. Overall, the identification of novel biomarkers of infection severity, such as sialic acid, can improve triage of severe dengue cases and may provide important insights into mechanisms of DENV pathogenesis and contribute to the development of new therapies.

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HERD IMMUNITY TO ZIKA VIRUS MAY CAUSE CHANGES IN THE DYNAMICS OF DENGUE INCIDENCE IN THE AMERICAS

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We have previously suggested that Zika virus (ZIKV) immunity may afford protection to dengue virus (DENV) infection. This hypothesis was raised based on analysis of long-term surveillance data showing a substantial reduction in dengue case detection after the ZIKV epidemics in Salvador, Brazil. We have therefore undertaken a broader explorative investigation covering DENV-endemic American countries that have recently undergone ZIKV epidemics to assess whether they also presented a decline in dengue occurrence. We analyzed the historical series (2008 to 2017) of dengue cases reported to World Health Organization (WHO) and Pan American Health Organization (PAHO) for the 12 countries with autochthonous ZIKV transmission in 2015, and with available dengue count data for the period (Brazil, Colombia, El Salvador, French Guiana, Guatemala, Honduras, Martinique, Mexico, Panama, Paraguay, Puerto Rico, and Venezuela). In the post-ZIKV period (2016-2017), dengue reported cases declined in 11 of the 12 countries, with Panama, where the number of cases increased, being an exception. Prior to the ZIKV epidemics, all countries displayed a

pattern of periodic oscillations of number of dengue cases over time, with each cycle lasting 1-3 years. In three countries (Puerto Rico, Martinique, and French Guiana), the number of dengue cases began to decline before 2015, and remains low for >3 years now, making it the longest sequence of years with low occurrence of dengue cases detected in this historical series. For other countries, such as Brazil, Puerto Rico, and Venezuela, the number of reported dengue cases in 2017 was the lowest reported since 2008. Although our analysis uses secondary, countrywide data (which may not reflect the true picture for each country due to reporting errors or absence of spatial overlap between ZIKV and DENV transmission within each country), our findings do corroborate the hypothesis that ZIKV epidemics may play a role in the dynamics of dengue occurrence in the Americas. Further long-term prospective follow-up of dengue reports are needed to confirm these findings and to assess whether they represent a long-lasting phenomenon.

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FLAVIVIRUS NS1 INDUCES ENDOCYTOSIS AND PHOSPHORYLATION OF TIGHT AND ADHERENS JUNCTION PROTEINS, LEADING TO ENDOTHELIAL BARRIER DYSFUNCTION

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Flaviviruses cause systemic or neurotropic-encephalitic pathology in humans. Recently, we showed that nonstructural protein 1 (NS1) from different flaviviruses causes endothelial barrier dysfunction and vascular leakage in a tissue-dependent manner. This phenomenon depends in part on the integrity of the endothelial glycocalyx layer (EGL) lining the surface of endothelial cells. Besides the EGL, tight and adherens junctions (TJ/AJ) also help maintain endothelial barrier function. Here, we investigate the relative contribution of TJ/AJ proteins to the increased barrier dysfunction caused by different flavivirus NS1 proteins from dengue (DENV), Zika (ZIKV), West Nile (WNV), Japanese encephalitis (JEV), and yellow fever (YFV) viruses on human placenta (HUVEC) and brain (HBMEC) endothelial cells. Six hours post-treatment, only DENV and ZIKV NS1 proteins altered the normal distribution of ZO-1, VE-cadherin, and β-catenin in HUVEC monolayers, as determined by immunofluorescence assay. However, in HBMEC, this distribution was affected by all flavivirus NS1 proteins but YFV NS1. Western blot analyses showed no significant changes in expression of TJ/AJ proteins in both endothelial cell lines after NS1 treatment, except that DENV NS1 in HUVEC and WNV NS1 in HBMEC significantly reduced the expression of ZO-1. These results suggest that flavivirus NS1 proteins disrupt the integrity of TJ/AJ proteins by either altering their normal localization in the cell-to-cell contacts or reducing their expression. Mechanistically, we found that DENV NS1 induces clathrin/dynamin-dependent endocytosis of ZO-1 and VE-cadherin, as well as phosphorylation of β-catenin (Ser45) by CK-1 and GSK-3β kinases, resulting in the remodeling of the intercellular junction complex. Specific inhibitors of clathrin- and dynamin-dependent endocytosis and inhibitors of GSK-3β activity abrogated NS1-induced permeability of HUVEC monolayers, indicating that these mechanisms are also involved in the NS1-induced endothelial dysfunction. These findings add new insights into the modulation by flavivirus NS1 proteins of endothelial barrier function.

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TRACKING THE POLYCLONAL NEUTRALIZING RESPONSE TO A DENV1 SEROTYPE-SPECIFIC EPITOPE ACROSS TWO POPULATIONS IN ASIA AND THE AMERICAS

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Recent advances in the characterization of human antibody responses to primary dengue virus (DENV) infection indicate that highly neutralizing type-specific (TS) antibodies target quaternary epitopes on the DENV envelope protein. Within this class of potent TS neutralizing antibodies is 1F4, isolated from a donor with a primary DENV1 infection. Given the high neutralization capacity of 1F4, we created a recombinant DENV2 containing the 1F4 epitope (rDENV2/1) via structure-guided immunogen design and reverse genetics. Here, we use the rDENV2/1 chimeric virus to measure the proportion and kinetics of DENV1 TS neutralizing antibody (nAb) responses targeting this epitope in individuals living in two dengue-endemic areas. We analyzed convalescent sera of 10 individuals with primary DENV1 infection in Nicaragua and 10 in Sri Lanka to determine the prevalence and proportion of the DENV1 nAb response attributable to the 1F4 epitope. The nAb titers (NT_{50}) to the rDENV2/1 virus and its parental DENV1 and DENV2 viruses were obtained via a flow cytometry-based neutralization assay using human monocytic U937-DC-SIGN cells. To evaluate the kinetics of the nAb response to the 1F4 epitope, we analyzed samples collected 3 and 18 months post-infection from the same 10 individuals enrolled in a longitudinal dengue hospital-based study in Nicaragua. In preliminary data from the Nicaraguan and Sri Lankan populations, the polyclonal responses of 80% (8/10) and 90% (9/10) individuals, respectively, track to various degrees with the 1F4 epitope. The average proportion of the DENV1 nAb response attributable to the 1F4 epitope was 36% (3 months) and 56% (18 months) in Nicaraguan and 55% (1 month) in Sri Lankan samples. Longitudinal samples from additional individuals in Nicaragua are being analyzed from the hospital study as well as a cohort study over 4 years post-infection. Characterization of epitope-specific nAb responses in natural DENV infections in Asian and American populations can help elucidate the antibody repertoire to DENV epitopes, with implications for vaccine design.

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EPIDEMIOLOGY OF DENGUE IN INDIA - OUTCOME OF A FACILITY BASED ACUTE FEBRILE ILLNESS (AFI) SURVEILLANCE

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Dengue is a major public health problem potentially affecting 50 million people worldwide. The epidemiology of dengue in India is complex and has substantially changed over the years in terms of circulating serotypes, severity, and geographic spread. As the available data regarding dengue is mostly from tertiary care settings, through the Global Health Security supported hospital-based acute febrile illness (AFI) surveillance in India, we

tested all consenting eligible AFI cases for viruses, bacteria and parasites in 32 district / sub-district hospitals across 10 states. We enrolled in-patients with documented or reported fever <15 days duration and collected demographic, clinical, and epidemiological data; we also collected blood samples. A case with either NS1Ag ELISA, IgM ELISA, PCR positivity in acute or IgM /IgG seroconversion in convalescent serum was defined as dengue. We enrolled 27,431 patients between June 2014 to July 2017 and 14,134 (51.5%) had a definitive diagnosis. The major etiologies were influenza (16.6%), dengue (9.7%), scrub typhus (8.6%), leptospirosis (8.1%), malaria (4.7%) and KFD (2.2%). The percentage dengue positivity among AFI cases varied by state from 1.7 to 18.6 and was highest in Tamilnadu (18,6%), Assam (14.4%) and Kerala (9.9%). Based on presence of IgG antibodies in acute serum, 43% cases were secondary dengue. As per WHO classification, 10% had severe dengue, 25% had warning signs. The median age was 26 years (IQR: 17, 38) and 60% were male. Predominant clinical symptoms included abdominal pain (27.5%), retro-orbital pain (30.2%), and rash (1.2%). Thrombocytopenia was evident in 44.6% cases. Referral to higher centres occurred in 159 (6.5%) cases and 3 cases died. Circulating dengue virus serotypes were DENV1, DENV2, and DENV3. Dengue was prevalent throughout the year with increased activity May to December. Prevalence of dengue among AFI cases varied geographically and multiple serotypes co-circulated. Availability of diagnostic facilities at district / sub district hospitals may lead to more rapid diagnosis and these data can be used to help develop clinical guidelines for managing AFI cases in India.

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THE RISING IMPACT AND COMPLEX ECOLOGY OF DENGUE IN RURAL MALAYSIA

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In South East Asia, dengue epidemics have increased in size and geographical distribution in recent years. In Malaysia, regular outbreaks occur across the country, causing significant morbidity and economic burden. While the majority of control efforts have focused on preventing dengue in urban areas, transmission dynamics are poorly understood in more rural states, or where urban and rural areas overlap. This study examined the epidemic characteristics and spatiotemporal distribution of reported dengue cases in the predominantly rural state of Sabah, in Malaysian Borneo. Our analyses of state-wide notification data over a 7-year period (2010-2016) indicated wide variability in the pattern of high-risk clusters detected over space and time, with a mean annual incidence rate of 49/100,000 people. The largest ever recorded outbreaks occurred in both urbanised cities and rural villages from late 2014 to 2016. The highest incidence rates were in people aged between 10-49 years, with a median age of 25. Severe dengue cases were geographically localised to two administrative divisions in the eastern area of the state: Tawau and Sandakan. Active surveillance of dengue case residences during the outbreaks identified the typically sylvatic vector mosquito *Aedes albopictus* as the dominant species breeding in all locations (comprising 65% of larvae identified in case residences). Our findings challenge the notion that human population density is the primary driver of dengue disease patterns, and provide a platform to further assess the complex interplay of local and regional climatic, ecological and anthropogenic factors that underlie dengue risk.

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DISCRETE PHENOTYPIC SIGNATURES OF CD8+ T CELLS DURING DENGUE VIRUS INFECTION

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Dengue virus (DENV), a mosquito-borne flavivirus, is estimated to cause up to 390 million infections per year, of which 100 million cases are symptomatic. The recently approved dengue vaccine has demonstrated only partial protection, despite optimal induction of neutralizing antibodies towards all DENV serotypes, thus highlighting the need for an improved definition of the correlates of protective immunity towards DENV. In particular, the nature and role of DENV-specific T cells remain unclear. Initial studies suggested a contribution of suboptimal cross-reactive CD8+ T cell responses to the development of severe disease during secondary infection. In contrast, more recent work suggests a key protective role of a vigorous DENV-specific T cell response restricted to "protective" HLA alleles. To better define the nature of the dengue-specific T cell response restricted to HLA molecules that are commonly expressed in South-East Asia, we performed an in-depth study of dengue-specific CD8+ T cells restricted to HLA-B*58:01, HLA-A*24:02 and HLA-A*11:01 using multiplex combinatorial tetramer staining and Time of Flight Mass Cytometry (CyTOF). T cells specific for >100 distinct epitopes from DENV 1-4, Epstein-Barr virus (EBV) and Influenza virus (Flu) were simultaneously analyzed for expression of 30 phenotypic and functional T cell markers during acute, post-febrile and convalescent dengue infection. High-dimensional phenotypic profiling reveals a distinctive and highly homogenous signature of DENV-specific T cells during acute infection, which was not affected by HLA restriction or epitope immunodominance. In contrast, at convalescence DENV-specific T cells diverged into T cells with two opposing phenotypic signatures characterized either by high expression of markers of terminal differentiation or of markers suggesting long-term memory persistence, the latter cells resembling those specific for EBV and Flu. Current efforts in our laboratory are aimed at investigating the influence of antigenic exposure in primary versus secondary dengue infection in driving these two opposing memory T cell signatures.

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A NOVEL PROSPECTIVE FAMILY COHORT STUDY OF DENGUE VIRUS TRANSMISSION IN KAMPHAENG PHET, THAILAND BASELINE IMMUNOLOGICAL AND SUBCLINICAL SEROCONVERSIONS FROM THE FIRST YEAR

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We describe results from the first year of a novel prospective study in Kamphaeng Phet, Thailand, which follows multigenerational family units for dengue virus (DENV) infection over several years. 368 families (2428 individuals) were enrolled between September 2015 and June 2016 with a median of 6 individuals per family and an age range of 0 to 93 years (median 21 years). Each family comprised of at least a pregnant female, her newborn child, one child aged below 18 years old, and one grandparent. Blood specimens were collected at enrollment; 73 families (416 individuals) underwent a first scheduled follow up blood collection

in mid-2016 (7-9 months post-enrollment). Flow-based neutralization antibody testing (FlowNT50) was used to characterize baseline serological profiles and to detect seroconversions, defined as a ≥3-fold rise in titer to one or more DENV type. Among newborns (as measured in cord blood), 85.9% had multitypic profiles, 2.8% monotypic, and 11.3% had no detectable DENV antibodies. Baseline seropositivity to DENV was lowest in children aged 0 to 5 years (26.8% multitypic, 19.5% monotypic, and 53.7% DENV-naïve). Above 30 years of age, 100% of individuals had multitypic DENV profiles. 53 seroconversions were identified by comparison of FlowNT50 titers from scheduled specimens, for a seroconversion rate of 12.7%. There was significant clustering of seroconversions within family units ($p<0.01$ by χ^2 testing) and, among families with one or more seroconversions, the median seroconversion rate was 25% (range: 8.3-100%). The maximum seroconversion rate was observed in individuals aged 16-20 years (34%), followed by children aged 0 to 5 years (20%). The lowest seroconversion rate was in adults aged 31-50 years (3%). This study demonstrates ongoing high incidence of DENV infection in north-central region of Thailand with clustering at the household level and continued risk affecting all ages.

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EPIDEMIOLOGICAL SIGNIFICANCE OF DENGUE VIRUS GENETIC VARIATION IN MOSQUITO INFECTION DYNAMICS

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The kinetics of arthropod-borne virus (arbovirus) transmission by their vectors have long been recognized as a powerful determinant of arbovirus epidemiology. The time interval between virus acquisition and transmission by the vector, termed extrinsic incubation period (EIP), combines with vector mortality rate and vector competence to determine the proportion of infected vectors that eventually become infectious. However, the dynamic nature of this process, and the amount of natural variation in transmission kinetics among arbovirus strains, are rarely taken into account in epidemiological models. Here, we combine empirical measurements *in vivo* and outbreak simulations *in silico* to assess the epidemiological significance of genetic variation in dengue virus (DENV) transmission kinetics by *Aedes aegypti* mosquitoes. We found significant variation in the dynamics of systemic mosquito infection, a proxy for EIP, among eight field-derived DENV isolates representing the worldwide diversity of recently circulating type 1 strains. Using a stochastic agent-based model to compute time-dependent individual transmission probabilities, we predict that the observed variation in systemic mosquito infection kinetics drives significant differences in the probability of dengue outbreak and the number of human infections. Our results demonstrate that infection dynamics in mosquitoes vary among wild-type DENV isolates and that this variation can contribute to the risk and magnitude of dengue outbreaks. Our quantitative assessment of DENV genetic variation in transmission kinetics contributes to improve our understanding of heterogeneities in arbovirus epidemiological dynamics.

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SPATIOTEMPORAL HETEROGENEITY OF DENGUE TRANSMISSION INTENSITY IN COLOMBIA

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With the rapid development of new dengue control options, such as the release of *Wolbachia*-infected mosquitoes and a second-generation of dengue vaccines, characterising dengue transmission intensity at fine spatiotemporal resolution is essential for the optimal design of control programmes. Serological studies are considered the gold standard to quantify dengue transmission intensity as they capture both symptomatic and asymptomatic infections. However, they are often expensive to conduct and hence not widely available. Previously, we have shown that dengue transmission intensity can be inferred by fitting catalytic models to age-stratified case notification data, and that these estimates are largely comparable to those obtained from seroprevalence data. Here we apply these methods to estimate the average and time-varying dengue force of infection and reporting probabilities from age-stratified case-notification data reported to National Institute of Health Colombia in 2010-2016. Analysis was conducted at the first and second administrative unit levels (department and district-level, respectively). We found large spatial and temporal heterogeneity in transmission intensity within and between departments, with the highest transmission intensity clustered in the North and Central-Western parts of Colombia. The estimated variation in transmission intensity at small spatial scales suggests that future control measures may need to be targeted at the district rather than the department level. Reporting rates also varied substantially between and within departments, suggesting differences in healthcare accessibility, coverage and health-seeking behaviour. Although additional, currently ongoing analyses are needed to calibrate force of infection estimates obtained from serological data with those obtained from case-notification data, routinely collected age-stratified dengue incidence data represent a valuable source of information and a unique opportunity to quantify global and local trends in dengue transmission intensity.

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COMPARISON OF CLINICAL MANIFESTATIONS AND DISEASE CLASSIFICATION OF ADULT AND PEDIATRIC DENGUE IN A PROSPECTIVE STUDY OF HOSPITALIZED DENGUE IN THAILAND

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Although dengue is considered a childhood disease recent evidence has demonstrated increasing numbers of adult dengue. Comparisons across studies have reported differences in clinical features of adult and pediatric dengue. However, these differences may be due to other factors, such as virus transmission and host genetic differences. To better define characteristics of dengue in adults and children in the same geographical location, we conducted a prospective study of hospitalized suspected dengue cases in Kamphaeng Phet, Thailand. Suspected dengue cases were enrolled and their daily clinical, laboratory, and imaging information was collected following a standardized protocol. Between March 2016 and September 2017 seventy-nine cases were enrolled, 64 were confirmed dengue cases: 24 adults, 40 children. The mean ages of the adults and the children were 29.1 (2.6) and 11.5 (.5) years, respectively. Nine (37.5%), 6 (25%), and 9 (37.5%) of adults were diagnosed with dengue fever (DF), dengue hemorrhagic fever (DHF) grade I, and DHF grade II. Twenty six (65%), 7 (17.5%), 6 (15%), and 1 (2.5%) of children had DF, DHF grade I, grade II, and grade III. Classification using the World Health Organization 2009 scheme resulted in 50%, 42%, 8% of adults, and 35%, 62.5%, 2.5% of children diagnosed as dengue (D), dengue with warning signs (DWS), and severe dengue (SD), respectively. A significant correlation between the diagnosis of DHF and DWS/SD was observed in children but not in adults. The enrollment clinical findings that were associated with the diagnosis of DHF or DWS/SD were abdominal pain in both adults and children. Only 33% of adult DHF cases had evidence of hemoconcentration (defined as at least a 20% increase in

hematocrit) compared to 71.4% of children with DHF and the degree of hemoconcentration was significantly lower (18% in adults and 28.6% in children). These findings suggest that adult and pediatric dengue have different clinical features and disease progression and that the current case classification and management schemes which were developed primarily from children are not ideal for adults with dengue.

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NK CELL DEGRANULATION ASSAY FOR ASSESSING DENGUE VIRUS SPECIFIC ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY ELICITING ANTIBODIES IN INFECTED AND VACCINATED INDIVIDUALS

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Clinical development of a safe and efficacious dengue vaccine is a DoD force health protection priority. Traditionally, it was assumed that dengue vaccine efficacy would correlate with neutralizing antibody (NAb) titers. However, recent data from Phase II/III trials of a DENV vaccine product demonstrate a discordance of vaccine efficacy with NAb titers. Therefore, the development and testing of alternative assays for measuring and assessing functional antibody activity against dengue virus is warranted. The aim of this study was to assess cellular opsonization and develop an ADCC assay based on the level of NK cell degranulation elicited using DENV-infected CEM-NK^R-DC-SIGN cells (targets). CEM-NK^R-DC-SIGN cells are resistant to non-ADCC NK cell killing, devoid of surface FcRs, and permissive to DENV infection. CEM-NK^R-DC-SIGN target cells demonstrated permissiveness to all four DENV serotypes and surface expression of low levels of structural proteins and high levels of NS1. These cells also demonstrated opsonization with IgG from the sera of DENV-infected subjects. Opsonized target cells were then co-incubated with PBMCs (at 1:1 Effector: Target ratio) isolated from a healthy donor and consequently stained with an antibody cocktail (APC-CD56, PerCP-CD3, FITC-CD107a, and PE-CD16). The expression of CD107a, which correlates with both cytokine secretion and NK cell-mediated lysis of target cells, was used to assess degranulation and determine an ADCC-Ab titer. The assay was then used to assess vaccine-induced ADCC activity in the context of the TDEN-001 LAV/LAV (WRAIR #1151) vaccine trial. We determined that opsonization and antibody-dependent NK cell degranulation increased dramatically following receipt of the vaccine for all four serotypes of DENV. Assessing vaccine-induction of these different types of Ab are critical for understanding the full breadth of the immune response directed against DENV. This study demonstrates the utility of the CEM.NK^R-DC-SIGN cells as target cells to assess serum Ab activity capable of eliciting ADCC and other responses.

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ANTIGEN-SPECIFIC B CELL ACTIVATION AFTER IMMUNIZATION WITH A TETRAVALENT DENGUE PURIFIED INACTIVATED VACCINE (DPIV)

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Dengue is a growing global health issue for which development of a protective vaccine has proven challenging. While neutralizing antibodies (NAbs) are thought to contribute to protection from disease, dengue infection still occurs in some individuals with measurable NAb titers. It is therefore postulated that both NAbs and cell-mediated responses are important for the generation of protective immunity. In a previously described Phase 1 study of a tetravalent dengue purified inactivated vaccine (DPIV) candidate (NCT01666652), we demonstrated the persistence of dengue-specific memory B cells up to 1 year post-vaccination despite waning NAb titers. Here we present data on vaccine-induced plasmablasts (PBs) and use fluorescently-labeled viruses to track antigen-specific cells in the periphery in a subset of subjects given a third booster dose of either the 1µg+AS01$_E$ (n=6) or the 4µg+Alum formulations (n=3). We observed robust expansion of these cells 7 days post-vaccination with discernible, multivalent binding of fluorescent viruses, which revealed a more distinct population compared to phenotyping PB alone. Freshly isolated peripheral blood mononuclear cells (PBMCs) were comparable to cryopreserved PBMCs with respect to the frequency of PBs detectable by ELISPOT, although spot size was notably smaller after cryopreservation. Isolated PBs were analyzed using Atreca's Immune Repertoire Capsule technology. Examination of over 500 heavy and light chain antibody pairs per subject revealed similar levels of overall somatic hypermutation between the two different vaccine formulations, although the antibody sequences with the highest mutation levels were observed in 1µg+AS01$_E$ recipients. Evidence of usage bias for particular heavy chain V genes was observed with minor differences between the two different vaccine groups. IgG1 was the most predominant IgG subclass used amongst isolated PBs, and similar levels of repertoire diversity were also noted. Overall, DPIV vaccination induced a distinct antigen-specific PB response. Future analyses to determine correlations of the PB response to memory B cell and NAb responses are planned.

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THE IMPACT OF WEATHER ON THE SPATIO-TEMPORAL TRANSMISSION DYNAMICS OF DENGUE ACROSS SOUTH EAST ASIA

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Drivers of mosquito population dynamics have significant impact on the transmission of Dengue virus. Climatic variables, such as temperature, influence the development and survival of mosquitoes in complex and non-linear ways, which in turn influences ongoing transmission. Standard approaches to incorporate weather into transmission models identify, for each weather variable, at most a few lags by which to stagger the relationship between the climatic driver and transmission dynamics. Distributed lag non-linear models (DLNM) allow for the assessment of all possible non-linear lagged relationships within a single framework. Here, we combined a DLNM and a generalized additive model to adjust for seasonality within a TSIR transmission framework to assess the influence of weather on monthly dengue transmission from 1993 to 2010 across 300 locations within 8 countries in South East Asia. We found significant impact of day and night temperature, precipitation, humidity, total column water vapor, and enhanced vegetation index. Due to collinearity in these drivers, we analyzes their impacts in isolation and in parallel. The univariate analyses were able to recreate some expected relationships such as the inverted-U shaped relationship between temperature and transmission. Interestingly, other drivers' influence on transmission was not as straightforward, with, for example, heavy rainfall being both bad for transmission in the near past, but also beneficial a month before. The final model that incorporated all the significant distributed lag effects explained 76% of the variance in the case data. Dengue transmission is both local and focal, and even in hyperendemic locations, poorly predicted. Accurately quantifying the role of extrinsic drivers such as weather is a vital step to understanding when and where an outbreak is most likely. The models presented here have many applications from correctly understanding the expected number of cases during control trials to targeted interventions during a dengue season.

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NATIONALLY REPRESENTATIVE SEROSTUDIES FROM BANGLADESH SHOW LARGE-SCALE HETEROGENEITY IN DENGUE RISK

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Nationally representative serostudies are invaluable for answering a wide range of broadly generalizable questions on population-level immunity to pathogens, historic circulation patterns, and risk factors for infection, but are rare because they are resource-intensive. Such studies are particularly needed for dengue where undetected outbreaks are common. We present the first nationally representative study of dengue in Bangladesh. We visited 70 randomly selected communities twice (in 2014 and 2015) and recruited >80 individuals of all ages per community. We collected questionnaire data on potential risk factors, collected sera that were tested for dengue IgG antibodies (PanBio), trapped and speciated mosquitoes. We used multilevel logistic regression to explore the role of individual- (e.g., age, sex, travel), household- (e.g., mosquito control) and community-level (e.g., rural/urban, *Aedes aegypti/albopictus* presence) factors on seropositivity. We used catalytic models to estimate the annual number of infections and built predictive maps of seropositivity for the country. Out of 5,866 participants, 24% had evidence of prior infection, ranging from 3% for locations in the north to >80% in urban centers of Dhaka and Chittagong. While all communities had at least one seropositive individual, 41 (59%) of locations had <20% seropositivity. Being male (adjusted odds ratio [aOR]: 1.8, 95% confidence interval [95%CI]: 1.5-2.1), recent travel (aOR: 1.3, 95%CI:1.0-1.7) and urban setting (aOR: 2.0, 95%CI:1.1-3.7) were associated with increased seropositivity. *Ae. aegypti* was found in 23 (33%) communities and its presence was significantly associated with seropositivity (aOR: 2.3, 95%CI 1.3-4.0), whereas *Ae. albopictus* presence was not (p=0.92). We estimate 2.4 million annual infections and that 34 million in the population have ever been infected. Our findings show that outside urban centers, the majority of Bangladesh remains susceptible, with the limited presence of *Ae. aegypti* a key mitigating factor for occurrence of outbreaks. Changes in climate and land use that promote its habitat could increase future outbreak risk.

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DEVELOPMENT AND IMPLEMENTATION OF A UNIVERSAL DENGUE VIRUS SEQUENCING ASSAY FOR GENOTYPING AND GENOMIC SURVEILLANCE

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The global expansion of dengue virus (DENV) has driven the divergence of genotypes and other sub-genotypic lineages, which have been associated with epidemic potential and more severe clinical manifestations. Knowledge of virus diversity and phylogeny in the Americas is limited, largely affected by the lack of genomic surveillance and substantial underrepresentation in the public genome repositories. In order to address this deficiency, we collaborated with the Pan American Health Organization (PAHO) to develop the Dengue Genomic Surveillance Project in the Americas (ViGenDA) to facilitate the systematic study of DENV genomic diversity integrated to molecular and epidemiological surveillance. Thereupon, we developed a partial genome sequencing assay capable of sequencing directly from clinical specimens and is readily adaptable to any dengue molecular surveillance laboratory. Serotype-specific oligonucleotides were designed to detect contemporary genotypes with public health relevance. The CDC Dengue Virus E Gene Sequencing Assay is serotype-specific and targets the envelope (E) glycoprotein gene, which is amplified by a single RT-PCR protocol and sequenced with a bi-directional terminator dye Sanger method. Consensus sequence of the complete E gene is assembled with a minimum 2x coverage achieved through this assay. To determine if the assay can amplify and sequence global strains of DENV, we tested a panel of 25 DENV strains isolated from diverse global regions representing a variety of genotypes within the 4 serotypes. We obtained E gene contigs of sequence with ≥ 2x coverage from all strains except for a sylvatic isolate whose target sequence varies significantly from the rest of the contemporary strains and only partial sequence was obtained. To date, this assay has been transferred to over 14 laboratories, partners of the network of arbovirus laboratories of the Americas (RELDA) through a series of laboratory workshops. More than 4 participating laboratories currently implement this assay as part of their surveillance and efforts are underway to harmonize dengue genomic surveillance across the Americas.

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ZIKA VIRUS AND TRAVEL IN THE NEWS - A MEDIA CONTENT ANALYSIS

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The purpose of this study was to understand what information the media communicated about Zika virus (ZIKV) and travel in 2016 and 2017. We obtained a stratified, random sample of English-language, U.S. print newspaper and television news coverage about ZIKV and travel from April 5, 2016 to March 31, 2017. We developed a coding scheme to assess key messages in the news, including how and where ZIKV is transmitted, symptoms and outcomes of ZIKV, and recommended preventive behaviors. Media coverage peaked in August 2016, when the Centers for Disease Control and Prevention (CDC) issued a travel advisory for Miami, FL. Almost all news stories mentioned mosquito-borne transmission (96.8%) and most stories mentioned pregnant women as an at-risk group (89.7%). News stories mentioned sexual transmission (55.3%) or men as an at-risk group (35.3%) less frequently. News stories were more likely to talk about outcomes (78.8%) than ZIKV symptoms (40.6%). CDC was the most frequently mentioned information source (79.5%). Although the media communicated key messages about ZIKV, more nuanced messages were missing. The study offers unique insights into what information the media provided the public about the risks of ZIKV and travel.

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USE OF MACHINE LEARNING IN SURVEILLANCE CASE IDENTIFICATION FOR THE ZIKA EMERGENCY RESPONSE

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Zika virus (ZIKV) infection during pregnancy is a cause of birth defects including microcephaly and other central nervous system anomalies. In 2016, CDC and public health partners established pregnancy registries to monitor health outcomes among pregnant women with ZIKV infection and their infants. During an emergency response, clinical data are collected rapidly and must be reviewed to identify cases of interest using standardized surveillance case definitions. This is a resource and time-intensive effort. Machine learning and natural language processing

methods were applied to available data from the US Zika Pregnancy and Infant Registry (USZPIR), collected from January 2016 to October 2017, to identify potential cases of interest algorithmically and to reduce the number of non-cases of interest needing review. Cases of interest are defined as pregnancies with evidence of Zika-associated birth defects. Data were collected through systems tailored for the US territories and freely associated states (territories) and for the 50 US states and District of Columbia (states). An ensemble method was developed for each data set separately based on manually determined case outcomes. The method aggregates several models including support vector machines, random forests, and gradient boosted trees to determine potential cases of interest. Across both data sets, the machine learning models exhibited high sensitivity for identifying cases of interest while proving potential time savings. Models developed for data collected from the territories would have averaged a time savings of 66% while maintaining 97% sensitivity in predicting cases of interest. Models developed for data collected from the states would have averaged a time savings of 50% while maintaining 96% sensitivity in predicting cases of interest. These models demonstrate the potential application of machine learning and natural language processing for augmented case identification and for reducing unnecessary clinical review volume during a public health emergency response.

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IS THERE A GENETIC BASIS FOR SALIVARY GLAND INFECTION AND ESCAPE BARRIERS TO ARBOVIRAL INFECTION AND TRANSMISSION?

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In almost all arthropod vectors of disease the salivary gland is the organ that ultimately transmits a pathogen from an infected mosquito into the vertebrate host. Salivary gland infection barriers (SGIBs) and salivary gland escape barriers (SGEBs) determine whether the saliva of a mosquito will contain sufficient numbers of pathogens to overcome the vertebrate immune system. SGIBs are manifest as the absence of detectable pathogen in the salivary glands of mosquitoes that have a disseminated infection. There are large numbers of studies that document SGIBs for bunyaviruses and flaviviruses. SGEBs on the other hand are manifest as the absence of detectable virus in the saliva of mosquitoes that have infected salivary glands (as determined by bioassay of dissected glands). In contrast to SGIBs, there are very few studies that document SGEBs in mosquitoes and those have only examined bunyaviruses. Consequently we have little understanding of whether there is a genetic basis for SGEBs or whether they are random, subject to a few or many "environmental" factors. Herein we used a half-sib analysis to examine the quantitative genetics of SGIB and SGEB for three viral species in a freshly colonized strain of *Aedes aegypti* from Mapastepec, Mexico. We wished to determine the relative contributions of genetic and environmental factors to variation and covariation in SGIB and SGEB for DENV2 (QR094), ZIKV(PRABC59) and CHIKV(R99659) . With DENV2, the narrow sense heritability (&lth2 &gt) of SGIB was 0.789 (P &lt= &gt3.35E-07) while &lth2 &gt for SGEB was 0.172 (P &lt= &g0.0443). With ZIKV, &lth2 &gt of SGIB was 0.153 (P &lt= &g 0.041632) while h^2 SGEB was 0.336 (P = 0.00024). With CHIKV, the h^2 of SGIB was 0.000 (P &lt= &g 1) while the narrow sense heritability for SGEB was 0.461 (P &lt= &g0.0005075). In infections with DENV2 there is a large genetic component associated with a SGIB but not with ZIKV or CHIKV. In ZIKV and CHIKV the SGIB &lth2 &gt was low because the salivary glands of all individuals became infected at uniformly high titers. The genetic contribution to SGEB In infections with ZIKV and CHIKV was a moderate but with DENV2 was very low.

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DEVELOPMENT OF A POINT OF CARE METHOD FOR DETECTION OF ZIKA VIRUS BASED ON LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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The recent emergence of Zika virus disease (ZVD), a febrile viral illness, highlight the need for point-of-care testing. One of the main problems is the lack of standardized diagnostic tools that involves minimal laboratory capacity which is urgently required to facilitate early detection of ZVD. We developed a novel method based on reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) to detect Zika Virus (ZikV). The ZikV RT-LAMP primers were designed based on the NS5 region using a multiple alignment of 64 ZikV complete genomes. Dried LAMP reagent was used for the reaction. Initial specificity and sensitivity assays were performed. Seven RNA templates of different arboviruses were analyzed by the RT-LAMP and only the samples positive for zika virus returned positive. Additionally, serial dilutions of one of the ZikV RNA templates we tested by RT-LAMP and real time RT-PCR (CDC-US). Comparison results showed that RT-LAMP is 1000 times more sensitive than real time RT-PCR; reveling detection limit as low as 0.0007pg. We also evaluated 250 serum samples using the RT-LAMP and the results were compared with the RT-PCR and obtained a sensitivity of 96.77, a specificity of 100, a positive predictive value of 100 and a negative predictive value of 99.2. the sensitivity, specificity, (PPV) and (NPV). Our novel ZikV RT-LAMP proved to have good performance and reliability. Currently we are running a field validation where the ZikV RT-LAMP dried reagent was proven useful because its transportation and storage do not necessarily require refrigerated or frozen conditions. Confirmation of the utility of this point-of-care testing provide a low-cost alternative for rapid diagnosis of ZVD in primary health care facilities or local hospitals with resource-limited laboratories.

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THE ROLE OF CROSS-REACTIVE DENGUE VIRUS ANTIBODIES IN SEXUAL TRANSMISSION OF ZIKA VIRUS

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In addition to being the first example of a teratogenic human arbovirus, Zika virus (ZIKV) is also the first example of an arbovirus that can be sexually transmitted. ZIKV is now co-endemic in Latin America with the closely related flavivirus, dengue virus (DENV). Since DENV is highly prevalent throughout this region, a large proportion of individuals at risk of ZIKV transmission previously have been exposed to DENV. Antibody responses between ZIKV and DENV are highly cross-reactive but do not cross-neutralize and provide minimal cross-protection from vector-borne transmission of these viruses. However, the effect of cross-reactive DENV antibodies on sexual transmission of ZIKV is unknown. IgG is secreted from systemic circulation into vaginal mucus, and is the predominant antibody in the vagina. Unlike IgG in plasma, vaginal IgG interacts with mucins, and the cumulative effect of multiple low-affinity Fc-mucin interactions can immobilize antibody-bound particles, even if those antibodies are non-neutralizing. This immobilization contributes to protection from sexual transmission of pathogens including herpes simplex virus-2 because the virus is prevented from accessing a host cell even while the particle remains infectious. We evaluated whether a similar mechanism could allow cross-reactive non-neutralizing DENV antibodies to protect against ZIKV sexual transmission. We labeled purified ZIKV virions with a fluorescent dye and measured particle mobility in vaginal mucus in the presence of cross-reactive monoclonal and polyclonal antibodies from DENV-infected individuals. We also used a mouse model of ZIKV intravaginal infection to determine whether DENV antibodies in the vagina can protect against ZIKV intravaginal challenge, and to compare the quantity and quality of

antibody responses in the vagina to those in plasma. Future studies will include determining the epitope binding and neutralization of ZIKV by IgG in vaginal mucus from DENV and ZIKV immune women in Nicaragua.

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DETERMINING AN IMMUNOCOMPETENT, SMALL ANIMAL SEXUAL TRANSMISSION MODEL FOR ZIKA VIRUS

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Zika virus (ZIKV) is a mosquito borne flavivirus that has been prevalent in Asia, parts of Africa, and Central and South America. The recent pandemic in South and North America resulted in over 1,000,000 cases. The most common symptoms of Zika virus disease are rash, arthralgia, conjunctivitis and headache, however compilations have been observed including Guillain-Barré syndrome and birth defects in infected newborns. Interestingly, ZIKV is the only known flavivirus to be able to transmit sexually. This study was undertaken to determine an immune-competent, non-primate, small animal model that would be valuable for understanding the mechanism of ZIKV sexual transmission. Previous published evidence showed that cottontail rabbits and guinea pigs seroconvert after inoculation with ZIKV, and other infection studies suggested that *Mastomys natalensis* are host for serval arboviruses. We are looking for evidence of infections in male tissues and body fluid of these three animal models; rabbits (NZW strain), guinea pigs (Hartley strain) and *Mastomys natalensis*. Groups of each species are being inoculated with ZIKV or uninfected cell culture media, and then blood, oral swab, urine and semen samples are collected and tested for ZIKV by q-RT-PCR and plaque assays. To date, we have determined that NZW rabbits are not a competent model for ZIKV sexual transmission. Additional results are pending. Once an animal model is determined we will use it to study if differences in virus tropism or disease pathology occur by different route of infections (needle inoculation, mosquito bite, and sexual transmission). By determining an immune-competent, non-primate, sexual transmission animal model for ZIKV we hope to begin addressing many unknown questions about this mode of transmission and its influence on ZIKV disease.

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IMAGINING ZIKA: EPIDEMIOLOGY, GLOBAL HEALTH, AND UNCERTAINTY

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On September 25, 2017, the headline of the Tampa Bay Times wondered, "What Ever Happened to Zika?". In coming to understand "what happened to Zika", it is crucial to first understand how our ideas about Zika were formed. This paper offers a first answer this question through a reexamination of the evidence that formed the basis of scientific predictions, retracing the ways that a picture of the virus emerged based on limited knowledge, best guesses, and public anxiety. In so doing, it takes a close look at how inconsistencies in Zika predictions emerged from a series of gaps in knowledge about fundamental features of the disease. It demonstrates how representations of the epidemic throughout the sciences were mired in uncertainty at every level, and that these uncertainties allowed scholarship on Zika to be partially guided by social and cultural forces. This paper further suggests that this process had concrete consequences for policy. In understanding how scientists "created" a picture of Zika even as they attempted to locate it, this paper further examines the ramifications for arboviruses and for tracking future emerging epidemics. It suggests that in the context of fundamental uncertainty about how a new disease works, scientists must learn to depart from the norms of scientific publication in order to make especially explicit the degree to which their findings are certain. Further, uncertainty is particularly underrepresented in attempts to communicate findings to those interested in creating policy. It is crucially important to critically examine methods of communication of uncertainty, as well as the potential consequences of action guided by uncertain evidence. However, this paper further proposes that by reexamining the assumptions behind epidemiology, there is an opportunity to creatively redesign scientific processes to better depict and predict disease in ways that will be more useful to those engaged in disease control.

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STRUCTURAL PROTEINS PRME DICTATE SEXUAL TRANSMISSION POTENTIAL OF ZIKV IN AN IMMUNOCOMPETENT MOUSE MODEL

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The sexual transmission potential of African genotype Zika virus (ZIKV) strains cannot be studied in models deficient of IFN response [type I/II receptor knockout (AG129) mice] due to rapid and high mortality rates after inoculation with African genotype viruses. To overcome this challenge, a longitudinal ZIKV study was carried out in immunocompetent C57bl/6 mice treated with a monoclonal antibody against type I IFN receptor. In C57bl/6 mice, following transient knockdown of type I IFN signaling, delayed and/or reduced mortality was observed with all ZIKV strains compared to similar inoculations of AG129 mice. The African and Asian genotype ZIKVs, with the exception of a 2015 Asian genotype isolate (PRVABC59), demonstrated similar sexual transmission potential in this C57bl/6 model. Similar proportions of ejaculates from C57bl/6 mice inoculated with either Asian or African genotype ZIKV strains contained both viral RNA and infectious virus; however, none of the ejaculates from mice inoculated with PRVABC59 virus contained virus. To determine if viral structural elements mediate sexual transmission potential differences observed between PRVABC59 and African genotype ZIKVs, the prME genes from MR766 (African genotype) were cloned into a cDNA clone of PRVABC59. Mice inoculated with a prME chimeric virus generated higher viral titers in the epididymis during acute infection compared to PRVABC59-inoculated mice. Furthermore, ejaculates from mice inoculated with the prME chimeric virus contained infectious virus at titers similar to ejaculates collected from mice inoculated with MR766 virus. Overall, all ZIKVs tested, except for a strain from the current outbreak, had the potential for sexual transmission in an immunocompetent mouse model. The potential significance of type II IFN for modulation of sexual transmissibility of ZIKV is evidenced by the lack of sexual transmission potential of the PRVABC59 Asian genotype virus in transient type I knockdown mice as opposed to sexual transmission in type I/II IFN receptor deficient mice.

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HUMAN TESTICULAR ORGANOID MODEL AS AN *IN VITRO* SYSTEM TO INVESTIGATE ZIKA VIRUS PATHOGENESIS

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Zika virus (ZIKV) is an arbovirus belonging to the *Flavivirus* genus of the *Flaviviridae* family. The 2015-16 ZIKV epidemic in South America resulted

in more than 1.5 million symptomatic cases. Traditionally associated with mild fever, the recent outbreak presented new severe features of ZIKV disease, including neonatal microcephaly and Guillain-Barre syndrome in adults. Further, sexual transmission of ZIKV emerged as a cause of disease spread to non-endemic regions, a unique finding not reported for other flaviviruses. ZIKV has been detected in semen for up to 188 days after symptoms onset and is shown to potentially affect fertility, indicating ZIKV establishes persistence in the testes. Our recent study demonstrated that ZIKV could efficiently infect human Sertoli cells (SC), an important cell type that contributes to the immune privilege environment of seminiferous tubules. However, due to lack of relevant animal models to study ZIKV pathogenesis and apparent limitations of monolayer cultures, an *in vitro* system that incorporates multiple human testicular cell types to partially recapitulate testis function is considered ideal to investigate pathogenic features of ZIKV infection in the human testes. Our collaborators recently developed a human testicular organoid (hTO) model, consisting of multiple testicular cell types, that produces testosterone continuously and partially supports early stages of spermatogenesis. Our objective here was to evaluate the hTO model as an *in vitro* system to study ZIKV pathogenesis. hTOs were infected with an epidemic ZIKV strain and then subsequently assessed for virus replication, viability, and function post-infection. We found that hTOs supported productive ZIKV replication, which resulted in reduced hTO survival and function. Our results also demonstrated induction of key antiviral genes *IFNB* and *IFIT1* and the pro-inflammatory cytokine gene *IL6* following ZIKV infection. Collectively, our results indicate that the hTO model can be used as a relevant *in vitro* system to study ZIKV infection of human testes, including cellular tropism, immune response, and potential effects on spermatogenesis.

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LOCAL AND REGIONAL DYNAMICS OF ZIKA VIRUS TRANSMISSION IN THE AMERICAS: THE ROLE OF MISMATCHED SPATIAL HETEROGENEITY

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Models of transmission dynamics are routinely fit to spatially-aggregated epidemiological time series even though transmission is typically heterogeneous at fine spatial scales. For example, numerous models have used national-level time series of Zika cases for forecasting and inferential purposes in the Americas since Zika virus was first reported in Brazil in 2015. Analyses of nationally aggregated time series data might bias model inference or prediction, leading to poorly targeted public health interventions. To examine whether modeling transmission dynamics below the scale of spatially-aggregated case reports could improve model fit and capture spatial heterogeneity in disease incidence, we fit an individual-based transmission model to case data from several Central and South American countries as follows: (1) single-patch national models fit to national data; (2) multi-patch national models fit to national data, where patches represent 1st-level administrative units; and (3) multi-patch sub-national models fit to sub-national data, where patches represent 2nd-level units. Model consistency with observed dynamics improved with increasing model granularity. For the majority of countries (7 of 10), the multi-patch national model better captured national-level temporal patterns than did the single-patch national model, reducing the weekly mean absolute error (MAE) (e.g., MAEs for El Salvador and Costa Rica were reduced by 19.9% and 11.4%, respectively). Furthermore, sub-national predictions of incidence benefitted significantly from incorporating information about the timing of introduction at the sub-national level into the fitting process. For example, the correlation between modeled and reported province-level incidence in Ecuador was 0.48 (95% CI: -0.02-0.93), significantly higher than a null model assuming uniform incidence across provinces (Wilcox signed rank test; $p<0.01$). Overall, more granular models performed better by better matching human populations with locally relevant risk, suggesting that these models can be used to estimate heterogeneity in incidence at policy-relevant spatial scales.

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LONG TERM PERSISTENCE OF ZIKA VIRUS IN RHESUS MACAQUE TISSUES

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Previous *in vitro* studies have demonstrated cross-reactivity of antibodies derived from dengue virus (DENV) and yellow fever virus (YFV) infections with Zika virus (ZIKV), as well as antibody-mediated enhancement of infection in cell culture. We evaluated the impact of prior heterotypic flavivirus infection on ZIKV titers in biofluids and tissues of rhesus macaques at multiple time points post exposure. Animals previously infected (≥420 days) with DENV2, DENV4, or yellow fever virus ("flavivirus-immune") were compared to flavivirus-naïve animals following infection with a Brazilian ZIKV strain. Both the flavivirus-immune and flavivirus-naïve groups exhibited similar ZIKV titers across most tested tissues through day 10 post exposure. Long after the biofluids no longer presented detectable ZIKV in all animals, the spleen and the draining lymph node nearest the inoculation site were consistently positive through day 56 in both groups, and positive in only the flavivirus-naïve group on day 112 post exposure. Overall, the results indicate that prior infection with DENV or YFV did not meaningfully alter ZIKV titers or tissue distribution during the viremic period, and suggest the potential for greater tissue persistence in the flavivirus-naïve animals at later time points.

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DURATION OF DETECTION OF ANTI-ZIKA VIRUS IGM ANTIBODY IN A PROSPECTIVE COHORT STUDY

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Understanding the duration of detection of anti-Zika virus (ZIKV) IgM antibody is necessary to inform diagnostic testing algorithms. CDC recommends testing serum specimens collected up to 12 weeks after symptom onset or ZIKV exposure for anti-ZIKV IgM. Using data from a cohort of patients with ZIKV disease in Puerto Rico confirmed by RT-PCR, we estimated the time to first detection and loss of detection of anti-ZIKV IgM antibody. Index patients were recruited following detection of ZIKV RNA in serum or urine specimens. Index-patients' household contacts were also tested, and if positive by RT-PCR were invited to join the cohort. Follow-up visits occurred every week for four weeks and at 2, 4 and 6 months post-enrollment. Serum collected at each visit was tested by anti-ZIKV IgM antibody capture enzyme-linked immunosorbent assay (MAC ELISA). Data were used to determine appearance and loss of anti-ZIKV IgM detection as a function of days post-symptom onset (DPO). We used repeated-measures logistic regression to model the proportion of specimens with detectable IgM over time, yielding the estimated times at which 50% and 25% of specimens were still IgM-positive. Of 279 symptomatic cohort participants, 135 (48.4%) were female, including nine pregnant women, and median age was 36 years (range: 9 months–83 years). Anti-ZIKV IgM antibody was detected in 271 (97.1%) participants. Of 22 specimens collected from 0–3 DPO, anti-ZIKV IgM was detected in six (27.3%). About 80% (61/77) of the specimens collected from 4–7 DPO had detectable ZIKV IgM. The estimated time when 50% and 25%

of participants still had detectable anti-ZIKV IgM was 97 days (95%CI: 91, 103) and 125 days (95%CI: 118, 134), respectively. Participants aged <18 years had a significantly shorter time to 50% detection than other participants (p=0.03). No significant differences were observed by sex. These data suggest nearly all symptomatic ZIKV-infected individuals develop anti-ZIKV IgM antibody, and half will still have detectable IgM antibodies about three months after symptom onset. These observed estimates are consistent with current CDC testing recommendations.

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EFFECTS OF AGE, BLOOD MEAL ACQUISITION, AND MORTALITY ON THE TRANSMISSION POTENTIAL OF ZIKA VIRUS

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Zika virus (ZIKV) is a mosquito-borne flavivirus currently circulating in South America. A widespread outbreak of the virus was first detected in Brazil in 2015, and has quickly spread throughout South and Central America. Unlike previous outbreaks, cases of ZIKV from the current outbreak have been linked to severe symptoms, including microcephaly in newborns and neurological complications in adults. There are currently no antiviral treatments or vaccines available, and prevention relies solely on vector control. Understanding the factors that affect viral transmission from mosquito to human is critical when designing vector control strategies. Specifically, we investigated the interactions of mosquito age, order of infectious blood meal, and mosquito mortality in order to assess the transmission potential of ZIKV. Cartons of *Aedes aegypti* were subjected to three different treatments to assess average time to viral dissemination. These treatments include a single ZIKV blood meal 3-5 days post-emergence, a single ZIKV blood meal 10-12 days post-emergence, and a non-infectious blood meal 3-5 days post-emergence, followed by a ZIKV blood meal 10-12 days post-emergence. Sampling was performed at multiple days post-exposure. Using qRT-PCR, abdomens and legs were tested separately for infection and dissemination, respectively. To assess average time to death, the same treatments were used, and dead mosquitoes were collected and counted daily for 23 days. We found no significant difference in average time to dissemination between the three treatments; however, the average time to death was significantly different among the three treatments. Our results suggest that interactions between age, order of infectious blood meal, and mortality change the transmission potential of ZIKV in *Aedes aegypti.* Furthermore, these observations provide insight into potential vector control methods for ZIKV-exposed mosquitoes.

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SAFETY AND IMMUNOGENICITY OF MEASLES, MUMPS, AND RUBELLA VACCINE [PRIORIX, GLAXOSMITHKLINE BIOLOGICALS] CO-ADMINISTERED WITH LIVE ATTENUATED SA 14-14-2 JAPANESE ENCEPHALITIS VACCINE [CD.JEVAX, CHENGDU INSTITUTE OF BIOLOGICAL PRODUCTS]

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Japanese encephalitis (JE) virus is the leading cause of viral encephalitis across temperate and tropical zones of Asia. The live attenuated SA 14-14-2 JE vaccine (CD-JEV) is one of several vaccines prequalified by the World Health Organization to prevent JE. When incorporating a new vaccine into a country's Expanded Programme on Immunization, it is important to show that the new vaccine can be administered concurrently with other routine pediatric vaccines without impairing the immune response or safety profile of the coadministered vaccines. This Phase 4 open-label study evaluated the safety and immunogenicity of measles, mumps, and rubella (MMR) vaccine co-administered with CD-JEV. The study randomized 628 healthy Filipino children aged between 9 and 10 months to receive MMR and CD-JEV concurrently or separately. JE immunogenicity was assessed 28 days after CD-JEV vaccination using plaque reduction neutralization test (PRNT). MMR immunogenicity was assessed 56 days after MMR vaccination using a measles PRNT, anti-mumps immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA), and anti-rubella IgG ELISA, respectively. Safety was assessed through observation for reactions immediately following immunization, solicitation of systemic adverse reactions occurring within 14 days of vaccination, and follow-up of serious adverse events during 7 to 8 months of study participation. During the study, no vaccine-associated encephalitis cases or serious adverse events were reported in either group, and concurrent immunization with CD-JEV and MMR vaccines was not associated with any unusual safety signals when compared with sequential immunization. Complete serologic results will be available by June 2018.

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DETECTION AND MOLECULAR CHARACTERIZATION OF ZIKA VIRUS IN SPECIMENS COLLECTED DURING 1998 TO 2017 FROM THAILAND

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The Department of Virology, AFRIMS has conducted surveillance for mosquito-borne viral pathogens for several years in Thailand. Following the WHO's declaration of Zika virus (ZIKV) as a public health emergency in 2016, AFRIMS added ZIKV testing to our current prospective dengue surveillance studies and outbreak investigations, and also began retrospectively testing specimens collected to assess for the extent of ZIKV circulation in Thailand. Since March 2016, ZIKV real-time RT-PCR has been performed on a total of 3,634 acute specimens collected in Thailand from 1998 to 2017. The specimens tested include whole blood, serum, plasma, and urine. Seventy percent of the specimens (2,528/3,634) had previously tested negative for dengue virus RT-PCR. The overall ZIKV positivity rate of detection by real-time RT-PCR in this study was 1.7% of total specimens tested (61/3,634). The positive detection rate by specimen type was 12% for whole blood (19/164), 0.5% for serum (14/2,796), 0% for plasma (0/62) and 4.6% for urine (28/612). ZIKV was detected in the specimens collected from five years including 2011, 2013, 2014, 2015, and 2017. We were able to isolate a total of 9 ZIKV viruses from RT-PCR positive specimens including 8 new isolates reported here and 1 previously reported isolate from 2014. Complete genome sequences of the 8 new isolates, obtained from specimens collected from the five years, were generated and analyzed with other sequences from GenBank. Phylogenetic analysis showed that all of the isolates were clustered close

to and belonged to the Asian genotype. The single mutation in the prM protein that has been suggested to contribute to fetal microcephaly was not found in our ZIKV sequences. The sequences of binding sites for the primers and probes used in ZIKV real-time RT-PCR were also observed in order to monitor mutations that might impact to the sensitivity of the used assay. Mismatch at five positions were found. Continued investigation and analysis of the molecular characteristics of ZIKV circulating in Thailand will provide more accurate and up-to-date information about ZIKV transmission and its clinical impact on global public health.

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LABORATORY ANALYSIS OF ARBOVIRUS SURVEILLANCE IN SOUTHERN THAILAND FROM 2012-2015

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Since 2012, the Armed Forces Research Institute of Medical Sciences (AFRIMS), in collaboration with Prince of Songkla University (PSU) has conducted arbovirus surveillance in Southern Thailand in an effort to identify and characterize dengue (DENV), chikungunya virus (CHIKV), Japanese encephalitis Virus (JEV), and Zika virus (ZIKV) circulation. Patients presenting with undifferentiated febrile illnesses and dengue-like symptoms were enrolled at PSU Hospital and the surrounding local hospitals. Acute and convalescent samples were collected for flavivirus nucleic acid testing and when possible a 14 day convalescent sample was taken. Semi-nested DENV/JEV/CHIKV RT-PCR and Zika real time RT-PCR conducted on acute samples identified 219 DENV (219/970), 3 CHIKV (3/679), 0 JEV (0/755) and 3 ZIKV (3/337) positive specimens. Serological testing on acute and convalescent samples included DENV/JEV EIA and Hemagglutination Inhibition (HAI) for DENV and CHIKV. DENV HAI seroconversion in DENV PCR positive (211 acute and 202 convalescent) and DENV PCR negative (696 acute and 676 convalescent) ranged from 58-78% and 58-81%, between 2012-2015 and both peaked at 78% and 81% in year 2013, respectively. Both DENV PCR and HAI revealed that DENV was highly endemic in Southern Thailand. CHIKV HAI seroconversion in DENV PCR positive samples was 42%, 35%, 23%, and 24% in 2012, 2013, 2014, and 2015 whereas in the DENV PCR negative group, rates were 36%, 30%, 23%, and 19% in years 2012, 2013, 2014, and 2015, respectively. A downward trend of CHIKV HAI seroconversion is seen in later years following the CHIKV outbreak in 2006-2008 but CHIKV appears to still be circulating in the area as asymptomatic cases. Plaque Reduction Neutralization Titers confirmed all 105 pairs of CHIKV HAI seroconversion. Our data showed that HAI seroconversion is useful tool to predict the endemicity of DENV and CHIKV in both DENV and CHIKV PCR positive and negative patients. This data will help us to understand arbovirus transmission and disease burden in a population living in rural and urban areas of Southern Thailand.

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A RETROSPECTIVE STUDY USING A SEROSURVEY TO DETECT ZIKA VIRUS IN MOMBASA, KENYA

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Zika virus (ZIKV) has not been confirmed in Kenya and WHO classifies Kenya as having a competent vector but no past or current documented infection. Previous ZIKV serology studies demonstrated evidence of ZIKV in Kenya using outdated immunoassays with limited specificity. We used specimens collected from a dengue seroincidence study to retrospectively test for the recent presence of ZIKV in Kenya. The dengue seroincidence study was conducted amidst a dengue outbreak to determine the incidence of dengue amongst residents of Tudor Mombasa, Kenya from May 3 to May 11, 2013 where 1500 residents from 986 randomly selected households consented to participate in the study. A questionnaire was administered to each household and a venous blood specimen was collected and tested for DENV by real-time reverse transcription polymerase chain reaction (rRT-PCR) and anti-DENV IgM. From these specimens, there were 746 determined to be DENV negative with sufficient volume to test for ZIKV by rRT-PCR, and anti-ZIKV IgM. To confirm the specificity of the results from the anti-ZIKV IgM antibody capture enzyme linked immunosorbent assay (MAC-ELISA), all positive (positive to negative ratio (P/N) ≥3) and equivocal (P/N=2-2.99) specimens are being tested with the 90% plaque reduction neutralization test (PRNT90) for ZIKV and DENV. From the 746 specimens tested to date, none were ZIKV rRT-PCR positive, 35 (4.7%) were anti-ZIKV IgM positive and 23 (3.1%) anti-ZIKV IgM equivocal. The PRNT testing is in progress however from the four specimens that were tested by PRNT, two were confirmed as a ZIKV cases, defined as ZIKV PRNT90, positive with a 4 fold higher titer for ZIKV compared to DENV. This is the first study that confirms that ZIKV infections occur in Kenya. The results suggest that similar to DENV, ZIKV may be endemic in the coastal region of Kenya and should be included in the differential diagnosis especially amongst pregnant women.

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SEROPREVALENCE, RISK FACTOR, AND SPATIAL ANALYSES OF ZIKA VIRUS INFECTION AFTER THE 2016 EPIDEMIC IN MANAGUA, NICARAGUA

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In 2015, a Zika epidemic in Brazil began spreading throughout the Americas. Zika virus (ZIKV) entered Managua, Nicaragua, in January 2016 and caused an epidemic that peaked in July-September 2016. ZIKV seropositivity was estimated among participants of pediatric (n=3,740) and household (n=2,147) cohort studies, including an adult-only subset from the household cohort (n=1,074), in Managua. Seropositivity was based on a highly sensitive and specific assay, the Zika NS1 blockade-of-binding ELISA, which can be used in dengue-endemic populations. Overall seropositivity for the pediatric (ages 2-14), household (ages 2-80) and adult (ages 15-80) cohorts were 36%, 46%, and 56%, respectively. Trend, risk factor, and contour mapping analyses demonstrated that ZIKV

seroprevalence increased non-linearly with age and that body surface area was statistically associated with increasing seroprevalence in children. Seropositivity to anti-ZIKV antibodies was higher in females than in males across almost all ages, with adjusted prevalence ratios in children and adults of 1.11 (95% CI: 1.02-1.21) and 1.14 (95% CI: 1.01-1.28), respectively. No household-level risk factors were statistically significant in multivariate analyses. A spatial analysis revealed a 10-15% difference in the risk of ZIKV infections across our 3km-wide study site, suggesting that ZIKV infection risk varies at small spatial scales. To our knowledge, this is the largest ZIKV seroprevalence study reported in the Americas, and the first in Central America and in children. It reveals a high level of immunity against ZIKV in Managua as a result of the 2016 epidemic, making a second large Zika epidemic unlikely in the near future.

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CHANGES IN BEHAVIORS ASSOCIATED WITH TRANSMISSION AMONG A COHORT OF ZIKA VIRUS INFECTED PARTICIPANTS IN PUERTO RICO

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Little is known about behaviors associated with Zika virus (ZIKV) infection and transmission to either mosquitos or humans after infection, including how they may change after diagnosis with ZIKV disease. We evaluated changes in repellent use and sexual activity using data from the Zika virus Persistence (ZiPer) cohort study in Puerto Rico that began in May 2016. After presentation to healthcare facilities, ZIKV RNA-positive patients were offered study participation, including a questionnaire and testing to detect: a) ZIKV RNA in serum, urine, saliva, and vaginal fluid or semen; and b) anti-ZIKV IgM antibody in serum. All study participants were educated at enrollment about routes of ZIKV transmission, and the risks due to mosquitos and unprotected sexual contact. Questionnaires and specimens were collected weekly for the first month and at 2, 4, and 6 months. Among ZIKV RNA-positive participants, we used x^2 tests to assess changes in the percentage of participants reporting repellent use in the past 30 days, sexual activity and condom use in the past 7 days, by day post-illness onset (DPO). Among 295 RNA-positive participants, 63% reported repellent use at enrollment. In prospectively followed symptomatic participants, repellent use was 33% at 0-7 days DPO, 69% at 8-14 DPO, and 51% by ≥125 DPO. Of 226 adults, at enrollment 52% were sexually active, of whom 19% reported condom use. Sexual activity was 71% at 0-14 DPO and 50% at ≥125 DPO. Condom use among sexually-active participants was low and peaked at 34% at 46-60 DPO. Of 96 men with semen specimens, 62% were sexually active and of them 19% reported condom use at baseline. During follow-up visits, 55% were sexually active and 44% of these periods involved condom use. Between men with and without detectable RNA in semen, differences in sexual activity trends and condom use were not significant. In summary, although reported repellent use was higher after symptom onset, it declined over time. Condom use overall frequencies were low, including among men with ZIKV RNA in semen, illustrating the risk of sexual transmission. Counselling of patients with ZIKV disease should emphasize protective behaviors.

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NEUROLOGICAL ASSESSMENT OF CHILDREN BORN TO PREGNANT WOMEN RETURNING FROM ZIKA AFFECTED AREAS: RESULTS FROM SURVEILLANCE IN A NON-ENDEMIC AREA

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Zika virus (ZIKV) epidemic has dramatically impacted the health of mothers and infants in affected areas. Research on ZIKV has focused on the characterization of the congenital ZIKV syndrome and the severe clinical manifestations of affected children. The long-term consequences of ZIKV infection in pregnancy on children health remain unclear. We aimed to assess the physical, neurological and psychomotor development of children born to pregnant women returning from ZIKV affected areas with a possible ZIKV infection (probable or confirmed). Active surveillance of pregnant travellers, potentially exposed to ZIKV was established at the Hospital Clínic of Barcelona, Spain, that included follow up of their children until 18 months of age. Follow up consisted of ZIKV screening at birth, weight, length, head circumference measurements, neuroimaging, ocular, hearing and psychomotor assessment, as well as the Bayley Scale for development at 18 months of age. All variables were collected using standardized questionnaires. Here, we describe a cohort of 43 children born to pregnant women with probable or confirmed ZIKV infection. All children tested negative for ZIKV at birth by PCR. Serological analysis revealed placental transfer of ZIKV maternal IgG antibodies. No growth retardation or delay in the psychomotor development was found among study children during follow up. Cerebral ultrasound did not reveal major pathological findings, however two children were found with choroid plexus cysts and with cerebral ventricular asymmetry, respectively, as a variant of normality. In addition, a study infant was diagnosed with a non-affiliated hepatitis at two months of age. Data from follow up of healthy children exposed to ZIKV in uterus is scarce, almost inexistent. Prospective studies to comprehend the neurological impact of ZIKV in these children in the long-term and its clinical and developmental consequences are crucial.

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CHARACTERIZING THE IMMUNE RESPONSE TO ZIKA VIRUS USING EPITOPE MAPPING, REPORTER VIRUS PARTICLES, AND ANTI-ZIKV ANTIBODIES

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We have characterized the immune response to ZIKV infection and vaccines by epitope mapping over 50 anti-ZIKV MAbs at amino acid-resolution, using a comprehensive ZIKV prM/E library of 672 single alanine mutants expressed in human cells. A published study described epitopes of MAbs isolated from a Brazilian patient, including a highly neutralizing MAb protective in animal models of ZIKV fetal disease. The epitope location suggested that the MAb acts by binding across adjacent protein E dimers, preventing the rearrangements necessary for ZIKV infectivity. The epitope maps obtained also reveal which epitopes are specific for ZIKV or are common to DENV, information that can be used to create better vaccines and therapeutics. We have also identified mutations that

increased ZIKV RVP budding, and which may be applicable to the design and production of anti-ZIKV vaccines, by screening each individual ZIKV library prM/E variant for ZIKV particle budding and infectivity. To provide critical reagents, we have isolated anti-ZIKV MAbs for ZIKV-specific immunodetection, diagnostic applications, and ZIKV neutralization studies. MAbs were isolated after immunization with DNA and sub-viral particles, and phage library panning with ZIKV reporter virus particles (RVPs) that we have developed, capable of one round of infectivity with luminescent or fluorescent readout. We isolated 48 conformational MAbs specific to prM/E from ZIKV, but not DENV, including a MAb that potently neutralized ZIKV RVPs (IC_{50} 45 ng/ml) and for which mapping identified a quaternary epitope spanning adjacent E proteins. We have also used ZIKV RVPs to identify new cellular receptors and attachment factors that enable ZIKV entry. ZIKV RVPs were tested on our Membrane Proteome Array (MPA), comprising 5,300 unique human membrane proteins individually expressed in live human cells. Known receptors and attachment factors were identified (validating the approach), as well as a number of membrane proteins not previously known to enable ZIKV entry. These newly identified proteins help explain viral tropism and pathogenesis, and may be useful as therapeutic targets.

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CHARACTERIZATION OF POPULATION EXPOSURE (SEROPREVALENCE) TO ARBOVIRUSES AFTER RECENT OUTBREAKS IN COLOMBIA: DENGUE, CHIKUNGUNYA AND ZIKA

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The 2015-16 Zika (ZIKV) epidemics in Latin America took the world by surprise, much as did the chikungunya (CHIK) epidemics in 2013-14. Nevertheless, the extent of exposure among the population at risk is still uncertain. Based on probabilistic population sampling, between October and December 2016 we conducted household-based seroprevalence studies in four different cities in Colombia, South America (Cúcuta, Neiva, Sincelejo and a sector of the city of Medellín). Over 2400 participants were enrolled, provided a blood sample and answered a questionnaire assessing risk factors for exposure. Prior infection by dengue (DENV), ZIKV and CHIK was ascertained using a multiplex recombinant antigen-based microsphere immunoassay measuring IgG against the three viruses. We found large variation in seropositivity, suggesting large variation in the rate of infection between the four cities. For DENV, seropositivity varied from 48.9 (CI 44.8 - 53.0) to 88.9 % (CI 86.1 - 91.3); for CHIK, seropositivity varied from 7.1 % (CI 5.2 - 9.4) to 72.3 % (CI 68.7 - 75.8); while for ZIKV, seropositivity varied from 6.7% (CI 4.8-9.0%) to 65.9 (CI 62.0 - 69.6). The increase in DENV seropositivity with age confirmed the endemic nature of its transmission, whereas exposure to both ZIKV and CHIK appeared unrelated to age confirming their epidemic nature. Among the four cities, Medellin had the lowest seroprevalence for all the three viruses measured. Interestingly, history of DENV or ZIKV disease in the past was not associated with antibody presence against these viruses. In contrast, having been previously clinically diagnosed with CHIK was strongly associated with being seropositive to CHIK (OR 10.32; 95% CI 7.20 - 14.79) and 41.16% of individuals who tested positive for CHIK IgG reported presenting symptoms associated to this infection in the past. This study provides the first estimation of population attack rates and exposure levels to two emerging arboviral infections in Colombia, a Dengue endemic country, and provides key elements towards the general understanding of the transmission patterns of arboviral infections.

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EVALUATING DIFFERENCES IN ZIKA VIRUS DIAGNOSTIC TEST RESULTS BY SPECIMEN TYPE AMONG PREGNANT WOMEN DURING THE ZIKA VIRUS OUTBREAK, PUERTO RICO 2016

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Zika virus (ZIKV) infection during pregnancy can cause adverse outcomes such as microcephaly, brain abnormalities, eye abnormalities, and other problems at birth. To help women and infants, early identification of ZIKV infection is essential. To evaluate the difference between ZIKV diagnostic tests by different specimen types and inform an optimal strategy for diagnosis of ZIKV infection among pregnant women to identify those needing additional monitoring. From October 1st to November 4, 2016, 532 pregnant women with no prior positive ZIKV test were recruited during prenatal visits and provided serum, urine, and whole blood for ZIKV nucleic acid testing (NAT) and immunoglobulin M (IgM) testing. For NAT, specimens were tested by the Trioplex Real-Time RT-PCR assay; if positive, specimens were tested using the Singleplex ZIKV assay to confirm. A positive by NAT or IgM indicates a recent ZIKV infection. Data were collected on patient age, trimester of pregnancy, ZIKV symptoms, and days since symptom onset. Women were considered symptomatic if ZIKV symptoms were present during specimen collection. Of 532 women, 495 (93.1%) were asymptomatic and 37 (7.0%) were symptomatic. Among asymptomatic women, 40/489 (8.2%) tested positive by IgM and 10/490 (2.0%) tested positive by NAT. Among positive NAT samples, 7 were positive in serum, 5 in whole blood, and 0 in urine. Among symptomatic women, 4/37 (10.8%) tested positive by IgM and 1/37 (2.7%) tested positive by NAT (serum and whole blood). Only one asymptomatic pregnant woman tested positive by both NAT (serum and whole blood) and IgM. There was little overlap in positive ZIKV test results by different diagnostic tests and specimen types. The majority of women tested were asymptomatic; however, positive ZIKV test results were identified among 10% (49/490) of women by at least one diagnostic test and specimen type. This data may inform guidance for testing pregnant women living in areas with ongoing risk of ZIKV infection by both NAT and IgM tests to identify pregnancies that require additional monitoring.

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UNEXPECTED PROPERTIES OF A ZIKA NS2B EPITOPE SUGGEST ITS POTENTIAL AS A BIOMARKER FOR DIAGNOSIS AND PROGNOSIS

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Latin America and the Caribbean are facing an epidemic of three co-occuring arboviruses - Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV). Major efforts have been made to develop reliable diagnostic tools, capable of differentiating between Zika and other Flavivirus infections. However, clinical overlap and cross-reactivity in serological assays between Flavivirus family members remain a main challenge. We postulate that ZIKV-specific epitopes can allow for a specific serological detection. Therefore, we translated the proteomes of 15 different ZIKV and 211 DENV strains (retrieved from NCBI database) into overlapping linear 15-mer peptides on high-density peptide arrays. To identify antibody response profiles differentiating between ZIKV and DENV infections, 84 clinically well-characterized patient and control serum samples were screened with peptide arrays. Comparison groups included (1) acute *vs.* convalescent adults, stratified by previous DENV background, (2) mother-infant pairs, and (3) neuro-Zika patients. Our data shows specific IgG responses towards peptides from the NS1 protein of DENV and peptides from the NS2b protein of ZIKV in convalescent samples, whereas background signal in control samples was low. Interestingly, no differences in the ZIKV-specific antibody profiles were observed, when comparing samples with or without DENV background. We could identify a 20mer epitope in the NS2b protein region, which may serve as a biomarker for specific diagnostics and a candidate target for drug development. In our initial microarray-based study, this marker shows high sensitivity and specificity. The reactivity of this NS2b epitope was also evaluated against a sample set with confirmed DENV or ZIKV infection only, whereas 71% of subjects possessed NS2b-specific antibodies. The affinity of this epitope to the respective human IgG antibodies is currently being assessed by microscale thermophoresis and a fusion protein was engineered, which will be used to elicit neutralizing antibody responses in future vaccination approaches. [Eur. patent registration EP17209651.3; 21 Dec 17]

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NEUROLOGICAL SEQUELAE ASSOCIATED WITH ACQUIRED ZIKA VIRUS INFECTION IN CHILDREN

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Although studies have evaluated biochemical pathways for congenital Zika virus (ZIKV) infection and related severe neurodevelopmental fetal and neonatal outcomes, research on health outcomes related to postnatally acquired ZIKV infection in children is lacking. Reports of acute central nervous system disease in adults and adolescents secondary to ZIKV infection suggest that acquired ZIKV infection may impact neurological health (e.g., neuropsychological deficits, long-term fatigue, and spinal cord lesions with paresthesia and muscle weakness). To better understand the epidemiology of neurological sequelae of acquired ZIKV infection in children, we conducted a cohort study of 36 ZIKV-infected and 35 uninfected children in León, Nicaragua. Children were evaluated for neurological symptoms (e.g., self-reported history of fatigue, headaches, seizures, memory loss, changes in vision and hearing; and observed visual acuity, facial and limb strength, reflexes, cold touch and vibration response) using a clinical neurological exam at baseline and for neurodevelopmental functioning (e.g., executive function, visual-spatial thinking, processing speed, attention, behavior) 6 months later. At baseline, 14 children were aged 2-6, 28 aged 7-12, and 29 aged 13-17, and 56% percent of children were female. ZIKV-infected children were slightly younger than uninfected children, but the sex distribution was similar. We found no statistically significant differences in neurological symptoms or neurodevelopmental function by ZIKV status. However, several characteristics were more prevalent among infected vs. uninfected children: decreased speed of thought (35% vs. 28%); aggressive behavior (45% vs. 33%); attention problems (45% vs. 27%); rule-breaking behavior (38% vs. 21%); limited cognitive efficiency (86% vs. 64%); and limited verbal ability (27% vs. 9%). Of note, somatic problems and lower non-verbal IQ were more common among uninfected children. Although limited by a small sample size, this study is the first to systematically compare short-term neurological sequelae among children with and without acquired ZIKV infection.

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REPURPOSING NOVOBIOCIN AS A FLAVIVIRUS PROTEASE INHIBITOR

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Flaviviruses such as yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and Zika virus (ZIKV) continue to be global public health threats. Currently, there are limited treatment options for medically important flavivirus infections. We previously reported the *in vitro* and *in vivo* antiviral activity of novobiocin against the closely related ZIKV through inhibition of the ZIKV NS2B-NS3 protease. In this study, we expanded from these established results to investigate whether novobiocin also inhibits the protease activity of other medically important flaviviruses. The *in vitro* antiviral activity of novobiocin against DENV, WNV, and JEV was assessed in Vero and Huh-7 cells using cytopathic effect inhibition, viral load reduction, and plaque reduction assays. A fluorescence-based protease inhibition assay was used to assess the anti-flaviviral protease inhibitory activity of novobiocin. Molecular docking was performed to predict the binding between novobiocin and the proteases of DENV, WNV, and JEV. Novobiocin inhibited DENV, WNV, and JEV in Vero and Huh-7 cells in cytopathic effect inhibition, viral load reduction, and plaque reduction assays at low micromolar concentrations. The functional protease inhibition assay showed that novobiocin inhibited the protease activity of all three flaviviruses. Molecular docking predicted that novobiocin bound to the viral proteases with high stability. Our study showed that novobiocin inhibited medically important flaviviruses *in vitro*. The likely mechanism is through inhibition of the viral proteases. Further studies should be conducted to assess whether novobiocin possesses similar antiviral activity through inhibition of viral protease activity of other medically important flaviviruses. Clinical trials should be considered to assess the treatment effects of novobiocin in patients infected with these flaviviruses.

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FORECASTING ZIKA INCIDENCE USING DENGUE SURVEILLANCE DATA FROM COLOMBIA

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Effective public health response to infectious disease outbreaks stands to benefit from accurate forecasts. The recurring seasonal nature of vector-borne disease incidence in countries where diseases such as dengue are endemic has resulted in the use of time series to inform models for forecasting future disease incidence. However, time series analyses are limited in their ability to make accurate predictions when there is little or no prior data available, such as during the recent 2015-2016

Zika epidemic in Colombia. In this increasingly common context of a disease emergence event, data from diseases with similar ecology and epidemiology could be used to form a basis of prior understanding for forecasting the emerging disease of interest. Similar to Zika virus, dengue virus is transmitted primarily by the *Aedes aegypti* mosquito and has epidemiological characteristics, such as incubation period and duration of immunity, similar to Zika. Here, we fitted time series models to weekly dengue incidence data from 2007-2015 at the departmental level in Colombia to predict peak time, peak height, and cumulative incidence of the Zika epidemic during the 2016 season. Individual models and predictions were made for each department, accounting for geographic differences in transmission. We compared our predictions to Zika incidence data and categorized our results based on the strengths of seasonality in the data. Our results suggest that model performance was heavily dependent on the strength of seasonality in departmental incidence. Models fitted to data from departments with stronger seasonality performed better at forecasting peak height and cumulative incidence than those fitted to data from departments with more irregular seasonal patterns of dengue incidence. However, the models we used were unable to accurately predict peak week, irrespective of seasonality in a region. Our results show some promise for formalizing the use of data from historically endemic diseases in forecasting emerging diseases, but at the same time they highlight limitations about the contexts in which doing so may or may not be successful.

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DURATION OF INFECTIOUS ZIKA VIRUS

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Zika virus (ZIKV) has recently caused a large epidemic in the Americas associated with birth defects. Although ZIKV is primarily transmitted by Aedes spp. mosquitoes, ZIKV RNA is detectable in blood and semen of infected individuals for weeks or months, during which time sexual and other modes of transmission are possible. However, viral RNA is expected to be detectable for longer than infectious virus is present. We determined the frequency of isolation of infectious virus from semen and serum samples prospectively obtained from a cohort of patients in Puerto Rico. Positive isolation was confirmed by cytopathic effect, increase in virus genome copy equivalents (GCE), immunofluorescence, and quantitation of infected cells by flow cytometry. These criteria confirmed infectious virus in semen from 8 of 97 patients for up to 38 days after initial detection when virus loads are higher than $1.4x10^6$ GCE/mL. Two serum isolates were obtained from 296 patients. One of these came from an aymptomatic individual. These findings can help inform important prevention guidelines for persons that may potentially be infected and transmit ZIKV sexually.

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CHARACTERIZATION OF ATTENUATING AND COMPENSATING MUTATIONS IN DOMAIN III OF THE WEST NILE VIRUS ENVELOPE PROTEIN

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West Nile virus (WNV) is a mosquito-borne flavivirus that can cause severe neuroinvasive disease often resulting in death or long term neurological sequelae. WNV is a global pathogen that is endemic in the United States, and for which no licensed vaccines or therapeutics are available for human use. The envelope (E) protein of flaviviruses has three structural domains and is the major component of the virion surface. Domain III of E (EIII) is the putative receptor-binding domain, and contains epitopes recognized by virus-specific potently neutralizing antibodies, although all three domains are targets for neutralizing antibodies. Mutagenesis of surface exposed EIII BC loop residues (aa 329-333) has been shown to affect the antigenicity and/or infectivity of WNV. Several non-conservative mutations at the highly conserved amino acid residue G331 were not tolerated. However, mutation to G331A yielded a virus that had altered antigenicity, moderately restricted growth in Vero cells, and a highly attenuated phenotype in mouse models of neuroinvasive disease, including in immunodeficient mice. Recovery of viruses from mice that succumbed to G331A WNV infection has identified putative compensating mutations that independently partially restore a neuroinvasive phenotype. This study aims to identify the potential mechanism(s) of attenuation that may be associated with G331A mutation. Recombinant WNV EIII and live infectious clone-derived WNV will be used to assess the effects of G331A and putative compensating mutations on structure and stability of WNV and WNV EIII, and whether the mutations affect WNV interactions with target cells *in vitro* and/or *in vivo*.

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METAGENOME ANALYSIS OF WEST NILE VIRUS AND OTHER ARBOVIRUSES IN CULEX MOSQUITOES FROM THE SAN GABRIEL VALLEY, CALIFORNIA

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California has its unique warm climate and landscapes favorable for the proliferation of disease transmission vectors. Vector-borne pathogens including arthropod-borne viruses (arboviruses) remain a serious threat to the public health requiring constant vector control and disease surveillance. In June to September of 2017, 1,522 pools of Culex mosquitoes (11,559 specimens) from 23 cities in the San Gabriel Valley, California were collected and tested for arboviruses. Approximately 10% of the pools were positive for West Nile virus (WNV). Random reverse transcription, PCR amplification and next-generation sequencing (NGS) were conducted for 81 WNV positive pools for a metagenome analysis of WNV and other arboviruses. Moreover, methods of whole genome amplification and enrichment using pan-viral-pathogen probes were applied to acquire WNV genome sequences for genome-wide phylogeny and variation determination. The sequence-based taxonomic classification identified eukaryotic, bacterial, viral and unclassified genetic material in the Culex mosquito pools. Additional viruses, other than WNV, were found in 50 out of the 81 mosquito pools (61.7%). These viruses shared various degrees of nucleotide sequence similarity with viral sequences in GenBank, which included sequences from Alphamesonivirus, Biggievirus, Bunyavirus, Cordoba virus, Culex flavivirus, Marafivirus, Houston virus, Negevirus, etc. WNV genome analysis revealed that these viruses belong to the genetic lineage I, which is the same as for the clinical WNV isolates in the United States. The genome sequence that encodes a polyprotein with the expected length of 3,433 amino acids is genetically homologous with WNV genomes from mosquitoes, birds and humans in the U.S., with a small number of nucleotide differences and a few amino acid substitutions. Further genetic, phylogenetic and taxonomic characterization of these arboviruses, and the deep analysis of genetic variation of the mosquito-borne WNV and their relationship with the WNVs in avian and clinical specimens will enhance knowledge-based disease surveillance and control of arboviral infections.

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ORAL SHEDDING OF JAPANESE ENCEPHALITIS VIRUS IN INFECTED SWINE SPECIES

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Japanese encephalitis virus (JEV) is a zoonotic mosquito-borne flavivirus maintained among swine and avian species as amplifying hosts in the Asian Pacific region. Infection of pigs with JEV can cause rapid onset of viremia, which supports the infection of competent mosquitoes. Systemic infection that allows viral dissemination into various organs has also been reported. Although the detection of JEV in oronasal tissues of experimentally infected pigs has been attributed to the transmission of JEV in the absence of arthropod vectors under laboratory conditions, quantities and duration of viral shedding during the infection process of JEV in pigs remains unclear. In this study, shedding of JEV was monitored in two JEV challenge models based on domestic pigs and miniature pigs with feral swine phenotypes. Oral fluids were collected from infected animals using a noninvasive rope-based technique followed by quantitative detection of the viral genome. Maximal viral shedding coincided with peak viremic titers at the acute phase of infection. Presence of JEV genomes in oral fluids persisted after the clearance of detectable viremia. Consistent with the detection of JEV in the lymphoid tissues at the convalescent phase of diseases, prolonged periods of JEV oral shedding into the environment provides the basis for our understanding of persistent infection of JEV in infected swine species and its transmission in the absence of competent vectors. The detection of viral genomes also demonstrated the feasibility and advantage of using oral fluids as a noninvasive sampling approach to improve existing diagnostic and surveillance methods in endemic regions.

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CROSS REACTIVITY BETWEEN WEST NILE VIRUS AND ST. LOUIS ENCEPHALITIS VIRUS ASSAYS IN FLAVIVIRUS NEUROINVASIVE DISEASE CASES IN LOUISIANA

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Serolologic assays are widely utilized in the diagnosis of flavirviral clinical disease and in public health surveillance. Previous studies have shown high rates of cross-reactivity between closely related members of the Flavivirdae family. We examined cross-reactivity between West Nile and St Louis Encephalitis serologic assays in 616 cases with presumed or confirmed flaviviral neuroinvasive disease in Louisiana. A total of 291 of 458 cases (63.5%) tested for both viruses had cross-reactivity. Of the 444 West Nile virus neuroinvasive disease cases tested, 286 (64.4%) had positive serologic tests for St Louis Encephalitis. Five of the 14 (35.7%) St Louis Encephalitis cases tested had positive serologic tests for West Nile virus. Cross-reactivity was high for both IgM and IgG tests. Cross reactivity in our large number of cases was higher than many previous estimates. As flaviviruses continue to spread, clinicians, researchers and public health personnel should be aware of the limitations of serologic assays in diagnosis and surveillance, especially in areas of the world with co-occurring viruses.

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EXPOSURE OF FREE RANGING BIRDS TO ST. LOUIS ENCEPHALITIS AND WEST NILE VIRUSES (FLAVIVIRUS) IN AGRICULTURE AND CATTLE RAISING LANDSCAPES IN ARGENTINA

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Anthropogenic changes affect biological communities of host and vectors driving arbovirus activity. In general, urban and agricultural landscapes host less avian and mosquito diversity and frequently these are dominated by few species. The St. Louis encephalitis (SLEV) and West Nile viruses (WNV) are infectious agents that have emerged and re-emerged worldwide. The aim of this work was to evaluate activity of SLEV and WNV in bird communities in agricultural and cattle raising landscapes. Field work was carried out in four different sites in La Pampa province (Argentina) between February and June 2017 (summer and fall). Avian sera samples were analyzed by plaque reduction neutralization test. Generalized Linear Models were performed to determine the effect of bird species and sampling site on the infection of SLEV/WNV. Neutralizing antibodies against SLEV were found in 45 out of 348 samples (12.9%) while WNV seroprevalence overall was 3.4% (12/348). House Wren (*Troglodytes aedon*), House Sparrow (*Passer domesticus*), Monk Parakeet (*Myiopsitta monachus*), Rufous Hornero (*Furnarius rufus*), Rufous-collared Sparrow (*Zonotrichia capensis*) and Picui Ground-dove (*Columbina picui*) were the most exposed avian species to SLEV. On the other hand, Shiny Cowbird (*Molothrus bonariensis*), Rufous Hornero (*Furnarius rufus*), Monk Parakeet (*Myiopsitta monachus*), and Picui Ground-dove (*Columbina picui*) showed the highest seroprevalence values for WNV. The sampling site was a significant variable affecting the chance to detect an infected bird by SLEV/WNV. Sites located in northern area showed higher seroprevalence values than those located southern. Our data confirm the geographic expansion of WNV in Argentina from center region to southern latitudes. Northern area of La Pampa is subject of intense agricultural activities (soy, corn, wheat) whilst southern area has a mixed of agricultural and cattle raising activities. These preliminary data indicate a potential positive effect of agricultural activities over the SLEV/WNV activity.

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DETECTION AND QUANTIFICATION OF WEST NILE, RIFT VALLEY FEVER, AND DENGUE FEVER VIRUSES FROM DRIED BLOOD SPOTS TO IDENTIFY ZOONOTIC POTENTIAL

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Wildlife, non-human primates (NHPs) in particular, can serve as reservoirs for arboviruses such as Dengue Fever, West Nile Fever, and Rift Valley Fever Viruses, which can all be found in human populations in Madagascar. To better understand the zoonotic potential of Malagasy wildlife species to act as reservoirs of these viruses, methods for rapid, minimally invasive sample collection and preservation are necessary. With this study we aim to (1) validate the use of dried blood spots on TropBio cards as a method for detecting RNA viruses, (2) to determine the prevalence of these viruses in wild NHP species (lemurs of Madagascar). To validate the protocol, house mouse (*Mus musculus*) blood was spiked with non-infectious viral RNA or inactivated viral particles in a dilution series. The spiked blood and applied to TropBio cards, dried, and preserved at room temperature over several time-points. RNA was isolated from the cards and the target viral RNA was detected by qPCR. Lemur blood samples were collected from

intact forest and deforested regions of Madagascar. We are testing these samples for each of the viruses using qPCR and the prevalence will be presented.

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IVERMECTIN-TREATED BIRD FEED TO CONTROL WEST NILE VIRUS TRANSMISSION

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Arbovirus transmission control with insecticides sprayed into the environment are often reactive interventions and inadequate for localized control during outbreaks, can be poorly-targeted, and often face opposition due to environmental and toxicity concerns. In this study, we developed endectocide-treated feed as a systemic endectocide for wild birds to target blood feeding *Culex tarsalis,* the primary West Nile virus (WNV) vector in the Western United States, and we conducted preliminary tests on the effects of deploying this feed in the field. In lab tests, ivermectin (IVM) in a powder formulation was the most effective endectocide tested against *Cx. tarsalis*. Chickens and wild Eurasian collared doves fed solely on treated bird feed concentrations up to 200 mg IVM/kg feed exhibited no signs of toxicity, and most *Cx. tarsalis* that blood fed on these IVM-fed birds died compared to control birds. Mosquito survivorship following blood feeding correlated with IVM serum concentrations at the time of blood feeding, which dropped rapidly after the withdrawal of IVM feed. Two pilot field trials were conducted during the summers of 2016 and 2017. The 2016 season was designed with three field sites, each with a central IVM-treated bird feeder with mosquito traps at 10 m and 150 distances from the feeder. Results from 2016 showed that wild birds frequently visited the IVM-treated feeders and there was an observable trend where the 150 m traps that are expected to be beyond the zone of control had more WNV-positive *Cx. tarsalis* pools compared to 10 m traps. The 2017 trial was designed with three control and three IVM sites, where each site had an array of three bird feeders around a central mosquito trap. Results from 2017 demonstrated that 87% of birds captured around IVM bird feeders had detectable levels of IVM in their blood, and entomological data suggested the treatment may have reduced WNV transmission around treated bird feeders. With further development, deployment of ivermectin-treated bird feed might be an effective hyper-localized WNV transmission control tool.

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INTRAHOST GENETIC DIVERSITY OF WEST NILE VIRUS IN HORSE BRAIN AND PLASMA

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In 1999, a highly virulent West Nile virus (WNV) was introduced to North America resulting in more than 28,012 equine infections. WNV has been shown to be genetically diverse within the mosquitoes in nature. These genetic variations are essential in the adaptation of flaviviruses in a new and changing environment and host. A genetically diverse virus population would seem to have an advantage on adaptation because the virus has pre-existing variants which are better adapted to a new and changing environment. Other than in the mouse, there is limited information regarding viral diversity, tissue tropism, manifestation, and severity of this disease in mammalian tissues. Little is known regarding viral evolution within the natural vertebrate host. A wide variety of vertebrate hosts are infected with WNV, but only humans and horses develop significant neurological clinical disease and much can be derived by studying pathogenesis in the horse. The goal of this project was to sequence the WNV genes to investigate the hypothesis that different viral variants are present in plasma and brains of WNV infected horses. Five brain tissues and plasma of two horses were sequenced using Pacific Biosciences (PacBio) sequencing to investigate genetic variation in the NS4B gene of the WNV. The NS4B gene was chosen for this preliminary sequencing run because of the size, signal noise, and conserved sites. The mean read quality of the WNV NS4B gene sequences obtained from PacBio sequencing was 99.18% with 22 average passes. Brain had the highest number of total virus variants with most of the variants were in the cerebrum of the horses. DNA sequencing showed significant difference in sequence variation and reads in different days post infection. This preliminary finding indicated that WNV could adapt in different tissues and generated more variation overtime in the horses. More WNV gene sequences and phylodynamic analysis are on the way to further analyze the WNV diversity in horse brain and plasma.

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INFLUENCE OF EARLY INFLAMMATION, TRACE ELEMENTS, AND ANTIBIOTIC EXPOSURE ON IMMUNE RESPONSES TO ROTAVIRUS VACCINATION

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Rotavirus (RV) is the leading cause of diarrheal mortality in children globally. Oral RV vaccines are less effective in resource-limited settings, where the greatest RV disease burden occurs. Immunological responses to the RV vaccine may be influenced by systemic inflammation or malnutrition, such as trace element deficiencies. Antibiotic use, through perturbing the gut microbiome, is also hypothesized to impact RV vaccine responses. We tested these possible mechanisms in a RV vaccine immunogenicity study of 305 infant-mother pairs from El Alto, Bolivia. Infants received two doses of the monovalent RV vaccine (at ~2 and 4 months of age) and were followed from 2 weeks to 12-18 months of age. RV-specific IgA titers were measured in infant serum at 2 and 6 months, and seroconversion was defined as a four-fold or greater increase in geometric mean titers (GMT). Systemic inflammation (α-1-acid-glycoprotein [AGP], C-reactive protein [CRP]) and trace element concentrations (copper, iron, zinc, magnesium, and calcium) were measured in serum at 2 and 6 months by ELISA and inductively coupled plasma atomic emission spectrometry. Six-month serum copper was positively correlated with AGP ($\rho = 0.54$; $p < 0.01$) and CRP ($\rho = 0.48$; $p < 0.01$), but serum iron was negatively correlated with AGP ($\rho = -0.19$; $p < 0.01$) and CRP ($\rho = -0.26$; $p < 0.001$). The fold change in rotavirus IgA GMT between pre- and post-vaccination was negatively associated with 6-month serum CRP ($\rho = -0.16$; $p = 0.005$) and copper ($\rho = -0.14$; $p = 0.01$), but positively associated with 6-month serum iron ($\rho = 0.11$; $p < 0.05$). Antibiotic prescriptions prior to 6 months were extracted from clinical chart reviews for a subset of infants (135). Thirty-three (24%) received at least one course of antibiotics. Antibiotic prescription was not significantly associated with RV IgA seroconversion (OR = 1.1; 95% CI = 0.5 - 2.6; $p = 0.85$). We hypothesize that inflammation is driving the association between trace elements and RV vaccine immune responses. It will be important to test this hypothesis to assess the relative contributions of both inflammation and trace elements on impaired RV vaccine responses in global infant cohorts.

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MELITTIN AS REGULATOR OF APOPTOSIS LATE IN CHRONIC CELL LINE INFECTED WITH HTLV-1 ADULT T-CELL LEUKEMIA / LYMPHOMA

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The human T-cell leukemia virus type-1 has in Perú a prevalence of 5% to 10% people infected globally. Perú an endemic area of which only 2% -6% of infected develop Adult T Cell Leukemia / Lymphoma (ATLL), a malignant neoplasm that has been seen to have a high proliferation of infected cells. As HTLV-1 is an important risk factor for development of ATLL; we raised the question if Melittin (compound extracted from bee venom) plays a role in the proliferation of a chronic cell line infected with HTLV-1.Differents non-lytic concentrations of commercial Melittin [0.5 mM to 2.5mM] were used in cell culture exposing on the logarithmic phase both MT-2 cell line as K562 cell line (not infected with HTLV-1). The viability of these lines was observed for 48 hours and was measured with cell tracker Calcein AM Green, Anexin V and 7-AAD, to Trypan Blue Exclusion and cytometer. No increase in apoptosis late between MT-2 without treatment and at 2.5 mM melittin (0.1% to 9.5% 12% to 28% of cells in apoptosis late) with no statistically significant ($P < 0.05$) between different melittin concentrations; whereas with the uninfected cell line, a higher apoptosis increase is observed (12% to 28% of cells in apoptosis late). Our results showed that Melittin for apoptotic late K562 that control the cellular proliferation but the use of melittin in non-sublitic doses same with MT-2 no significance in apoptosis late.

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GENETIC DIVERSITY OF CIRCULATING NOROVIRUS, SAPOVIRUS AND ASTROVIRUS IN A PEDIATRIC HOSPITAL AT LIMA-PERÚ

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The most common etiological viral agents of gastroenteritis are Norovirus, Rotavirus, Sapovirus, and Adenovirus after the introduction of rotavirus vaccine. This study evaluated the genetic diversity and prevalence of Norovirus (NoV), Sapovirus (SaV), and Astrovirus (AstV). 1778 Fecal and swab samples were collected from children less than 5 years old presenting WHO's criteria for diarrhoea that attend the Hospital del Niño in Lima Peru between December of 2013 and April 2015. Control samples were collected from children attending the hospital for reasons other than diarrhoea. Samples were individually tested for NoV, Sav and AstV using RT-qPCR. Positive samples were amplified by conventional PCR and the amplification product was sequenced. Phylogenetic analysis was performed to establish genetic relationships with reference strains. Among diarrhoea cases, Norovirus was the most predominant virus (36%) followed by Sapovirus (8%) and Astrovirus (5%). We were able to identify: *(i)* among NoV positive samples, 4 and 13 genotypes from genogroups GI and GII respectively; GI.3 and GII.4 were the most predominant genotypes in each genogroup; *(ii)* among SaV positive samples the genotype GI.2 as the most predominant, *(iii)* among the AstV positive samples, the genogroups MastV1, MastV6, MastV8 were detect and MastV1 as the most predominant genogroup.Viral agents showed a high diversity among children with diarrhoea that seeks medical attention. Surveillance needs to be implemented to identify genotype changes among those viral agents, especially for vaccine development.

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USING COMPLEX DATA AND DEEP LEARNING TO PREDICT RIFT VALLEY FEVER OUTBREAKS

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Disease outbreaks are difficult to predict because they rely on the alignment of multiple complex factors and circumstances, many of which have not yet been identified or are not well understood. Rift Valley fever virus (RVFV) is a vector-borne zoonotic phlebovirus that primarily infects livestock and humans via transmission by mosquito or direct contact with contaminated bodily fluids. RVFV is a Category A biodefense pathogen because it can cause life-threatening hemorrhagic fever, meningoencephalitis and organ failure in humans, and has the potential to result in catastrophic economic harm to livestock. Previous models of RVFV outbreaks in Kenya have often focused on a single data source, such as climate, or estimated the contributions of a specific metric, such as rainfall, genetic diversity, or severity of El Niño effects. Such models have identified key factors affecting outbreaks but have not delivered effective outbreak prediction. In this study, we utilized a deep learning platform trained on the history of outbreaks in Kenya. A variety of data on socio-economic and behavioral traits in multiple communities from various regions of Kenya was part of the training set. Traditional machine learning approaches, such as clustering, decision trees, and naïve Bayes were used to analyze these epidemiological data on RVFV outbreaks, but failed to provide sufficient predictive power. Deep Learning (DL) using recurrent neural networks allowed us to incorporate climate data spanning 100 years and data from the 2009 census. The resulting DL model predicted about ~80% of Kenyan outbreaks to date. The epidemiology of RVF, massive periodic outbreaks and continued spread to new areas, suggest that increased efforts and expanded data sources are needed to predict, prevent, contain, and control this economic, veterinary, and public health threat.

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NON-SECRETOR HISTO-BLOOD GROUP ANTIGEN PHENOTYPE DOES NOT IMPAIR SUSCEPTIBILITY TO INFECTION WITH ROTARIX VACCINE AMONG INFANTS IN BANGLADESH

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Rotavirus (RV) is the leading cause of infectious diarrhea among infants worldwide. This occurs despite availability of oral, live-attenuated vaccines, which underperform in low-income settings. Children with non-secretor histo-blood group antigen phenotype (HBGA) have reduced susceptibility to RV diarrhea (RVD), raising concerns that non-secretors may also be less susceptible to oral, live-attenuated RV vaccines. However, direct evidence of susceptibility (or lack thereof) to vaccine strains among non-secretors is lacking. It also remains unknown if HBGA-mediated differences in RV susceptibility is limited to RVD or extends to asymptomatic infection, as seen in oral vaccination. Therefore, we investigated associations between secretor status, asymptomatic RV infection, and post-vaccination fecal vaccine shedding in PROVIDE, a Rotarix vaccine trial conducted among infants in Bangladesh. Secretor phenotyping was performed on saliva. Eleven surveillance stool specimens were collected over the first year of life, and a subset of vaccinees had additional specimens collected within

one week following both Rotarix doses. Stools were tested for RV by real-time RT-PCR; positive specimens underwent Sanger sequencing for strain confirmation. To assess HBGA in asymptomatic RV infection, we evaluated unvaccinated infants who were RVD-free through one year. Among 112 such infants, significantly fewer non-secretors experienced asymptomatic infection (12%) compared to secretors (42%; P<0.0001). We then evaluated for Rotarix shedding. Among 182 vaccinated infants, 115 (63.2%) shed Rotarix following either dose. No significant differences were seen in the proportion of non-secretors who shed Rotarix (65%) compared to secretors (60%). Non-secretors had decreased risk for natural RV infection but are successfully infected with vaccine during Rotarix challenge. These results provide the first direct evidence that susceptibility to RV vaccine is unaffected by secretor status, and that HBGA-mediated resistance to oral RV vaccines is thus an unlikely mechanism of vaccine underperformance.

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EVALUATION OF ZIKA VIRUS ISOLATION PROCEDURES USING MOSQUITO INOCULATION COMPARED TO *IN VITRO* C6/36 CELL CULTURE

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Zika virus is a single stranded RNA virus and a member of the family *flaviviridae*. It is transmitted to humans by mosquito bite, sexual transmission or during pregnancy to the fetus. A large outbreak of zika disease emerged in Yap State, Polynesia in 2007 and again in French Polynesia in 2013. The latest outbreak in Brazil in 2015 produced severe syndrome of microcephaly in infants. Real-time Reverse transcription Polymerase Chain Reaction (rRT-PCR) is the primary method used to detect zika virus in samples. Being able to isolate virus is also very useful and allows analysis of virus evolution, epidemiology, molecular markers of virulence or attenuation and other factors that may be implicated in disease pathogenesis and/or protection from the disease. In this study, two procedures of viral isolation are described using various types of clinical specimens, e.g., sera, plasma, whole blood, urine. Specimens that were rRT-PCR positive (Ct value ≤38) were used for virus isolation using *Toxorhynchitis splendens* mosquito (*Tox*) and tissue culture (C6/36, *Aedes albopictus*) inoculation. The specimens originated from various sites in Thailand and the Philippines. All specimens were collected during year 2014-2018. From zika virus PCR positive serum samples, 9/13 (69%) were successfully isolated using *Tox* while only 1/13 (7.6%) was able to be cultured in C6/36-3 blind passages. We were unable to isolate zika virus from any of the PCR positive plasma samples using *Tox* or C6/36-3 (0/4 tested). From whole blood, 2/15 (13%) were isolated by *Tox* but none were successfully isolated using C6/36 cells. Only 1/25 (4%) urine PCR positive samples were successfully isolated in C6/36-3 but none by using mosquito inoculation. These data indicated that *Tox* mosquito inoculation provides a high success rate for zika virus isolation over C6/36 cells when using serum or whole blood samples.

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PREVALENCE OF DENGUE VIRUS, CHIKUNGUNYA VIRUS, AND ZIKA VIRUS DETERMINED THROUGH ENHANCED SURVEILLANCE OF EMERGING INFECTIOUS DISEASES IN MALAYSIA

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Peninsular Malaysia has been the site of an increase in the ability to rapidly detect disease outbreaks and accurately diagnose infectious agents, a capacity that is paramount to any public health surveillance program. The viral pathogens for dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV), cause diseases of particular medical importance in that they have been detected in Malaysia previously adding to other notable historic outbreaks in Reunion Island and Latin America. Besides these viruses, there are other unique vector-borne pathogens found in Malaysia. Proper evaluation of these pathogens enables tracking of emerging infections at their potential origin sites. Implementation of syndromic surveillance protocols has enabled the surveillance and laboratory-based diagnostic testing for uncommon diseases in patients presenting to select healthcare facilities in peninsular Malaysia, such as members of the indigenous Orang Asli community and rural farm/agricultural workers living and working at the animal-human interface in the forest fringe areas. Surveillance and laboratory-based diagnostic testing for asymptomatic members of the community were performed to screen patients using serological assays and molecular detection, respectively. Patients were recruited from settlements and nearby public health clinics. By November 2017, 730 blood samples had been collected from patients that were either febrile (144) or asymptomatic (586). DENV and CHIKV were found in 10 (6.9%) and 6 (4.2%) of the samples, respectively, that were collected from febrile patients. DENV (41.6%), CHIKV (32.9%) and ZIKV (20.9%) were also found to have the highest seroprevalence among all the pathogens screened. In addition to DENV, CHIKV, and ZIKV fevers are likely to be endemic in communities living in the forest fringe areas of Malaysia but presently under-detected. These latter infections are probably not recognized due to the high prevalence of DENV, which may mask identification of the infections. Further studies with higher sample sizes from other localities would aid in obtaining a better estimate of the extent of these infections.

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INFLUENZA A AND B VIRUS EPIDEMICS IN BHUTAN, CAMBODIA, NEPAL, PHILIPPINES AND THAILAND ARE CHARACTERIZED BY REPEATED INTRODUCTIONS AND LIMITED PERSISTENCE OF CIRCULATING STRAINS

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Asia plays a major role in the global transmission of influenza A and B viruses, yet the epidemiology of influenza in tropical and subtropical Asia is not entirely clear. We therefore evaluated the temporal epidemic dynamics of influenza viruses in five Asian countries over an entire decade. Human respiratory specimens derived from year-round influenza-like illness surveillance activity in Bhutan, Cambodia, Nepal, Thailand and the Philippines during 2007- 2016 underwent complete HA segment sequencing for all subtypes. These data were collated with public HA sequences available on the Influenza Research Database (www.fludb.org) to yield curated datasets for Bhutan (n=252), Cambodia (n=73), Nepal (n=373), Philippines (n=393) and Thailand (n=1,449). The datasets were further aligned with curated public HA sequence data from all other countries to yield global alignments for sH1N1 (n = 1,110), pdm2009H1N1 (n = 9,694), H3N2 (n=7,655), B Yamagata [B Yam] (n=2,932) and B Victoria [B Vic] (n = 2,191). Maximum-likelihood phylogenetic trees were constructed for each subtype using RAxML or PhyML, and used to estimate subtype strain persistence for the five countries. Across all subtypes, there were frequent new strain introductions which had short median persistence times in Bhutan (sH1N1 = 18 days, pdm2009H1N1 = 14.5 days, H3N2 = 30 days, B Yam = 20.5 days, B Vic = 37 days), Cambodia (sH1N1 = 53 days, pdm2009H1N1 = 20 days, H3N2 = 3 days, B Yam = not detected (n=0), B Vic = 170 days), Thailand (sH1N1 = 34.5 days, pdm2009H1N1 = 1 day, H3N2 = 2 days, B Yam = 54 days, B Vic = 70 days), Nepal (sH1N1 = 63 days, pdm2009H1N1 = 27 days, H3N2 = 23.5 days, B Yam = 19 days, B Vic = 65 days) and the Philippines (sH1N1 = 78 days, pdm2009H1N1 = 32.5 days, H3N2 = 11 days, B Yam = 50 days, B Vic = 124.5 days). Influenza A and B virus epidemics in Bhutan, Cambodia, Nepal, Philippines and Thailand are characterized by repeated introductions and limited persistence of circulating strains, similar to influenza viruses in temperate regions. The longer persistence of influenza B may represent differences in epidemic dynamics between the influenza A and influenza B subtypes and warrants further study.

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DISCOVERY OF NOVEL VIRUSES IN MOSQUITOES FROM THE UNITED STATES AND MEXICO

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Metagenomics was used to identify novel and previously discovered viruses in mosquitoes from the United States and Mexico. To facilitate the identification of viruses in the U.S., 1432 mosquitoes (8 species) from Iowa were sorted into 54 pools, homogenized, filtered and inoculated onto monolayers of *Aedes albopictus* (C6/36) cells. Another 37 pools, comprised of 1359 mosquitoes (7 species) from the Yucatan Peninsula of Mexico were also processed as described above. A second blind passage was performed then total RNA was extracted from all cultures showing cytopathic effect as well as select cultures that did not and analyzed by Ion Torrent sequencing. Three novel and two previously recognized viruses were detected in the mosquitoes from Mexico. The first novel virus is a Negevirus recovered from several pools of *Aedes taeniorhynchus*. The second is a member of the *Tymoviridae* family that was isolated from *Culex quinquefasciatus*. The final novel virus is most closely related to an unclassified RNA virus known as Hubei noda-like virus 5 and was isolated from *Psorophora ferox*. The two recognized viruses identified in the Mexico collections are Culex flavivirus (family *Flaviviridae*) and Houston virus (family *Mesoniviridae*), isolated from *Culex quinquefasciatus* and *Ae. taeniorhynchus*, respectively. Culex flavivirus and Houston virus were also isolated from mosquitoes originating from Iowa. The Iowa collections also yielded two novel orbiviruses and two recognized orbiviruses. Both of the novel orbiviruses were isolated from *Aedes trivittatus*. The two recognized orbiviruses, Umatilla virus and Koyoma Hill virus, were isolated from *Ae. trivittatus* and *Aedes triseriatus*, respectively. We are currently determining the *in vitro* host ranges of these viruses by assessing their ability to replicate in 11 cell lines of human, monkey, hamster, duck, mouse, moth and mosquito (*Aedes*, *Anopheles* and *Culex*) origin.

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EFFECT OF MASS ARTESUNATE-AMODIOQUINE DISTRIBUTION ON EBOLA-RELATED MORTALITY IN SIERRA LEONE

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There are no known treatments for Ebola Virus Disease (EVD). Although the mechanism is unknown, *in vitro* experiments have shown that the drug amodiaquine may inhibit Ebola virus activity. During the Ebola epidemic in Sierra Leone in 2014/15, the government implemented two mass drug administrations (MDA) of artesunate-amodiaquine (ASAQ), a 3-day oral malaria treatment regimen, to decrease the burden of malaria. Each MDA was implemented over a four-day period (Dec 5-8, 2014 and Jan 16-19, 2015) to chiefdoms with previously confirmed EVD cases. Mortality data for EVD+ patients admitted to three Ebola Treatment Units (ETUs) in Sierra Leone were analyzed retrospectively to evaluate the potential effect of ASAQ administration on mortality. All patients admitted to the ETUs during the days of the MDA until 10 days after (based on amodiaquine's half-life) from chiefdoms where the MDA was implemented were presumed to have received amodiaquine. The primary outcome was ETU mortality comparing EVD cases treated with ASAQ versus those not treated. Kaplan-Meier survival analyses were performed with significant differences assessed using log-rank tests. Cox proportional hazards models were used adjusting for patient age and malaria co-infection status to yield adjusted hazard ratios (aHR). In the cohort 1524 patients were treated of whom 254 had EVD. Overall mortality for EVD patients was 55.1%. EVD patients from MDA chiefdoms admitted to the ETUs during the presumed therapeutic effect window of amodiaquine had a 25% decreased risk of death compared to all other EVD+ patients, although this was not statistically significant (aHR 0.75; 95%CI 0.40-1.43). There was no evidence of reduction in mortality risk in EVD patients from MDA chiefdoms admitted during the presumed therapeutic window of amodiaquine as compared to EVD patients from non-MDA chiefdoms admitted during the same time period (aHR 1.05; 95%CI 0.29 - 3.72). Further investigation in patient cohorts known to have received amodiaquine at an individual level is needed to determine whether amodiaquine may directly modify mortality risk in EVD+ patients and the mechanism by which this may occur.

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EARLY INTRAVENOUS FLUID THERAPY AND MORTALITY OUTCOMES AMONG PATIENTS WITH EBOLA VIRUS DISEASE: A MULTISITE RETROSPECTIVE PROPENSITY-MATCHED COHORT STUDY

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This retrospective cohort study investigated the relationship between early intravenous fluid (IVF) therapy (initiated within 48 hours of care) and mortality among patients with Ebola Virus Disease (EVD) admitted to five International Medical Corps run Ebola Treatment Units (ETU) in two countries during the 2014/15 epidemic. Standardized treatments with antimicrobials and nutritional supplementation were provided to all patients, however IVF was administered based on clinical indication and resource availability. A propensity score incorporating factors associated with IVF exposure was derived and cases were matched using a caliper of <0.2. Post-matching absolute standardized bias was <0.25 for all parameters. The overall cohort was analyzed, and additionally, stratified analyses were performed based on cycle threshold (CT) values as low (>22) or high (<22), corresponding to high and low Ebola viral loads respectively. The outcome of interest was proportional mortality during care compared between cases exposed to early IVF and those not. Risk differences (RD) with 95% confidence intervals (CI) were calculated in comparative analyses. There were 424 cases analyzed, of which 257 (38.9%) received early IVF. In the propensity-matched analysis of the overall cohort mortality prevalence was 53.2% for non-IVF cases and 58.9% for IVF treated cases (RD=5.7%, 95% CI: -3.6%, 14.9%; p=0.23). In matched stratified analysis of cases with low CT values mortality prevalence was 71.1% for non-IVF cases and 70.2% for IVF treated cases (RD=0.9%, 95% CI: -10.8%, 12.6%; p=0.88). Among cases with high CT values mortality prevalence was 31.9% for non-IVF cases and 41.8% for IVF treated cases (RD=9.9%, 95% CI: -4.1%, 23.3%; p=0.17). Although mortality prevalence was greater with early IVF treatment in the overall cohort, and among cases stratified by viral loads, there were no significant differences in outcomes, suggesting minimal utility for a therapy requiring relatively high-resource allocation and greater provider risk in epidemic settings.

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DIFFERENCES IN THE PEDIATRIC ENTERIC VIROME ACROSS WATERY, LOOSE AND FORMED STOOL CONSISTENCY TYPES

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Stool consistency is an important diagnostic criterion in both research and clinical medicine. Most epidemiologic studies use stool consistency, specifically 'loose or watery stool', to define diarrhea disease. We examine the pediatric enteric virome across characterized stool sample consistency to evaluate differences in viral abundance community composition. We collected stool samples from children participating in a clinical trial evaluating a water improvement intervention in the Amhara region of Ethiopia. The consistency of each fecal sample was graded according to the modified Bristol Stool Form Scale for children (mBSFS-C) before a portion of stool was preserved for viral metagenomic analysis. The number of viral reads along with the taxonomic assignments and sample characteristics were assembled using the *phyloseq* package in R. Differential abundance was determined at the genotype level using negative-binomial modeling in the *DESeq2* package in R. Of 446 censured children who were eligible to participate, 317 children presented for the study visit examination and 269 provided stool samples. The mean age of children with stool samples was 2.7 years old. Stool samples were grouped into 29 pools according to stool consistency type. Species richness was highest in watery-consistency stool and decreased consistently as stool consistency became firmer (Spearman's r=–0.45, p=0.013). The median number of distinct viruses (richness) was 6.5 in Type 5 (watery) stool, 5.5 in Type 4, 4.0 in Type 3, 3.0 in Type 2 and 2.0 in Type 1 (hard pellets). The greatest $\log_2$ fold change in differential abundance comparing loose or watery to formed stool was for Norovirus GII (7.64) followed by Aichivirus A (5.92), Adeno-associated dependoparvovirus A (5.8), Enterovirus A (4.85), Human mastadenovirus C (3.84), Cosavirus A (3.20), and Anellovirus (3.09). In conclusion, we documented a difference in pediatric enteric viromes according to mBSFS-C stool consistency category, both in species richness and composition. The findings may support the content validity of using stool consistency as a proxy for viral infection.

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EPIDEMIOLOGY OF G12 ROTAVIRUS AMONG INDIAN CHILDREN IN A MULTI-CENTRIC HOSPITAL-BASED SURVEILLANCE FROM 2005 TO 2016

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G12 rotavirus strains were first isolated in India in 2001, and have emerged as a predominant strain causing diarrhea in the under-five age group. The Christian Medical College(CMC), Vellore, Child Jesus Hospital, Trichy from south India, and St. Stephen's hospital, Delhi from north India took part in hospital-based surveillance from December 2005 to July 2016 to evaluate rotavirus associated diarrhea in children <5 years of age. Stool samples were collected from children within 48 hours of admission after obtaining informed consent from parents/guardian. 6907 children were recruited across three sites of which, stool samples was not collected for 5 children, and for 10 children sample was inadequate for testing. All the samples were tested at CMC, Vellore. 6892 samples were tested and 2759(40%) were positive for rotavirus antigen by Enzyme Immunoassay (EIA). Rotavirus genotypes (VP7 and VP4) were determined by hemi-nested multiplex PCR for 2732 EIA positive samples. G12 strains were detected in 10.5% (286/2732) of the genotyped samples. The proportion of G12 was 18.7% (179/955) in north India and 6% (107/1777) in south India. Overall, G12P[6] (137/286, 49.7%) and G12 P[8] (82/286, 28.6%) were the common circulating genotypes. In northern India, G12P[6] (60%, 107/179) was the most common genotype, followed by G12P[8] (15%). In contrast, G12P[8] (50%, 54/107) was the most common genotype in south India, followed by G12 P[6] (28%). G12P[11] (1 isolate each from the north and south India), and G12P[4] (2 strains from north India) were the other uncommon genotypes isolated. In conclusion, G12 rotavirus infection was more common in north India and there was a difference in circulating G12 strains between northern and southern parts of India.

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VECTOR-HOST ASSOCIATIONS AND REASSORTMENT POTENTIAL OF TICK-BORNE ORBIVIRUSES

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Orbiviruses are segmented dsRNA viruses with a wide range of arthropod vectors and vertebrate hosts, including humans. Owing to the segmented nature of orbiviruses, species-level classification is often obfuscated. The International Committee on the Taxonomy of Viruses outlines a polythetic definition of orbivirus species, including but not limited to the reassortment of genome segments, conserved terminal nucleotide sequences, serological cross-reactions, comparison of homologous genome segments or proteins by sequence analysis, and host and vector ranges. With the advent and ubiquity of whole-genome sequencing, recent reports often rely solely on phylogenetic analysis of the conserved viral polymerase gene and other more variable segments. Bats have been identified as natural hosts for many high-profile zoonotic viruses. Four species of orbiviruses have been isolated from free-ranging bat species, two of which fall into the tick-borne orbivirus clade (Bukakata and Fomede). The viral polymerase genes of these viruses are genetically similar to one another and also to Chobar Gorge, an orbivirus isolated from a pool of ticks in Nepal. We have determined strong serologic cross-reactivity between polyclonal sera obtained from mice hyperimmunized with Chobar Gorge virus and both Bukakata and Fomede viruses. However, few studies have integrated whole genome sequencing and serology with *in vitro* segmental genetic reassortment potential. Ongoing work to further

define the species-level classification of this group of tick-borne orbiviruses includes pairwise co-infection of Bukakata, Fomede, and Chobar Gorge followed by serial passage on IDE8 cells (derived from *Ixodes scapularis*), Vero cells, and R06E cells (derived from *Rousettus aegyptiacus* bats). Plaque purification of isolates following serial passage will be analyzed for the occurrence of segmental reassortment utilizing RT-PCR and Sanger sequencing. Analysis of reassortment potential in cell lines derived from both the purported host and arthropod vector species will provide unique and valuable information to the study of orbivirus speciation.

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SENSITIVITY OF A RAPID DIAGNOSTIC TEST FOR INFLUENZA

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Influenza causes considerable morbidity and has significant operational impact in the military. Rapid influenza diagnostic tests (RDTs) which detect viral antigens of influenza are used as tools to screen patients with suspected influenza and are used to reduce inappropriate antibiotic use, decrease unnecessary diagnostic testing and to facilitate timely control measures prior to results of confirmatory tests during outbreaks or during field deployments where access to confirmatory tests is unavailable. Between Aug 2011 to Oct 2016, patients presenting with influenza-like illness (ILI) (n=2,333) at the V Luna Medical Center, Quezon City, Philippines were tested using Quickvue (QV) influenza A+B RDT and influenza real-time RT-PCR. Statistical tests of association were done to determine effect of age, viral titers (Ct values), and timing of collection on QV sensitivity/specificity to detect different influenza sub-types (Flu A/H3, Flu A/pdmH1, Flu A/H3+Flu A/pdmH1and Flu B). P value <0.05 was considered statistically significant. QV sensitivity, specificity, positive and negative predictive values can be seen in Table 1. Linear regression showed that QV sensitivity across all sub-types was significantly correlated with lower Ct values (higher virus titers) ($p<0.001$) and except for Flu A/H3 ($p=0.974$), was also significantly associated with timing of specimen collection ($p<0.05$). There were no statistically significant difference noted in QV sensitivity for Flu A/H3 ($p=0.130$), pandemic H1/N1 ($p=0.207$), Flu A/H3+pandemic H1/N1 ($p=0.341$), and Flu B ($p=0.103$) across different age groups but sensitivity of QV significantly differed ($p<0.001$) across the different influenza sub-types. The findings highlight the need to develop more sensitive influenza RDTs to detect circulating influenza strains.

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SPATIAL AND TEMPORAL SPREAD OF ZIKA AND CHIKUNGUNYA VIRUSES IN COLOMBIA, A GRAVITY-MODEL BASED APPROACH

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Zika virus (ZIKV) and chikungunya virus (CHIKV) were recently introduced into the Americas resulting in significant disease burdens. Understanding the spatial and temporal dynamics of the recent outbreaks in Colombia is key to informing control strategies at the subnational level. We have analyzed anonymized line lists data on 100,000 ZIKV and 380,000 CHIKV cases reported to SIVIGILA, Colombia's national public health surveillance system, between 2014 and 2017. These data include both suspected and laboratory confirmed cases. We fitted gravity models to analyze transmission between cities based on population size and distance, utilizing the approach of Eggo et al. in their study of the 1918 influenza pandemic. We were able to track the spread of reported cases between more than 300 locations (administrative level 2) on a weekly basis. We assessed the extent to which transmission depended on population density and then examined the effects of altitude, temperature, and annual precipitation on ZIKV and CHIKV spread. These environmental factors affect mosquito development and could help explain observed disease trends. Modeling at such fine spatial scale will inform both surveillance and control strategies.

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ASSESSMENT OF POLIOVIRUS ANTIBODY SEROPREVALENCE IN HIGH RISK AREAS FOR VACCINE-DERIVED POLIOVIRUS TRANSMISSION IN MADAGASCAR

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Vaccine-derived polioviruses (VDPV) outbreaks typically occur in areas of low poliovirus immunity. Madagascar successfully eradicated wild poliovirus in 1997. However, multiple VDPV outbreaks have occurred since then, and numerous vaccination campaigns have been carried out to control the VDPV outbreaks. We conducted a survey of poliovirus neutralizing antibodies among Malagasy children to assess the performance of vaccination campaigns and to estimate the risk of future VDPV outbreaks. A random community survey was undertaken in children aged 6-11 months, 36-59 months and 5-14 years of age in four high-risk areas of Madagascar (Mahajanga, Toliara, Antsalova, and Midongy-atsimo) and in a reference area (Antananarivo). After obtaining informed consent, basic demographic and vaccination history, 2 mL of peripheral blood were collected. Neutralizing antibodies against all three poliovirus serotypes 1, 2, 3 (PV1, PV2, PV3 respectively) were detected by using a standard microneutralization assay. There were 1500 children enrolled and 1496 (>99%) provided sufficient quantity of blood for analysis. Seroprevalence for PV1 was >90% in all age groups and the study areas. PV2 seroprevalence ranged between 75-100% with the lowest found in the youngest age group in Midongy and Toliara. PV3 seroprevalence ranged between 79-100%. The seroprevalences in the reference area was not significantly different from that of high risk areas. Madagascar achieved high population immunity. In order to sustain the protection, routine immunization needs to be strengthened. Currently, the risk of new VDPV emergences in Madagascar appears low.

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CLINICAL DEVELOPMENT OF VIRAL VECTORED VACCINES AGAINST MERS-COV

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Viral vectors have been used as delivery platforms for vaccines against several pathogens including malaria, HIV, TB, influenza, Ebola and hepatitis and have demonstrated acceptable reactogenicity in all age-groups including neonates, as well as excellent immunogenicity. Of seven vaccine candidates in clinical development against ebolavirus disease (EVD) during the recent West African outbreak, six were based on viral vectored technology with rVSV-ZEBOV demonstrating high, short-term efficacy against disease in a Phase III ring vaccination study in Guinea. Chimpanzee adenoviruses (ChAd) are attractive as viral vectors due to ease of genetic manipulation, compatibility with high-yielding GMP production processes, a safety profile suitable for administration to infants as young as 1-week of age and induction of potent cell-mediated immunity and neutralising

antibody responses. Building on these features, we are undertaking clinical development of a number of vaccines against important outbreak pathogens identified by the WHO Blueprint for R&D preparedness. Using ChAd vectors, the Jenner Institute is currently evaluating vaccine candidates against CCHF, EVD and Marburg, Lassa Fever and Nipah in pre-clinical models. Vaccines against MERS-CoV, Rift Valley Fever, Chikungunya and Zika will enter Phase I clinical trials during 2018 and early 2019. MERS-CoV is a zoonotic virus that causes respiratory infection and severe disease in humans with a fatality rate as high as 35%. Camels are the animal reservoir for the virus and hence a vaccine with utility in both dromedaries and humans would be of significant public health benefit. We will present safety and immunogenicity data from this Phase I, dose-escalation, first-in-man trial of ChAdOx1 MERS describing humoral and cellular immune responses from the study, which started in March 2018. A second Phase I trial will begin in Saudi Arabia in late 2018. This reinforces the utility of ChAd vectors as vaccines for outbreak pathogens because of the capability to rapidly produce GMP vaccine stockpiles for pandemic preparedness.

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OXIDATIVE STRESS IN MALARIA ENDOTHELIAL PATHOLOGY

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Malaria remains a global burden worldwide, causing approximately 400,000 deaths annually. Blood stage malaria is characterized by a strong inflammatory response and also considered to be highly oxidative. Recent work in our laboratory indicates that oxidative stress could be an important driver of inflammation in malaria. In particular, we have found the host enzyme xanthine oxidase (XO) in combination with *Plasmodium falciparum*-infected erythrocytes induces a strong inflammatory response in human monocyte-derived macrophages. XO is an enzyme involved in purine catabolism, but is most noted for its ability to produce reactive oxygen species (ROS). XO has been shown to be elevated in African children with malaria and we have also found elevated activity of XO in adult Indian patients with uncomplicated or cerebral malaria. To further investigate the role of oxidative stress and disease pathology, precisely the role of XO in cerebral malaria, we studied the effects of XO alone on human brain microvascular endothelial cells (HBMECs). When we incubated HBMECs with XO, we observed a disruption in endothelial cell junctions. We will study the role of oxidative stress in cerebral malaria further by determining the effects of XO in combination with *P. falciparum*-infected erythrocytes on HBMECs and the signaling involved. Using mice models of malaria, we will also determine whether XO binds the endothelium during malaria infection, as described when this enzyme is in the circulation. Through these investigations we hope to obtain a better understanding of the interplay between oxidative stress and malaria endothelial pathology.

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ASSESSMENT OF ARTEMISININ COMBINATION REPEATED TREATMENT EFFECT ON BLOOD CELL LINES PARAMETERS DURING A TWO-YEAR FOLLOW UP INVOLVING 3ACT, ARTESUNATE-PYRONARIDINE (PYR), DIHYDROARTEMININ-PIPERAQUINE (DHA-PQ) COMPARED TO ARTESUNATE-AMODIAQUINE (ASAQ) IN THE TREATMENT OF THE UNCOMPLICATED ACUTE *PLASMODIUM FALCIPARUM* MALARIA CASES IN BANFORA HEALTH DISTRICT

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Artemisinin combination therapies (ACTs) still remains in Sub Saharan Africa, the most effective anti-malarial drugs for the malaria treatment in areas with multidrug resistant *Plasmodium falciparum* malaria. The use of these drugs to treat malaria may be associated with possible side effects on the blood cells. It was therefore the aim of this study to undertake a comparative effect of a repeatedly administration of artesunate-amodiaquine, dihydroartemisinine-piperaquine, and artesunate-pyronaridine on haematological parameters (RBC count, Hb, WBC count, Platelets count) in *P. falciparum* uncomplicated malaria patients living in Burkina Faso. Confirmed malaria infected patients (n=763) aged from 6months to 54 years of both sexes were included in the study carried out in Banfora Health District area in Burkina Faso. About 2ml of blood sample was collected into EDTA tube from the same patient before the ACT administration and after at day 3, 7 and 28 of the follow up for blood cell lines analysis at each malaria episode. The findings of this present study show that Artesunate-Amodiaquine, Dihydroartemisinine-Piperaquine and Pyronaridine-Artesunate have effects on the hematological parameters in *P. falciparum* malaria infected patients. The three ACTs significantly reduced the proportion of abnormal blood levels of white blood cells, neutrophils, eosinophils and lymphocytes without significant effects on the proportion on the abnormal level of basophils cell. The proportion of abnormal blood level of RBC, hb and platelets were significantly increased at the day 3 after treatment before decreasing at day 28. However a repeatedly treatment of these three ACTs had no significantly effect on the blood cell level.

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DEVELOPMENT OF RELIABLE MOSQUITO INFECTIONS WITH EAST AFRICAN *PLASMODIUM FALCIPARUM* STRAINS FOR HETEROLOGOUS CONTROLLED HUMAN MALARIA INFECTION

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Since 1985, the Walter Reed Army Institute of Research (WRAIR) has produced *P. falciparum*-infected mosquitoes for use in clinical trials to evaluate anti-malarial vaccines, drugs, and immunity. The vast majority of those &gt 110 experimental malaria challenges used Pf3D7 and its parent strain, PfNF54, but the recent progression of candidate anti-malarial products necessitates development of *P. falciparum* strains that are heterologous to Pf3D7. The standard methodology used to grow PfNF54 is insufficient to produce sporozoite infections with most other *P. falciparum* strains, therefore new methodology must be formulated to support heterologous strain production. In a multi-laboratory effort, the capabilities and resources of WRAIR and USAMRD-Kenya combine to form a *P. falciparum* development pipeline which has led to the advancement

of four new *P. falciparum* lines for use in Controlled Human Malaria Infections (CHMI) with over 30 additional lines still in various stages of progress. These lines were derived from parasitemic patient collections at hospitals or centers within the USAMRD-Kenya Surveillance Network and initially adapted to culture at USAMRD-K. Once received at WRAIR, strains were prioritized based on geographic diversity and CSP, MSP-1, and AMA1 sequence diversity, then individually optimized in two critical determinants of Pf mosquito infection: culture process and vector-parasite interactions. Strains initially propagated under PfNF54-like culture conditions were subjected to a suite of culturing protocol adjustments with assessment of gametocyte yield and exflagellation to measure progress. Strains with robust gametocyte yields were subjected to additional adjustments to the mosquito membrane feeding protocol and resulting infected mosquitoes were evaluated for oocyst and sporozoite burden. Using this workflow, we report 8 strains now produce sporozoites in anopheline mosquitoes; several produce infection levels similar to PfNF54. Over 25 additional Kenyan lines remain to be evaluated and this process is being extended to field isolated parasites collected by other DoD labs throughout the world.

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EVALUATING LIFE CYCLE PROGRESSION OF *PLASMODIUM FALCIPARUM* USING IMAGING FLOW CYTOMETRY

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Conventional flow cytometry (FC) has been used for evaluating a multitude of assays involving *Plasmodium falciparum* (Pf) red blood cell (RBC) infections. While FC allows characterization of Pf infected cells using fluorescent labeling (e.g., labeled antibodies for Pf-specific proteins), alone, this approach requires cell sorting to integrate image analysis for further assessment of parasite morphology and development. Imaging flow cytometry combines the power of fluorescence-labeling with visual inspection of individual cells. Using this technique, false-positive rate of a fluorescent signal can be minimized and higher resolution of probe localization achieved. Here, we studied erythrocyte invasion and development of the Pf transgenic strain, 3D7, expressing green fluorescent protein (GFP). Pf can be exposed to different perturbations (e.g., Cytochalasin D [CytoD], Heparin, and Phenylmethylsulfonyl fluoride [PMSF]). The underlying RBC invasion mechanism involves an actin-myosin motor termed "glideosome". CytoD inhibits actin polymerization and interferes with the glidosome, while PMSF, a serine protease inhibitor prevents the cleavage of the merozoite coat proteins through subtilisin-2 and thereby prevents a successful invasion. We hypothesized that CytoD and PMSF would strongly inhibit Pf RBC invasion based on our previous experiments. In addition, we hypothesized that Heparin would inhibit as well but to a lesser degree and the mode of action is unknown. By simply analyzing Hoechst positivity as a measure for infected cells, a surprising result was obtained with CytoD treatment. CytoD samples resulted in a significantly higher amount of parasites localized to the exterior of the RBC compared to the untreated sample, suggesting that parasites are trapped during their invasion step. These cells are marked as Hoechst positive although the parasite has not invaded the RBC and would not be able to maintain infectivity. The PMSF treated sample showed similar results, however with reduced parasite numbers (~85%). The remaining parasites seem to be trapped in late stages prevented from egressing the already infected RBC.

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PLASMODIUM VIVAX CIRCUMSPOROZOITE PROTEIN (CSP) IS UNABLE TO FUNCTIONALLY COMPLEMENT *P. FALCIPARUM* CSP

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Rodent malaria parasites in which the endogenous gene encoding circumsporozoite protein (CSP) has been replaced with csp genes of human malaria parasites, *Plasmodium falciparum* (Pf) or *P. vivax* (Pv), are currently being used as pre-clinical evaluation tools to assess CSP vaccines *in vivo*. These chimeric rodent parasites produce fully infectious sporozoites in Anopheles stephensi mosquitoes that are capable of infecting rodent and human hepatocytes. Here we describe the creation, using CRISPR/Cas9 gene editing methodologies, and evaluation of two chimeric Pf parasites where the Pf csp gene has been replaced by two major Pv csp alleles, either the VK210 or VK247. The availability of fully infectious Pf-Pv CSP chimeric lines would open up possibilities to perform Controlled Human Infections to examine the potency of different Pv CSP vaccines in small scale clinical trials, and overcome several limitations that currently prevent the rapid evaluation Pv CSP vaccines in humans. The two chimeric Pf-Pv CSP lines have normal asexual and sexual blood stage development *in vitro* and A. stephensi mosquitoes fed with Pf-Pv CSP gametocytes produce sporozoite-containing oocysts. However, most oocysts degenerate before sporozoites are released from oocysts. In contrast, when we examine Pf parasites in which we have disrupted Pf csp, we find that oocysts are produced but sporulation is absent. By immunofluorescence analyses we demonstrate expression of Pv CSP in oocyst-derived sporozoites and that Pf CSP expression is absent. The absence of chimeric sporozoites in A. stephensi salivary glands demonstrates that Pv CSP can only partially complement Pf CSP and that species-specific features of CSP govern full maturation and development of sporozoites of these two human malaria parasites.

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ME: HANDSOME MALARIA PARASITE. YOU: PUNCTUAL HOST THAT EXERCISES INFREQUENTLY AND LOVES DINNER. LET'S GET TOGETHER.

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How biological rhythms influence the interactions between organisms is poorly understood. That parasites and hosts display rhythms in behaviours and immunity, suggests that rhythms are important in host-parasite interactions. These interactions matter for the outcome of infections, and understanding why they have evolved and how they're regulated could lead to innovative disease control measures. Malaria parasites have cyclic developmental rhythms that last multiples of 24-hours. The survival and transmission of malaria parasites is determined by whether their developmental rhythms are synchronised to the host's circadian rhythms, but how parasite and host rhythms interact to shape host health is poorly understood. We address this using rodent malaria (*Plasmodium chabaudi*) infections of wild type (WT) and arrhythmic clock mutant (Per1/2 double knock out) mice. We compare parasite and host rhythms in WT mice kept in LD and DD, with rhythms observed in mutant mice in DD. Second, we compare parasite and host rhythms in group- and singly-housed mice in DD. Finally, we use the mutant mice and a restricted feeding regime to decouple host rhythms in feeding from body temperature and locomotor activity and examine the consequences for parasite rhythms.

We show that: (i) parasites maintain normal rhythms in WT and mutant mice in DD, ruling out a circadian oscillator entrained by light; (ii) parasite rhythms match the phase of the host's feeding rhythm but not the phase of rhythms in activity or body temperature; (ii) parasite development in food-restricted mice is more synchronous than in ad-lib fed hosts; and (iv) mutant mice suffer no additional health costs of infection thus the intact clock in WT mice provides little obvious benefit in this disease model. We discuss how host feeding and social interactions could shape rhythms in parasite development and co-evolutionary trajectories between hosts and parasites.

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VECTOR SPECIES SELECTION IS CRITICAL FOR OPTIMIZATION OF *PLASMODIUM FALCIPARUM* (7G8) INFECTION OF MOSQUITO SALIVARY GLANDS FOR CONTROLLED HUMAN MALARIA INFECTION

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Pf7G8 is a Brazilian strain of *Plasmodium falciparum* originally cloned from a patient sample at the Walter Reed Army Institute of Research over 25 years ago. Despite demonstrable mosquito infectiousness at that time, most attempts in the last decade to achieve robust Pf7G8 mosquito infections (oocysts and sporozoites) have been inconsistent and unreliable while the need to test pre-erythrocytic vaccines and drugs against *P. falciparum* strains that are heterologous to Pf3D7 has increased. Pf7G8 is an ideal heterologous parasite strain, given its genetic diversity, geographic origin, chloroquine resistance, and existing cryostocks prepared under GMP condition. However, to be useful for Controlled Human Malaria Infection (CHMI), strains must reliably produce robust infections on a precise timeline, which has been elusive for Pf7G8. Here, we report the reliability of Pf7G8 mosquito infections depends not only on growth conditions of the asexual stage culture but also on the mosquito vector species. Using parallel membrane feeding assays to evaluate the ability of three different Anopheles species to sustain CHMI-worthy sporozoite infections, we determined these species differentially give low, medium, and high infection rates, indicating the vector species is a primary determinate of Pf7G8 success. Using this optimized protocol, Pf7G8-infected mosquitoes were prepared for CHMI with oocyst and sporozoite infection prevalence and intensity equivalent to Pf3D7. Ongoing experiments are testing Pf7G8 as the second *P. falciparum* strain to produce oocysts and sporozoites in our cell-free continuous *in vitro* culture system.

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ENDOTHELIAL GLYCOCALYX DEGRADATION IS ASSOCIATED WITH SEVERE DISEASE AND FATAL OUTCOME IN ADULTS WITH *PLASMODIUM FALCIPARUM* MALARIA

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The pathogenesis of severe falciparum malaria is not fully understood. Microvascular sequestration of parasite erythrocytes and microvascular dysfunction/activation independently predict severity and outcome. The glycocalyx, a carbohydrate rich gel like layer lining the endothelium, is required for constitutive endothelial nitric oxide (NO) production and regulates endothelial activation and vascular permeability. We investigated the role of the glycocalyx loss in falciparum malaria pathogenesis. Inpatients (≥18 years old) with severe (SM, n=42) and moderately severe (MSM, n=56) falciparum malaria, and healthy controls (HCs, n=27) were enrolled at RSMM district hospital in Papua, Indonesia. Disease severity was assessed by WHO criteria. Glycocalyx degradation products (total glycosaminoglycans [GAG]) were measured on enrolment urine using a dimethylmethylene blue assay and normalised to creatinine. The GAG:creatinine ratio [GAG:Cr]) was related to disease severity and vascular NO bioavailability measured by reactive hyperemia peripheral arterial tonometry (RH-PAT). GAG:Cr was significantly higher in severe disease (mean 3.89; 95%CI 2.44-5.33) than in MSM (1.77; 1.26-2.28) and HCs (0.22; 0.06-0.39) p<0.001. In severe disease, GAG:Cr was higher in those with a fatal outcome (n=3; median 6.72, IQR 3.8-27.87) than in survivors (n=39; 3.1, 0.46-4.5) p=0.03. There was a significant association between parasite biomass (plasma HRP2) and GAG:Cr in MSM (r=0.48, p<0.001) and SM (r=0.47; p=0.002), and an inverse association between RH-PAT and GAG:Cr in SM (r=-0.4, p=0.007) and MSM (r=-0.27, p=0.05). The association between mortality and GAG:Cr remained significant after adjusting for parasite biomass (p=0.01) or lactate (p=0.02). The severity of endothelial glycocalyx breakdown is associated with impairment of endothelial NO, severe disease and a fatal outcome in adults with falciparum malaria. Glycocalyx degradation is likely to be a key process exacerbating endothelial NO deficiency, dysfunction and activation, parasite sequestration and disease severity, and predicts mortality in falciparum malaria.

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A BACTERIAL COMPLEX IS REQUIRED FOR PLASTID INTEGRITY IN *PLASMODIUM FALCIPARUM*

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The deadly malaria parasite, *Plasmodium falciparum*, contains a non-photosynthetic plastid known as the apicoplast, that functions to produce essential metabolites. Little is known about its biology or regulation, but drugs that target the apicoplast are clinically effective. Previous studies have identified several prokaryotic Clp (caseinolytic protease) genes, encoded by the *Plasmodium* genome. In bacteria, the evolutionary ancestors of the apicoplast, and in plants chloroplasts these proteins form complexes that degrade proteins in a proteasome-like manner to regulate key cellular processes, but their function in the apicoplast is completely unknown. Using phylogenetic analysis, we identified the Clp members that may form a regulated proteolytic complex in the apicoplast. We genetically targeted members of this complex and generated conditional mutants of the apicoplast-localized PfClpC chaperone, PfClpP protease and PfClpR inactive protease subunit. Conditional inhibition of the PfClpC chaperone resulted in growth arrest and apicoplast loss, and was rescued by addition of the essential apicoplast-derived metabolite, IPP. Moreover, cellular assays suggest that PfClpC inhibition interferes with the ability of the schizont stage parasites to divide and sort functional apicoplast organelles to daughter-merozoites. Using a double conditional-mutant parasite line, we discovered that the chaperone activity is required to stabilize the active protease, revealing functional interactions. Finally, bacterial Clp inhibitors were screened to test selective activity against *Plasmodium* Clp members. These data demonstrate the essential function of PfClpC in maintaining apicoplast integrity and its role in regulating the proteolytic activity of the Clp complex.

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PLACENTAL MALARIA INDUCES OXIDATIVE STRESS IN HUMAN SYNCYTIOTROPHOBLAST

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Placental malaria is characterized by accumulation of *Plasmodium falciparum*-infected erythrocytes and maternal inflammation in the intervillous space of the placenta. These features, in particular the latter, are associated with placental damage and fetal compromise. However, understanding of the mechanisms that lead to poor pregnancy outcome and interventions targeting excessive host responses to placental malaria are still lacking. The syncytiotrophoblast, a cell of fetal origin, is known to

be responsive to malaria-infected erythrocytes as well as the malaria toxin, hemozoin, but its susceptibility to oxidative stress and how this might contribute to placental damage and dysfunction has not yet been directly investigated. The characteristics and key drivers of the syncytiotrophoblast response to oxidative stress were investigated using *ex vivo* human placental tissues and primary trophoblasts isolated from healthy pregnant women. Primary syncytiotrophoblast was exposed to hemozoin and tumor necrosis factor, a critical inflammatory cytokine, to model conditions found in pathogenic placental malaria. The data show remarkable lipid peroxidation in human placental samples from a malaria endemic setting and increased markers of an anti-oxidative response and oxidative damage in syncytiotrophoblast exposed to hemozoin, tumor necrosis factor, and tumor necrosis factor combined with hemozoin. These results suggest that oxidative stress may be a key driver of trophoblast functional compromise in placental malaria and could be targeted therapeutically to mitigate the poor outcomes associated with this syndrome.

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LOW TO INEXISTENT KEY COMPONENTS OF NECROPTOSIS PROTECTS SYNCYTIOTROPHOLAST FROM DEATH RECEPTOR DEPENDENT PATHWAY OF NECROPTOTOSIS

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The syncytiotrophoblast is the main site of exchange of nutrients, waste products, gases, metabolites and drugs between the mother and the fetus. Efficient transfer of these products across the placenta is essential for healthy fetal growth and development. Dysfunction of the syncytiotrophoblast is implicated in poor birth outcomes in a number of syndromes, including placental malaria, but a complete understanding of the mechanisms by which this multinucleated cell is damaged and lost in the context of malaria is lacking. In particular, the susceptibility or resistance of the syncytiotrophoblast to necroptotic death is unknown. The objective of this study was to investigate how the syncytiotrophoblast reacts to necroptosis signals through death receptors, namely those detecting tumor necrosis factor, which has been linked to poor birth outcomes associated with placental malaria. Primary trophoblast and placental tissue explants were isolated from term healthy pregnancies and exposed to tumor necrosis factor and cyclohexemide after pretreatment with Z-VAD-fmk, a pan caspase inhibitor, conditions demonstrated to induce necroptosis in other epithelial cell types. Total proteins were isolated and subjected to western blot for the key components of the necroptosis and apoptosis pathways. A cell viability assay was used to assess cell death/viability on syncytialized primary trophoblast and immunofluorescence staining was used to localize proteins of interest. Results indicate that necroptosis through death receptor pathway is almost inexistent in primary trophoblast (cytotrophoblast and syncytiotrophoblast) as indicated by the very low expression of the receptor interacting protein kinase 3 and phosphorylated mixed lineage kinase domain-like, two key markers for necroptosis. In contrast, apoptosis was detected and confirmed by cell viability assay. These findings indicate that primary human trophoblast is resistant to necroptosis through death receptors and suggest that regulation of cell death pathways in syncytiotrophoblast is unique.

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INVESTIGATING THE ROLE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN IN THE INVASION OF DUFFY NEGATIVE RETICULOCYTES IN THE PACIFIC COAST OF SOUTH AMERICA

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Ecuador is focusing in elimination of malaria; nevertheless, outbreaks and infections of the disease persist in the Coast and Amazon of the country. *Plasmodium vivax* is the most prevalent malaria parasite in Ecuador and it is endemic in the coast and Amazon regions. The invasion of reticulocytes has been considered dependent of the interaction of the *P. vivax* Duffy binding protein (PvDBP) in the merozoite of the parasite with the Duffy antigen receptor of chemokines (DARC) in the erythrocyte. Recently we reported that Duffy negative individuals were infected by *P. vivax* in northwest Ecuador and that Duffy negativity does not seem to provide protection against vivax infection in the northwest of the country. Consequently, we are investigating the role of PvDBP in this process. For this purpose, we have sequenced the binding region of *PvDBP* in 50 Ecuadorian *P. vivax* samples from the Amazon and Coast of the country in addition to determine the *PvDBP* copy number in Ecuadorian samples and we are in the process of studying the differential binding ability of DBP naturally found polymorphisms to DARC genotypes present in Ecuadorian individuals. Our results so far indicate that *PvdbpII* is highly diverse: 45 polymorphic sites were found, 35 sustitutions were non synonymous and 28 haplotypes were identified. There was a very high genetic variability for *Pvdbp RII* (Hd= 0.940 ± 0.019) while nucleotidic diversity was low (ϖ= 0.00736 ± 0,00059). Most haplotypes were unique for each locality. As expected, the Amazon region (where more cases are reported) was more diverse for *P. vivax* than the Coast (where a small number of infections are recorded). Even though the number of reported cases in Ecuador is low, there is important variability in merozoite invasion ligands. Nucleotidic diversity of *PvDBPII* may be involved in differential binding ability of PvDBP to DARC negative erythrocytes.

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HOST ANEMIA INCREASES *PLASODIUM FALCIPARUM* GAMETOCYTOGENESIS *IN VITRO*

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Gametocyte formation is essential for transmission of the malaria parasite. Several stress factors can contribute to increased levels of sexual conversion of the RBC stage of the parasite, including such conditions as sickle-cell trait. We thus hypothesized that asexual stage parasite growth attenuation in RBCs from anemic donors may additionally impact the rate and magnitude of gametocytogenesis. Clinical and epidemiological studies, as well as our *in vitro* modeling of asexual blood stage *Plasmodium falciparum* infection, have revealed iron deficiency and anemia are protective against malaria infection. Studying the impact of anemia on *P. falciparum* sexual reproduction can provide important insight into parasite transmission in populations where anemia is common - thus much of the malaria-endemic world. To study the impact of host anemia on gametocytogenesis, in this pilot study, we collected RBCs from 10 anemic and 10 non-anemic, otherwise healthy, Gambian children between the ages of 6 mo-3 years. We used these donor RBCs to quantify gametocyte production *in vitro* in relation to anemia status. Gametocyte detection was facilitated using a transgenic parasite strain expressing a GFP-tagged gametocyte specific protein, Pfs16. This allowed for flow cytometry-based high-throughput and more accurate detection of gametocyte formation, particularly at the early stages when gametocytes are difficult to distinguish from trophozoites by standard Giemsa-based microscopy. The gametocyte conversion rate was directly related to host hemoglobin and ferritin using linear regression modeling. Understanding the relationship between host anemia and malaria transmission, may guide public health strategies aimed at eliminating both. (This parasite strain was a kind gift from the laboratory of Dr. David Fidock.)

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CRYOPRESERVATION OF *PLASMODIUM FALCIPARUM* GAMETOCYTES TO ENSURE VIABILITY AND INFECTIVITY TO MOSQUITOES

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Malaria afflicts over 200 million people worldwide, with the most fatal infections caused by *Plasmodium falciparum*. Efforts to understand and reduce transmission often rely on the study of the gametocyte stage of the parasite. This stage, *in vitro*, requires extensive culturing for up to two weeks, which may be difficult in resource limited areas and impede the study of this transmissible stage. Cryopreservation of gametocytes would allow for more rapid culturing, and consequent infection of mosquitoes, to better study gametocyte biology and malaria transmission. Successful cryopreservation must maintain gametocyte viability, and previous cryopreservation studies have had limited success. In *Plasmodium falciparum,* traditional cryopreservation techniques lead to low gametocyte viability, and generally less than 5% of mosquitoes are infected upon standard membrane blood feeding assays (SMFA). All cryopreservation techniques to date are based off of a sodium chloride based protocol that has not been modified to our knowledge. We aim to develop an assay to effectively freeze gametocytes to maintain viability and infectivity to mosquitoes. Our preliminary data indicate that a novel modification of the traditional *P. falciparum* cryopreservation protocol leads to improved viability of gametocytes post-thaw. We have observed exflagellation for day 14/15 gametocytes after cryopreservation and thawing, though day 16/17 gametocytes appear to have reduced viability via this measure. Current studies aim to examine the infectivity of these frozen cultures in *Anopheles* mosquitoes via SMFA to determine the correlation of viability measured by exflagellation to infectivity in mosquitoes. We are also working to understand the optimal ratio of male to female gametocytes in cryopreserved cultures for infectivity to mosquitoes. We plan to freeze cultures at different development time points, with different ratios of male:female gametocytes, to understand how these subpopulations may influence success of infectivity. This study will help improve accessibility to gametocyte studies in a variety of lab settings.

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THE EFFICACY OF ACTS IN KENYA; THE STATUS AT KWALE, KISUMU, BUSIA AND KISII COUNTIES' THERAPEUTIC EFFICACY STUDY SITES

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Artemisinin-based combination therapies (ACTs) and its derivatives are the most rapidly acting of all the current antimalarial drugs and recognition of their potential role as a component of combination therapy have led to several large trials aimed at assessing the efficacy of the different available drugs. This study was undertaken to evaluate the efficacy of fixed dose combinations, Artemether-lumefantrine and dihydroartemisinin-piperaquine in uncomplicated malaria patients in Kenya with an aim to inform malaria treatment policy and practice in the country. The study was a multi-site two arm blinded randomized clinical trial. In this are the outcomes of all the study sites evaluated for the efficacy of two artemisinin-based anti-malarial combination drugs, AL and DP, in four counties in Kenya. The *in-vivo* (clinical microscopy) treatment outcome was evaluated followed by molecular correction. Parasitological response at day 28 and 42, cure ratios in the two treatment arms, Fever Clearance Time (FCT), Asexual parasite clearance time (PCT), Gametocyte carrier rates and Adverse events were evaluated. A total of 352 children in Kwale, 315 in Kisumu, 334 in Busia and 314 in Kisii, aged between 6 months and 14 years were randomized and treated with either AL or DP . In this paper the uncorrected and corrected treatment outcomes at Day 28 and at day 42 in the Intention to treat (ITT) and the per protocol (PP) analysis populations are presented for each of the 4 sites, in different combinations and cumulatively. For Kwale and Kisumu combined, in the uncorrected Intention to treat (ITT) analysis, at day 42, 483 participants had ACPR, ACPR rate of 72%(95% CI 69-76%), 268 participants in arm A (ACPR rate of 77% (95% CI 72-81%)) and 215 in arm B (ACPR rate of 68% (95% CI 62-73%)); P-value 0.008. By day 28, participants with ACPR were 542, ACPR rate of 81% (95% CI 78-84%), 293 in arm A (ACPR rate of 84% (95% CI 80-88%)) and 249 in arm B (ACPR rate of 78% (73-82%)); P-value=0.06. In conclusion, the results from these study sites indicate a note worth trend of the ACT efficacy in the regions and calls for vigilance on the same for appropriate policy measures.

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INCREASING *EX-VIVO* TOLERANCE OF GAMBIAN *PLASMODIUM FALCIPARUM* ISOLATES TO PARTNERS IN ARTEMISININ-BASED COMBINATION THERAPIES

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Ex vivo drug susceptibility tests complement antimalarial efficacy studies by assessing the effect of individual components of antimalarial drugs on parasite survival. These can enable early detection of changes in parasite susceptibility to artemisinin-based combination therapies (ACTs) before the onset of clinical failure. This is crucial given the emergence of artemisinin-resistant *Plasmodium falciparum* in south-east Asia and the potential for spread into Africa. As National Malaria Control Programmes scale up efforts to control the disease, there is huge pressure on the parasites from exposure to antimalarials in the population and this could result in selection for resistant strains.We report on *ex-vivo* assays of samples collected from Gambian children with confirmed uncomplicated malaria over 4 transmission seasons; 50% inhibitory concentration (IC_{50}) (n=303; 2013-2015, 2017) and parasite survival assay (PSA) using 10 times the median IC_{50} (n=42; 2015), to determine *ex vivo* susceptibility to artemisinins and partner drugs. We also performed whole-genome sequencing of parasite isolates from 2008 to 2015 to determine candidate drug associated loci.*P. falciparum* isolates showed increasing tolerance to Lumefantrine over the 4 transmission seasons. The PSA results showed a substantial number of isolates with parasite growth and re-invasion following drug exposure. Additionally, whole genome sequencing of parasites showed temporal differentiation of SNPs on chromosome 7 undergoing directional selection, which had strong associations with increasing Lumefantrine tolerance.The data shows increasing tolerance and directional selection to Lumefantrine; a key component of the first-line ACT in The Gambia since 2008. Parasite survival following Lumefantrine exposure may be the effect of increased selection pressure from the temporal differentiation of SNPs on chromosome 7. Overall, parasite tolerance observed with both Dihydroartemisinin and Lumefantrine calls for robust and continuous surveillance for the efficacy of currently deployed ACTs.

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TRENDS OF ANTIMALARIAL EFFICACY, MOLECULAR MARKERS OF DRUG RESISTANCE AND PARASITE POPULATION VARIATIONS AFTER MORE THAN A DECADE OF USING ARTEMISININ-BASED COMBINATION THERAPY IN MAINLAND TANZANIA

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Following deployment of artemisinin-based combination therapies (ACTs) in most malaria endemic countries and the emergence and spread of resistance to artemisinins and partner drugs in South-East Asia (SEA), the World Health Organization (WHO) recommends regular therapeutic efficacy studies (TES) to monitor the performance of these drugs. This review assessed the trends of therapeutic efficacy of ACTs, molecular makers of artemisinins and partner drugs resistance (*K-13* and *Pfmdr1*), and parasite population variations after using ACTs for more than one decade in Tanzania. Data from published and unpublished TES and genetic studies of parasites and molecular markers antimalarial drug resistance were assembled and analysed. Efficacy data included TES of artemether-lumefantrine (AL), artesunate - amodiaquine (ASAQ), and dihydroartemisinin-piperaquine (DP); while data of *K-13*, *Pfmdr1* and other parasite genetic data were obtained from TES and cross-sectional surveys conducted in different parts of Tanzania from 2000 to 2017. Efficacy of AL was assessed in 14 studies and PCR corrected adequate clinical and parasitological response (ACPR) ranged from 95.1% to 100% between 2003 and 2017. Five studies tested ASAQ and ACPR increased from 88% to 100 in 2004 and 2017, respectively. Three studies assessed DP between 2014 and 2017, and the ACPR was 97 - 100%. Non-synonymous mutations in the *K-13* gene were <3% between 2006 and 2016 and none of these mutations have been associated with artemisinin resistance in SEA. For mutations in *Pfmdr1* associated with lumefantrine resistance, N86 was 6% - 10% between 2002 and 2006, but increased to 99.1% in 2016; and 184F increased from 6% in 2002 to 10% in 2006, and was 40 - 65% between 2011 and 2016. Analysis of spatial and temporal parasite population variations is being finalized and will be presented at the meeting. Although the ACTs are still efficacious with no known *K-13* mutations associated with artemisinin resistance, increasing *Pfmdr1* markers associated with lumefantrine resistance is a cause of concern and argue for continued surveillance for supporting TES and provide evidence-based teatmentt policies.

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IMPACT OF HOST IRON DEFICIENCYON MALARIAL ARTEMISININ RESISTANCE

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Iron deficiency anaemia has been shown to confer protection against malaria in endemic areas. Intracellular red blood cell iron concentrations may also have an effect on antimalarial drug (Artemisinin) activity. Artemisinin's activity relies on the cleavage of its endoperoxide structure and production of highly reactive free radicals. Iron deficient RBCs contain constitutively elevated levels of reactive oxygen species. This could create an environment high in oxidative stress that could potentially amplify the effect of artemisinin. Understanding whether iron deficiency impacts artemisinin activity is important as artemisinin is a first-line antimalarial in many areas where iron deficiency is common. For this pilot study, 11 anaemic and 18 non-anaemic children were recruited. Using *Plasmodium falciparum* strain MR4-1241 (originally from Cambodia and resistant to artemisinin) ring survival assay (RSA) was done using 700 nM dihydroartemisinin (DHA) to determine the different parasite survival rates between the anaemic and non-anaemic group. Conventional drug assays were also done to determine difference in Inhibitory concentration (IC_{50}) of DHA, Chloroquine (CQ), Lumefantrine (LUM) and Pyrimethamine (PYR). No significant differences in parasite survival were seen between the anaemic and non-anaemic group (median survival percentage= 2.2 and 2.5 respectively; p value =0.1 Mann Whitney's test). Furthermore, no significant difference (p value = 0.2, 0.1, 0.3) was seen in median IC50 between the two groups with the drugs DHA, CQ and LUM respectively. There was however a higher median IC50 value observed in the anaemic group for PYR (p value = 0.02). There is no difference in artemisinin activity, as measured by either the RSA or conventional IC50 assays, against *P. falciparum* parasites grown in RBCs from anaemic and non-anaemic RBCs. Additional work is needed to assess the impact of host iron status on lower concentrations of artemisinin.

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COMPARING MALARIA TRANSMISSION IN TWO MALARIA STUDY SITES OF MALI

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Previous studies showed that malaria parasite clearance time following artesunate monotherapy was longer in Faladje than in Bougoula-Hameau. The objective of this study was to measure and compare malaria transmission in these two villages.The mosquitoes were caught using the catch spray method in 180 cases at each passage in Bougoula-Hameau and Faladje. Malaria vectors identified morphologically were treated with ELISA for determination of infection rate. *Anopheles gambiae s.l* was the only vector species of malaria found during our study in both villages. During the dry season, the density was 0.13 mosquitoe / hut in Bougoula vs. 0.02 in Faladje. No mosquito was infected in both villages. At the end of the rainy season, the density was 2.76 mosquitoes / case vs. 2.51. The aggressivity was 9 bites / person in Bougoula vs. 7.04 in Faladje The infection rate was 1.16% in Bougoula vs. 6.86 in Faladje, $p < 10^{-3}$. The entomological inoculation rate was 0.14 infective bite / person / month in Bougoula vs. 0.48 in Faladje. Parasite infection rate and entomological inoculation rates were both significantly higher in Faladje as compared to Bougoula-Hameau.

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PHARMACOKINETIC PROPERTIES OF RECTAL ARTESUNATE IN AFRICAN CHILDREN WITH SEVERE MALARIA

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Parenteral artesunate is the treatment of choice for severe *falciparum* malaria, but is still not provided in several malaria endemic areas. Many children with severe malaria die before or just after reaching a treatment facility capable of administering parenteral drugs. The rectal formulation of artesunate has been shown in very large community-based trials to reduce malaria mortality in children. But there is concern about artesunate toxicity as the absorption of rectal artesunate is erratic and the dose given is four times larger than the parenteral dose. This was a randomized, open labelled, 2-arm, cross-over, pharmacokinetic clinical trial in children admitted to hospital with severe malaria in the Democratic Republic of the Congo. Children received either 1 dose of rectal artesunate (10 mg/kg) followed 12 hours later by intravenous (IV) artesunate (2.4 mg/kg) (Arm 1) or vice versa (Arm 2). Artesunate (ARS) and dihydroartemisinin (DHA) concentration-time profiles were analysed using a standard non-compartmental approach. From Jul to Oct 2015, 82 children were enrolled. Twenty-seven children were malnourished (33%) and of those, 9 (11%) were severely malnourished. Children with 6 to 12.9 kg body weight

received, on average, 9.67 mg/kg (SD 2.05) of rectal ARS; those weighing 13 to 23.9 kg received 12.52 mg/kg (SD 1.98), and those between 24 to 34 kg received 10.79 mg /kg (SD 1.13). In the first 12 hours, T(max) and C(max) for both DHA and ARS was significantly higher in the IV group than in the rectal group. There was no significant difference in the total drug exposure (area under the concentration-time curve). Elimination half-life was significantly shorter in the IV group for both DHA and ARS. Of interest, the pattern of rectal absorption varied substantially between patients. The median estimated time for parasitaemia to decrease by half was 2.16 hours in Arm 1 (range 1.27 - 7.57) and 2.45 hours in Arm 2 (range 1.2 - 11.99, p=0.64). One patient in the arm that received rectal administration first died 10 hours after enrollment. Pre-referral artesunate should be given to any child suspected of having malaria who cannot reliably take oral medications.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE AND DIHYDROARTEMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AMONG CHILDREN IN WESTERN KENYA

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Parasite resistance to antimalarials is a major threat for global malaria control. The World Health Organization (WHO) recommends monitoring local susceptibility patterns to inform national treatment guidelines by following a standard protocol for therapeutic efficacy studies carried out at regular intervals, with a change in recommended therapy considered if the polymerase chain reaction (PCR)-corrected treatment failure exceeds 10%. We evaluated the efficacy of the first-line antimalarial, artemether-lumefantrine (AL), and the second-line antimalarial, dihydroartemisinin-piperaquine (DP), for treating uncomplicated *Plasmodium falciparum* malaria in children 6-59 months old in western Kenya. From June 2016 to March 2017, children with *Plasmodium falciparum* mono-infection presenting to the outpatient clinic at Siaya County Referral Hospital, Bar Agulu and Mulaha dispensaries were enrolled in an *in-vivo* efficacy trial per WHO protocol. Children were randomized to treatment with a 3-day course of AL or DP and followed for 42 days.The main outcome was PCR-corrected adequate clinical and parasitological response (ACPR) rate measured at 28 days for AL and 42 days for DP after treatment initiation. A total of 340 children were enrolled in the study (166 in AL arm and 174 in DP arm). There were no early treatment failures in either arm, and all children cleared parasites by day 3. Based on per-protocol analysis limited to children with complete follow up, on day 28 PCR-uncorrected ACPR was 67% (91/135) in the AL arm and 95% (142/149) in the DP arm ($p<0.0001$). PCR-corrected ACPR at day 28 was 91% (95% CI: 85–95) in the AL arm and 99% (95% CI: 95–100) in the DP arm ($p<0.003$), and at day 42 it was 88% (95% CI: 81–92) in the AL arm and 94% (95% CI: 89–97) in the DP arm ($p=0.06$). Intention-to-treat analysis produced similar results. Both drugs were well tolerated. In summary, both AL and DP remain efficacious and well tolerated for treatment of uncomplicated malaria in western Kenya. The longer half-life of piperaquine relative to lumefantrine might provide a prophylactic effect, accounting for the lower reinfection rate in the first 28 days after treatment in the DP arm.

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RATES OF SEVERE CUTANEOUS ADVERSE REACTION AMONG WOMEN TAKING SP-CONTAINING REGIMENS DURING PREGNANCY: A SYSTEMATIC REVIEW

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Malaria in pregnancy (MiP) is a preventable cause of maternal and infant morbidity and mortality; at least three doses of intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) are recommended to prevent poor outcomes. An estimated 30% of pregnant women in sub-Saharan Africa received adequate IPTp-SP in 2016. Severe cutaneous adverse reactions (SCARs), particularly Stevens-Johnsons Syndrome and Toxic Epidermal Necrolysis, can occur with use of SP. We performed a systematic review to update estimates of SCAR associated with IPTp-SP. Multiple databases were searched in accordance with PRISMA guidelines for systematic reviews. We included studies of IPTp-SP with adverse events (AEs) recorded. . The proportion of women with AEs was compared between those taking SP and non-SP containing IPTp regimens using Chi-squared test. Among 1,180 records identified from the search, data from 26 full-text articles published between 2001 and 2016 were included following assessment of title, abstract, and full-text eligibility. Among 67,572 women who had any SP-containing regimen (SP alone or in combination with piperaquine, azithromycin, or amodiaquine) there were no reported cases of severe rash, 347 hospitalizations (51.4 per 10,000), and 20 deaths (3.0 per 10,000). In the subset of studies with a non-SP containing arm, 12,575 women taking SP as compared to other regimens (N= 12,090) had higher rates of pruritus (171.8 vs 97.6 per 10,000, $p<0.01$) and lower rates of hospitalization (129.6 vs 210.9 per 10,000, $p<0.01$). Rates of death were not significantly different between the two groups (12.7 vs 17.4 per 10,000, $p=0.35$). There were no cases of severe rash. IPTp-SP was not associated with higher risk of SCAR or other severe AEs compared to the non-SP regimens. IPTp-SP remains an important tool to prevent adverse outcomes of MiP; our data highlight the safety of IPTp-SP. As malaria transmission declines, the risk-benefit ratio of IPTp-SP may shift. Continued monitoring of IPTp-SP efficacy and better estimates of incidence of drug related AEs will clarify the need to re-evaluate use of IPTp-SP in the future.

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THE *EX VIVO* GROWTH PROFILE OF ARTEMISININ-RESISTANT PARASITES AFTER ARTESUNATE TREATMENT IN THE INDUCED BLOOD STAGE MALARIA MODEL

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Artemisinin resistance was first detected by observing the effect of antimalarial drugs on parasite clearance in malaria patients. However, characterising artemisinin resistance in patients in the field is challenging for a number of reasons, including the confounding effect of human immunity on interpretation of parasite clearance. We recently established an artemisinin-resistant induced blood stage malaria (IBSM) model in which malaria-naïve subjects are inoculated with *Plasmodium falciparum* artemisinin-resistant parasites (K13; R539T). The aim of this study was to use samples from the novel artemisinin-resistant IBSM model to characterize the *ex vivo* growth profile of the K13 isolate. Subjects enrolled in a comparative randomized trial (ACTRN12617001394336) were inoculated with either ~2,800 viable K13 artemisinin-resistant parasites

(Day 0, n=7) or 3D7 artemisinin-sensitive parasites (Day 1, n=3). On Day 9 subjects received a single oral dose of artesunate (~2 mg/kg). Parasitemia was monitored by quantitative PCR targeting the *P. falciparum* 18S rRNA gene. Blood samples were collected pre-treatment and 2, 4, 8, 12, 16, 20, 24, 48 and 72 hours post-treatment from the two subjects from each group (K13, n=2; 3D7, n=2) who had the highest pre-treatment parasitemia. Samples were immediately cultured *ex vivo*. Parasite growth *ex vivo* was assessed by flow cytometry and microscopy for 20 days. In blood samples obtained 8 hours after treatment *ex vivo* parasite growth above 0.5% occurred earlier in K13 parasites (7-12 days post-incubation) than in 3D7 parasites (14-19 days post-incubation). Parasite multiplication rates during *ex vivo* growth and *in vivo* recrudescence will be compared to elucidate differences in drug response between artemisinin-resistant and artemisinin-sensitive parasites. Understanding the differences in recrudescence profiles between K13 and 3D7 parasites will provide greater insight into artemisinin-resistance.

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CHROLOQUINE, SULPHADOXINE-PYRIMETHAMINE RESISTANCE MARKERS PREVALENCE IN AREAS OF EARLY ELIMINATION IN SELECTED PARTS WESTERN AND SOUTHERN ZAMBIA

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One of the key factors in malaria infection management is having an effective drug to promptly treat malaria infections. Antimalarial resistance is one of the challenges in the fight against malaria and is a threat to achieving the malaria elimination goal. *Plasmodium falciparum* has developed resistance to most antimalarials due to drug pressure and other factors. This study sought to investigate the prevalence of Chloroquine and Sulphadoxine pyrimethamine(SP) molecular resistance markers in Southern Zambia. Data from a subset of individuals from a cross-sectional household survey, conducted during peak malaria transmission season in April and May 2017 was used. The survey collected socio-demographic information on households members,coverage of malaria interventions. In addition, malaria testing was done on all ages and dried blood spots were collected. Photo-induced Electronic Transfer - Polymerase Chain Reaction (PET-PCR) was used to analyse the filter papers for malaria species, the non-falciparum species will be excluded. High resolution melt (HRM) PCR will be used to analyse the resistant markers. Quintuple dhfr/dhps for SP and two PfCRT markers will be analysed. Samples from 1567 individuals were analyzed from the surveyed areas in Southern and Western provinces, of which304 were PET-PCR positive; and 266 were falciparum mono-infections. The results are not ready as analysis of the samples is currently under way. The findings will be available at the time of the conference. In conclusion,the results from the study will provide the national malaria program information on the current availability of resistance markers for chloroquine, ACTs and SP. While markers for resistance do not equate with clinical efficacy, these markers can be a sign that pressure for parasite selection is increasing and additional treatment options may need to be explored.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN NORTHWEST ETHIOPIA

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Artemether-lumefantrine (Coartem) is used for the treatment of uncomplicated falciparum malaria in Ethiopia. So far, treatment failure with artemisinin has not been observed in the country. Previously, we found a unique mutation in kelch 13 propeller domain (R622I) and anecdotal evidence for day-3 positivity by microscopy. In this study, we conducted a WHO 28-day efficacy trial to investigate the efficacy of artemether-lumefantrine (Coartem) for the treatment of uncomplicated *P. falciparum* malaria in Northwest Ethiopia (Negade Bahir and Dembia districts). We also assessed if co-infection with other parasites played a role in reducing the efficacy of antimalarial treatment. A total of 95 uncomplicated *P. falciparum* malaria patients were enrolled and completed the 28-day efficacy trial. We found out that artemether-lumefantrine is 100% efficacious with day-3 positivity detected by microscopy. We found that malaria patients were co-infected with other hemo- and intestinal parasites. Leishmania donovani, Schistosoma mansoni, and Ascaris lumbricoides were detected in 4.2%, 42.1%, and 6.3% of malaria-infected patients in this study, respectively. We conclude that artemether-lumefantrine is still efficacious in Northwest Ethiopia. Further studies will evaluate the presence of mutations in the kelch 13 gene, antibody levels to merozoite surface protein (msp), and the kinetics of parasite clearance using molecular amplification technologies.

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MONITORING OF PHARMACOVIGILANCE DURING THE SEASONAL MALARIA CHEMOPREVENTION CAMPAIGN IN SENEGAL, 2013 TO 2017

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In 2012, Senegal National Malaria Control Program (NMCP) implemented seasonal malaria chemoprevention (SMC), an intervention recommended by the World Health Organization (WHO) in 2012 in areas of seasonal malaria in which at least 60 % of cases occur over a period of four months. In Senegal, SMC was implemented in a door to door campaign by community health volunteers administering a dose of sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) under directly observed therapy to children three months to ten years and leaving an additional two doses of AQ for the guardian to administer. In 2013, the campaign took place in four districts with almost 60,000 children in the target age group, and was accompanied by communications, advocacy, and social mobilization activities. While the drugs used in SMC are generally considered safe and effective, they can cause adverse events that may be minor, moderate, or in very rare cases, severe. During the SMC campaign, notices of adverse events were collected by the NMCP and processed by the Anti-Poison Center. In 2013, the treatments of SP+AQ administered to 59420 children under 10 years, 33 adverse events notifications were sent to the NMCP. In 2014, 290 adverse events notifications to 614785 children, in 2015, 2054 adverse events notifications to 620877 children, in 2016, 479 adverse events notifications to 644830 and in 2017, 117 adverse events notifications to 627067 children. Adverse effects reported were mostly minor: abdominal pain, nausea, vomiting, urticaria, etc. But in 2014, 02 severe adverse events were notified, in 2015, 03 severe adverse events and 2016, 02 severe adverse events. Most of the notifications were made by health post nurses. The Anti-Poison Center, which is responsible for determining imputability, judged that imputability was certain, possible,

improbable and uncategorized. In 2018, SMC will be implemented in 16 districts in the four regions of Kedougou, Tambacounda, Kolda and Sedhiou, targeting nearly 600,000 children. The pharmacovigilance system will be strengthened to ensure that adverse events will be notified and tracked.

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NATIONWIDE MOLECULAR SURVEILLANCE FOR *PFCRT, PFDHFR,* AND *PFDHPS* GENE MUTATIONS WITHIN THE HAITIAN *PLASMODIUM FALCIPARUM* POPULATION

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Previous reports from the island nation of Haiti have indicated little evidence of resistance to chloroquine (CQ) based therapies in *Plasmodium falciparum*, and therefore CQ remains as a first line treatment for malaria, with sulphadoxine-pyrimethamine (SP) being second-line treatment. Therapeutic efficacy studies of CQ are impractical to implement in this low malaria prevalence setting, thus a molecular surveillance system was established in eleven health facilities throughout the country to monitor the emergence of genetic resistance markers for CQ and SP. In 2016 and 2017, 780 persons positive by malaria rapid diagnostic test were enrolled and patient samples assayed for putative resistance mutations to *Pfcrt, Pfdhfr,* and *Pfdhps*. Of these, 742/780 (95.0%) were found to contain *P. falciparum* DNA, and all specimens were found to be carrying *Pfcrt* wild-type allele CVMNK (codons 72-76). Sequencing for *Pfdhfr* found 257 (34.6%) of isolates harboring the S108N single mutation, as well as one isolate with the N59I and one with the C59R mutation. One isolate from the Ouest department was found to have triple *Pfdhfr* mutation (N51I, C59R, S108N). Sequencing *Pfdhps* in these isolates identified only one point mutation (A437G) in the Sud department. These results show and a predominant circulating wild-type *Pfdhps* genotype and no indication of emergence of *P. falciparum* CQ resistant *Pfcrt* alleles in Haiti. Though a significant percentage of *Pfdhfr* S108N genotypes were found, this alone has not been associated with SP resistance. This observation along with the evidence for lack of *Pfdhps* resistant markers (except in a single sample with a single mutation) suggests low SP resistance in Haiti. These data are consistent with the current recommendation of CQ and SP for treatment of malaria in Haiti. Nevertheless, continued annual molecular surveillance will be necessary to determine if and when resistant alleles emerge in the population.

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THERAPEUTIC EFFICACY OF ARTESUNATE-AMODIAQUINE AND ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN UNDER FIVE YEARS IN GUINEA

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Artemisinin-based combination therapy (ACT) is the first-line antimalarial treatment for uncomplicated *Plasmodium falciparum* infection in Guinea. This study prospectively evaluated the efficacy and tolerance of two ACTs used in the management of uncomplicated *P. falciparum* malaria cases in children under five years of age in two sites in Guinea. We conducted a two-arm, randomized therapeutic efficacy study evaluating artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) during a 28-day follow-up period. Data were collected at two health centers (located in Labé and Mafèrinyah Health Districts) between August 2015 and April 2016. Parasites from pre-treatment and day-of-failure samples from late treatment failures were genotyped and compared using neutral microsatellite markers to distinguish recrudescences from reinfections. A total of 421 subjects were enrolled, including 212 in the ASAQ arm and 209 in the AL arm; eight patients were lost to follow-up. Seven adverse events were reported, including two deaths that were not drug related. No patients had detectable parasitaemia on day 3. Of 22 late treatment failures, 20 were classified as reinfections and 2 as recrudescences. Across both sites, the therapeutic efficacy before molecular correction was 96% (95% confidence interval: 92-98%) for ASAQ and 93% (89-96%) for AL, and after molecular correction it reached 100% (96-100%) for ASAQ and 99% (97-100%) for AL. The two therapeutic combinations were effective for the treatment of uncomplicated *P. falciparum* malaria in children under five years in Guinea. Surveillance should continue to detect early antimalarial drug resistance in Guinea's fixed sentinel sites.

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PLASMODIUM MALARIAE- IF AT FIRST YOU DON'T SUCCEED, TEST, TEST AGAIN

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Plasmodium malariae is widely distributed in Africa, South America, Southeast Asia and Oceana but causes less than 2% of malaria cases worldwide. However, the proportion of *P. malariae* cases reported in travelers is increasing, due in part to improved PCR based diagnosis. *P. malariae* often has low levels of parasitemia and poor sensitivity of both rapid antigen tests and light microscopy. Long incubation periods may also decrease clinical suspicion. We report a case of a male in his early thirties presenting 10 weeks after returning from central Africa with cyclical fevers, chills, and severe myalgia despite atovaquone/proguanil prophylaxis. He appeared ill with fevers to 103.1F, tachycardia to 121 beats/ minute but no focal findings. His labs were significant for leukopenia 2900 cells/uL, thrombocytopenia 82000 cells/uL, elevated aspartate aminotransferase 316 units/L and alanine aminotransferase 400 units/L. Due to his remote travel history and high level of suspicion, *P ovale* or *P malariae* infection were considered. The first three malaria smears and rapid tests were negative. However, a fourth test of rapid diagnostic antigen and light microscopy identified *P. malariae*. Although there are reports of breakthrough late onset fevers despite adherent atovaquone/proguanil prophylaxis, subsequent treatment has been paradoxically successful with the same medication. This case highlights increasing recognition of *P. malariae* infection despite long senescent periods, prior appropriate chemoprophylaxis, and tests with poor sensitivity. It also illustrates the importance to have high level of clinical suspicion as well as the question if this represents case of drug resistance versus an alternative mechanism of prophylactic efficacy.

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BALANCED IMPACTS OF FITNESS AND DRUG PRESSURE ON THE EVOLUTION OF *PFMDR1* ALLELES IN *PLASMODIUM FALCIPARUM*

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Antimalarial drug resistance may be balanced by loss of fitness in parasites with decreased drug sensitivity. We are interested in evaluating the relative fitness of *Plasmodium falciparum* containing resistance-mediating mutations with and without drug pressure. We therefore evaluated the impacts on *in vitro* fitness of two common mutations in the putative drug transporter *pfmdr1*, 86Y, which is associated with decreased sensitivity to chloroquine and increased sensitivity to lumefantrine, and 184Y, which has uncertain impacts on drug sensitivity. We co-cultured NF10 *P. falciparum* engineered for varied sequence at these two alleles and followed genotypes over time with and without drug pressure. Wild-type (WT) and mutant parasites with the *pfmdr1* 86Y and/or 184F mutant haplotypes were co-cultured 1:1 in competitive growth experiments over 60 days. The *pfmdr1* sequence was assessed every 4 days by pyrosequencing, allowing quantification of alleles. In the absence of drug pressure, N86 and Y184 parasites overgrew mutant parasites (after 60 days cultures contained ~76% WT N86 and >95% WT Y184, respectively). However, double mutant 86Y/184F parasites were not overgrown by WT (after 60 days cultures contained ~56 % WT). Culturing in the presence of 80 and 160 nM (¼ and ½ IC_{50}) chloroquine favored mutant parasites (~20% and 5% N86 after 60 days, respectively). Culturing in the presence of 0.5 and 1.0 nM (¼ and ½ IC_{50}) lumefantrine favored WT parasites (>95% N86 by day 24). Culturing in the presence of 9 nM (¼ IC_{50}) piperaquine favored mutant parasites (~30% N86 after 60 days). Our results and prior data suggest that the *pfmdr1* 86Y mutation engenders some cost in parasite fitness, but that the 184F mutation compensates for this fitness loss, affording growth of the double mutant equivalent to that of WT parasites. However, low level drug pressure favors genotypes with decreased sensitivity to the selecting drug. These findings highlight the interplay between drug pressure and fitness that is guiding the evolution of resistance-mediating *P. falciparum*. Studies of impacts of other resistance-mediating mutations are underway.

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CHARACTERIZATION OF PFCRT F145I IN PIPERAQUINE-RESISTANT *PLASMODIUM FALCIPARUM* ISOLATES FROM CAMBODIA THROUGH ZINC-FINGER NUCLEASE-MEDIATED GENE EDITING

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The emergence of multidrug-resistant *Plasmodium falciparum* in Southeast Asia poses a threat to the control and elimination of malaria. We have previously shown that the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) F145I mutation is associated with reduced susceptibility to piperaquine, and confers resistance above that associated with amplified *plasmepsin II (pfpm2)* alone. In this study, we have used a gene editing approach to remove PfCRT F145I from Cambodian field isolates and have characterized the effect of this mutation on parasite susceptibility to piperaquine. Parasite isolates were collected as part of a survey of antimalarial drug resistance conducted in a region with significant clinical piperaquine resistance (Anlong Veng, Oddar Meanchey) in Cambodia. After adapting the isolates to *in vitro* culture, we genotyped them at the PfCRT F145I locus (via pyrosequencing) and estimated both *pfpm2* and *pfmdr1* copy number. All three isolates had multiple copies of *pfpm2* and the PfCRT F145I mutation but only one copy of *pfmdr1*. Using the piperaquine survival assay, the survival rate of the field isolates in all concentrations of piperaquine was greater than that of a piperaquine-sensitive lab strain (Dd2), and ranged from 25 to 60%, well above the 10% cutoff for piperaquine resistance. Transfection studies are underway and results will be presented.

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NOVEL QUANTITATIVE POINT-OF-CARE G6PD TEST FOR SAFE TREATMENT OF *PLASMODIUM VIVAX* MALARIA

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Currently, primaquine is the only registered drug for the radical cure of *Plasmodium vivax, but* such 8-aminoquinoline class compounds present moderate to severe hemolytic risk in subjects with glucose-6-phosphate dehydrogenase (G6PD) deficiency. This risk represents a major barrier to wide-scale adoption of radical cure. To ensure the availability of an accurate and robust G6PD test and discriminate hemizygous-deficient males and heterozygous-intermediate females from normal, PATH has supported the development of the STANDARD G6PD Test by SD Biosensor. The test provides quantitative output of two analytes: G6PD enzymatic activity (U/g Hb) and hemoglobin concentration (g/dL) from single fresh whole blood specimen across a broad temperature-humidity range with a quick turnaround of two minutes. The STANDARD G6PD Test was evaluated at the PATH laboratory in two consecutive studies using 110 venous blood specimens and 100 paired venous and capillary specimens to determine the precision, accuracy, and linearity at ambient temperature and 32°C at 50% relative humidity, as per target product profile performance criteria. No statistical difference was observed between results generated at 22°C and 32°C, and the combined data sets had a correlation coefficient (R^2) value of 0.81 for G6PD and an R^2 value of 0.88 for hemoglobin against the reference assays. No bias was observed for either venous or capillary specimens. Furthermore, the STANDARD G6PD Test showed sensitivity and specificity of 100% (95% confidence interval [CI] 84-100%) and 99% (95% CI 97-100%) at 30% normal activity; 93% (95% CI 81-99%) and 90% (95% CI 85-95%) at 70% normal activity; and 86% (95% CI 75-94%) and 87% (95% CI 81-92%) at 80% normal activity. Next, the STANDARD G6PD Test will be evaluated in malaria-endemic regions to determine the World Health Organization prequalification dossier requirement and support the wider adoption of *P. vivax* radical cure.

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ASSESSMENT OF DRIED BLOOD SPOTS FOR IDENTIFYING MALARIA INFECTION BY MULTIPLEX ELISA

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Low-density malaria infections, which are common across a range of transmission settings, are poorly understood. A better understanding of these sub-microscopic infections in terms of antigenemia will inform development of a new generation of diagnostics that will greatly facilitate the eradication of malaria. An improved enzyme-linked immunosorbent assay (ELISA) test that enables quantification of low levels of multiple *Plasmodium* antigens in blood samples, including histidine-rich protein 2 (HRP2) and pan-specific lactate dehydrogenase (Pan LDH), was recently developed. Dried blood spot (DBS) is the most operationally feasible form of collecting blood samples in surveys in malaria-endemic settings. Here, we aimed to determine the feasibility of application of DBS analysis in the

monitoring of malaria infections. Methods for antigen elution from DBS and multiplex ELISA were optimized to achieve maximal elution efficiency and maximal sensitivity. Analytic performance of this assay was evaluated using matched blood and DBS samples prepared from *P. falciparum* culture strain and collected from the field studies. The results showed a correlation coefficient of 0.98 for HRP2 and Pan LDH. Using the polymerase chain reaction test as a reference, preliminary DBS analysis showed sensitivity of 85% and specificity of 100% for HRP2, and sensitivity of 95% and specificity of 100% for Pan LDH. This easy-to-use, less-expensive, high-throughput assay, combined with DBS, can be tremendously useful in mass screening for malaria infections.

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THE PERFORMANCE OF A HIGHLY SENSITIVE RAPID DIAGNOSTIC TEST COMPARED TO POLYMERASE CHAIN REACTION

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Efforts to accelerate malaria elimination are limited by current diagnostic tools. Rapid diagnostic tests (RDT) are generally unable to detect parasitaemia below 50 parasites/µL (p/µL) while polymerase chain reaction (PCR) is often unavailable and doesn't allow for immediate treatment. Moreover, in a previous study we found that RDTs have a low sensitivity (18%, vs PCR) to detect low parasitaemia. However, recently a highly sensitive RDT (HSRDT), with claimed limit of detection 2-5 p/µL, was launched. Here, we present the first large-scale evaluation in field conditions; we evaluated the performance compared to PCR during pro-active case detection (ProACD) activities in Northern Cambodia from Oct 2017 - Mar 2018. ProACD entails the voluntary screening and treatment of asymptomatic at-risk individuals in 8 villages. Screening was done using the HSRDT (Alere Malaria Ag Pf) and PCR. HSRDT results were verified by a blinded double reading and the laboratory conducting the PCR was blinded to the HSRDT result. Moreover, for all PCR *Plasmodium falciparum* (Pf) + cases, the estimated parasitaemia was determined by PCR. We screened 2008 individuals, of which 38 were Pf+ using PCR (~2% prevalence). Of these, 13 were not detected using the HSRDT, resulting in a sensitivity of 66% (95% CI: 49-80%). Of the 1970 PCR negative results only two were HSRDT positive, giving a specificity of 99.9% (95% CI: 99.6-99.9%). During the double reading, only one was discordant, which was confirmed as positive by a third reader. The estimated parasitaemia was statistically significantly lower among the 13 false negatives (mean: 65 p/µL, SD: 128 p/ µL) compared to the 25 true positives (mean: 11366 p/µL, SD: 28213 p/µL), with 8 false negative individuals having a parasitaemia at/below the limit of detection (<2-5 p/µL). Further molecular analyses (HRP2 deletion and concentration) and comparison with standard RDT are currently ongoing. We show the feasibility of incorporating HSRDT in case finding strategies. The HSRDT has a high specificity, while the benefit of immediate treatment can be expected to compensate for the lower sensitivity.

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EVALUATION OF A NEW SEROLOGY RAPID DIAGNOSTIC TEST FOR IDENTIFYING INDIVIDUALS WITH RECENT *PLASMODIUM FALCIPARUM* INFECTION IN A UGANDAN COHORT

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Eliminating *Plasmodium falciparum* transmission is an increasing focus of anti-malaria efforts, and is now becoming feasible in countries throughout the world. As transmission reduces, prevalence of infection becomes heterogeneous, with *Foci* of persistent transmission within large populations with otherwise little or no transmission. With current tools, it is difficult to identify these populations and target resources appropriately since infection rates are low and infected people may be asymptomatic. A low-cost test is needed that can identify recently infected individuals with enough specificity to give assurance of the presence or absence of infection. To address this need, we developed a new serology-based rapid diagnostic test (RDT) capable of identifying individuals who have become parasite-positive within the past half year. The detection window is wide enough to enable reliable identification of transmission *Foci* without the need to test all individuals within a community, and short enough to reflect changing epidemiology patterns. The antigen in the assay is early transcribed membrane protein 5 (ETRAMP 5) that has previously been reported as a top predictor of recent *Pf* infection. To evaluate performance, we are currently testing the RDT in a longitudinal community-based study of malaria in Nagongera, Uganda (PRISM) that follows over 400 participants, aged 2-80 years and tests them every month for asymptomatic parasitemia using blood smears and quantitative PCR. To date, 158 samples have been tested from individuals where time since last parasite positive was known. Results show that the serology RDT is able to classify individuals with/without parasitemia in the past 180 days across a broad age range: < 5 years (n-43, 19/95% sens/spec), 5-15 years ((n=85, 25/97% sens/spec), > 15 years (n=30, 47/71% sens/spec), everyone (n=158, 25/93% sens/spec). We are awaiting the conclusion of enrollment to update results. This assay could also serve as a screening test for traveling individuals or are donating blood. It could also simplify the current WHO protocol for certification of national malaria elimination.

1009

FIELD EVALUATION OF THE MALACHITE GREEN LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AS A MALARIA DIAGNOSTIC IN A HEALTH POST IN RORAIMA STATE, BRAZIL

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Malaria is a debilitating parasitic disease that causes significant morbidity and mortality. Microscopic detection of parasites is currently the most widely used diagnostic, but this technique is limited in its ability to detect low-density infections and requires highly trained microscopists. More sensitive and user-friendly tools are needed to overcome these obstacles. We have shown previously that the molecular-based, colorimetric malachite green Loop-Mediated Isothermal Amplification (MG-LAMP) assay is an accurate tool for diagnosing malaria infection in a laboratory setting. In this study, we field evaluated this assay in a malaria diagnostic post in Roraima, Brazil. We prospectively collected 91 patient samples and performed microscopy, MG-LAMP, and real-time PCR (PET-PCR) to detect *Plasmodium* infection. Two independent readers were used to score the MG-LAMP tests to assess whether the sample waspositive (blue/green) or negative (clear). There was 100% agreement between the two readers (Kappa=1). Both the MG-LAMP and PET-PCR detected 6 and 7 more positive samples, respectively, than microscopy. The PET-PCR assay

detected 6 mixed infections (defined as infection with both *P. falciparum* and *P. vivax*) while microscopy detected only one and MG-LAMP detected two of these mixed infections. Microscopy did not detect any *Plasmodium* infection in 26 of the enrolled asymptomatic cases while MG-LAMP detected five and PET-PCR assay three positive cases. Overall, MG-LAMP provides a portable, user-friendly molecular method for malaria diagnosis and it is less subjective and more sensitive than microscopy. Importantly, MG-LAMP has the capacity to test at least 38 samples in one hour, allowing for the screening of large number of samples which is appealing when large scale studies are necessary e.g. in community surveillance studies. The current MG-LAMP assay was limited in its ability to detect mixed infection and extremely low density infections, but otherwise proved to be a powerful tool for diagnosing malaria in the field and opens news perspectives in the implementation of surveillance in malaria elimination campaigns.

1010

A MALARIA MICROSCOPY QUALITY ASSURANCE SYSTEM IMPROVES DIAGNOSIS FOR MALARIA ELIMINATION IN MYANMAR

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Myanmar's goal is malaria elimination by 2030. To accomplish this goal, the National Malaria Control Program (NMCP), National Health Laboratory (NHL) and partners are committed to strengthening microscopy diagnosis in malaria endemic areas. The Asian Development Bank's (ADB) Regional Capacity Development Technical Assistance works to improve laboratory quality through strengthened quality assurance (QA) systems. Through this program, University Research Co., LLC (URC) developed malaria microscopy standard operating procedures (SOP) and a QA manual to improve laboratory technicians' skills and strengthen microscopy in health facilities in 10 townships in Mon State and Sagaing Region. The SOP and manual were approved by the Ministry of Health and Sports (MOHS) and microscopy trainings were conducted for 43 technicians (24: Mon State, 19: Sagaing Region) by experts from the NHL and NMCP in January 2018. The trainings are an intensive two-week course providing core training modules including theory of malaria microscopy and practical slide reading. In Mon State, technicians scoring <80% on the pre- and post-test significantly decreased from 42% to 4%. Similarly, in Sagaing Region, technicians scoring <80% on the pre- and post-test decreased from 63% to 5%. The project also supports necessary equipment, coaching, monitoring and supervision to all supported facilities. In addition, the NHL conducts panel testing for laboratory technicians to improve malaria microscopy skills. In 2016, one hospital in Mon State and two hospitals in Sagaing Region participated in panel testing, which emphasizes parasite identification, species, stages and density. Among the three hospitals, only one received an excellent score while the remaining two scored poorly. In February 2018, the NHL conducted panel testing for an additional 24 hospitals after the two-week training and resulting scores were excellent. To further institutionalize these improvements, the NHL and NMCP, with support of ADB's Health Strengthening Program should refine policy on the role of malaria microscopy and a QA system in the context of malaria elimination.

1011

DEVELOPMENT OF QUANTITATIVE PCR FOR ACCURATE QUANTIFICATION OF *PLASMODIUM* PARASITES USING NOVEL PRIMERS/PROBE FOR 18S RRNA GENES

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Malaria, a mosquito-borne infectious disease resulted in ~216 million cases and 445,000 global deaths in 2016. Malaria is caused by parasitic protozoans belonging to the *Plasmodium* genus. Since the clinical sequelae of malaria largely result from asexual blood stage parasites, accurate assessment of parasitemia is vital for patient management, epidemiological surveillance, and evaluation of candidate malaria vaccine efficacy. DNA-based *Plasmodium* 18S rRNA quantitative PCR (qPCR) is a *robust method* for quantifying *Plasmodium* parasites, particularly when parasite densities are below the limit of detection with traditional microscopy and/or undetected with rapid diagnostic tests. Among the five 18S rRNA alleles in the genome of *Plasmodium*, three alleles have over 99% sequence identity, whereas the other two have with high sequence homology with each other. We determined that the widely used primer pair (Plasmo1-F/Plasmo1-R) amplifies a region with del/ins, yielding amplicons of two sizes: 157 bp and 173 bp. In contrast, our novel primer pair (Plasmo2-F and Plasmo2-R) yielded only a single amplicon (130 bp), representing all five *Plasmodium* 18S rRNA alleles. Using this novel primer set with a probe within the amplicon, we were able to quantify single parasites in as little as 3.125 nL of *Pf*3D7 cultures at a parasitemia of 1%. These findings warrant further validation of the accuracy of the novel approach for quantifying malaria parasitemia in clinical isolates.

1012

FALSE-NEGATIVE RAPID DIAGNOSTIC TEST RESULTS DUE TO *PLASMODIUM FALCIPARUM* HISTIDINE-RICH PROTEIN 2/3-NEGATIVE PARASITES ARE UNCOMMON AMONG SYMPTOMATIC SUBJECTS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Reports of *Plasmodium falciparum* parasites with *pfhrp2* and/or *pfhrp3* (*pfhrp2/3)* gene deletions are beginning to emerge in sub-Saharan Africa. These deletions can make *Plasmodium falciparum* parasites undetectable by PfHRP2-based rapid diagnostic tests (RDTs). Current recommendations suggest transitioning from PfHRP2-based RDTs to less sensitive, less heat-stable alternative RDTs when the frequency of these parasites among subjects with symptomatic falciparum malaria exceeds 5%. We previously reported a 6.4% national prevalence of *pfhrp2*-negative *P. falciparum* infection among asymptomatic children enrolled in the 2013-14 Democratic Republic of the Congo (DRC) Demographic and Health Survey. In order to guide decisions about optimal malaria diagnostic testing policies in the DRC, we performed a follow-up, cross-sectional study of symptomatic malaria caused by *pfhrp2/3*-negative *P. falciparum.* We have now measured the prevalence of these parasites in 3,628 symptomatic patients in three provinces, including two found to have high prevalence (Kinshasa and South Kivu) and one with low prevalence (Bas Uele) during our initial survey. Subjects underwent malaria diagnostic

testing using microscopy and a PfHRP2-based RDT, and dried blood spot samples were collected for molecular testing. Among the 427 subjects with microscopy-positive, RDT-negative parasitemia, 60 were *P. falciparum* lactate dehydrogenase (*pfldh)* PCR-positive. We tested for *pfhrp2/3* deletions in 25 of these isolates and 73 microscopy-positive, RDT-positive, *pfldh* PCR-positive controls. Eight symptomatic infections due to *pfhrp2/3*-negative parasites were identified, with microscopy parasitemias ranging from 1,900 to 35,000 parasites/uL. Our initial findings suggest that false-negative RDTs due to *pfhrp2/3*-negative *P. falciparum* are uncommon among subjects with symptomatic malaria in the DRC, and additional analyses are underway. These findings support ongoing use of PfHRP2-based RDTs in the DRC.

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CLINICAL APPLICATION OF MALARIAL RETINOPATHY DETECTION SYSTEM FOR HIGHLY SPECIFIC DIAGNOSIS OF CEREBRAL MALARIA

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Malarial retinopathy (MR) is a retinal manifestation of cerebral malaria (CM), a life-threatening clinical syndrome associated with malaria infection. The 2017 World Malaria Report estimates that malaria claimed the lives of 445,000 people worldwide, more than 75% of whom were African children under 5 years of age. Attributing mortality to CM on the basis of standard clinical features (depth of coma, the presence of parasitemia, absence of other obvious causes of coma) may misclassify up to 25% of deaths. If patients were found, on the basis of direct or indirect ophthalmoscopy, to have evidence of malarial retinopathy (MR), the specificity of the clinical diagnosis would exceed 90%. We have developed an artificial intelligence system, ASPIRE, for computer-based detection of MR that classifies patients as MR+ or MR-. The system integrates a fully automated retinal image analysis software with a portable retinal camera, Pictor-Plus. A clinical prototype was tested on the Pediatric Research Ward in the Queen Elizabeth Central Hospital (Blantyre, Malawi) in years 2017 and 2018. When compared with the ground truth (binocular indirect ophthalmoscopy), the specificity, sensitivity, and positive predictive value of ASPIRE for detecting MR were 100%, 62%, and 1.0, respectively. The clinical prototype received an encouraging feedback from the clinicians for its fully-automated functionality, portability, and user-friendly operability in the hands of a minimally -skilled user. In the next phase, we will redesign the system, improving the integration of the hardware/software components so that ASPIRE is affordable for clinical settings in Africa. Incorporating this technology into routine clinical care will improve the diagnosis of CM and will reduce the impact of disease burden in malaria-endemic areas.

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SCREENING FOR ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS IN MADAGASCAR: PERFORMANCE OF A HIGHLY-SENSITIVE RAPID DIAGNOSTIC TEST

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Low-density malaria infections are common in endemic countries and can persist undetected by microscopy and conventional rapid diagnostic tests (RDTs). We evaluated the performance of a new highly-sensitive malaria RDT by comparing rapid test results with detection of *P. falciparum* by polymerase chain reaction (PCR) among asymptomatic individuals in Madagascar. In December 2017, an Alere™ Malaria Ag Pf Ultra Sensitive RDT (uRDT) was added to two ongoing malaria detection surveys in six sites across Madagascar. The first, in four districts in the south and west, included screening all children aged six to 13 years in public and selected private schools; the second included screening all residents > six months of age in two districts in central Madagascar. Socio-demographic and clinical characteristics of participants were collected using a structured questionnaire; participants with a temperature <37.5°C who did not report taking antimalarials in the previous month were considered asymptomatic. Blood samples were obtained by finger prick for RDT (SD Bioline Malaria Ag *Pf* / Pan), uRDT, and dried blood spot (DBS) for PCR. DNA extractions from 20% of randomly selected DBS were tested for *P. falciparum* by nested PCR. Sensitivity calculations were done using PCR as the gold standard. Of 2,566 asymptomatic participants, 498 with PCR results were analyzed. The median age of these participants was 11 years (range: six months-70 years) and 219 (44%) were male. A total of 35 (7.0%) were positive for *P. falciparum* by RDT, 45 (9.0%) by uRDT, and 39 (7.7%) by PCR. Of the 45 positive by uRDT, 32 (71.1%) were PCR positive; of the 35 positive by RDT 29 (82.9%) were PCR positive. The sensitivity of the uRDT for detecting *P. falciparum* infection was slightly higher than that of the RDT: 82.1% (95% CI: 66.0-91.5%) *vs.* 74.4%, (95% CI: 57.8-86.0%), p=0.205). Specificity of the uRDT (97.2%, 95% CI: 95.2 - 98.4%) was similar to that of the RDT (98.7 %, 95% CI: 97.1-99.4%), p = 0.948. The uRDT detected a greater number of asymptomatic *P. falciparum* infections compared with the conventional RDT and might be useful for asymptomatic screening-and-treatment campaigns in Madagascar.

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LONG-TERM QUANTITATIVE ANALYSIS OF HRP2 PERSISTENCE POST-ANTIMALARIAL TREATMENT IN SENEGAL

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The *Plasmodium falciparum* Histidine Rich Protein 2 (HRP2) antigen is highly expressed, secreted and tend to accumulate in the blood during infections. This facilitates its detection by rapid diagnostic tests (RDTs) even when most of the parasitized red blood cells sequester away from the peripheral blood. Yet, HRP2 persistence can lead to false positive RDT results, whereby the antigen detected is not indicative of a current infection but is merely the slowly eliminating residue from a past infection. Most studies published to date on the subject are qualitative only and none were conducted past 42 days of follow-up. To address

this limitation, we report here a detailed long-term quantitative analysis of HRP2 persistence post-antimalarial treatment in Senegal, together with an evaluation of the time-to-negativity for a conventional RDT (CO-RDT) and high sensitivity RDT (HS-RDT). A total 120 individuals with an uncomplicated *P. falciparum* mono-species infection confirmed by microscopy or RDT were recruited at two sites. A capillary blood sample was collected at baseline, every second day until day 6, and every 4 days until day 62 for HRP2 quantification by Luminex, for infection detection by PCR, CO-RDT, and HS-RDT, as well as for *pfhrp2* and *pfhrp3* genotyping. A total of 107 (89%) individuals completed follow-up and 96 (80%) were included for data analysis. The parasitemia geometric mean and the HRP2 median at baseline were 5.435 p/µL and 103.7 ng/mL [IQR: 13.5 - 661.3 ng/mL]. Nineteen individuals (19.8%) still had residual HRP2 levels detectable by Luminex at day 62 (1.1 ng/mL average concentration). Preliminary statistical analyses indicated a median HRP2 half-life of 1.0 day [IQR: 0.48 - 3.1] and a median assay positivity survival time of 18 days for a CO-RDT, 30 days for a HS-RDT, while all individuals turned negative by PCR no later than day 4. These results demonstrate the long-term persistence of HRP2 by providing quantitative measurements thereof up to day 62. Our data also provide the first evaluation of the impact of improved HRP2 sensitivity on the assay positivity lag-time post-treatment.

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MULTI-ANTIGEN PROFILES CAN CHARACTERIZE *PLASMODIUM FALCIPARUM* INFECTION STATUS AND PARASITE GENOMIC COPIES IN SAMPLES FROM ANGOLA, 2016

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Surveys of *Plasmodium* infection in populations are key for planning and managing malaria control and prevention, allowing estimation of disease burden and assessing success of control and elimination programs. Traditionally, surveys have relied on rapid diagnostic tests (RDTs) and/or blood smear microscopy to determine malaria infection status at clinically-relevant parasite densities. However, RDTs do not provide information on parasite density, and high-quality microscopy is difficult to implement consistently for large surveys. Using 208 samples collected from outpatients attending 89 health facilities in Angola, we employed a bead-based immunoassay to detect and quantify the concentrations of three antigens: *P. falciparum* histidine-rich protein 2 (PfHRP2); pan-*Plasmodium* aldolase (pAldo); and pan-*Plasmodium* lactate dehydrogenase (pLDH). We also assayed the samples using ultra-sensitive qRT-PCR to detect and quantify *Plasmodium* 18S rRNA, from which we estimated a parasite density. For samples with no antigen detected, 16% (10/63) were positive for *P. falciparum* by qRT-PCR with a median parasite density amongst nucleic acid positive samples of 0.41parasites/uL. Of samples positive only for PfHRP2, 43% (43/100) were qRT-PCR positive with a median parasite density of 2.0p/uL. Of samples with the PfHRP2+/pAldo+/pLDH- profile, 96% (24/25) were qRT-PCR positive, and the median parasite density was 208p/uL. Samples positive for all three antigens were all qRT-PCR positive (100%, 20/20), with a median parasite density of 3,104p/uL. Concentrations of PfHRP2 (R^2 = 0.56), pAldo (R^2 = 0.64) and pLDH (R^2 = 0.64) increased linearly with increasing qRT-PCR-estimated parasite density. The multi-antigen profile predicted presence and amount of *P. falciparum* nucleic acids, suggesting the spectrum of the different kinds of parasite carriage in a population could potentially be characterized in an inexpensive and high-throughput manner simply by categorizing infections by multi-antigen profile. Determination of multi-antigen profile in additional populations and settings will provide data for assessing the method's utility.

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RAPID DIAGNOSTIC TESTS FOR MALARIA - SEVEN YEAR RESULTS FROM A PROFICIENCY TESTING SCHEME RUN BY UK NEQAS

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Rapid Diagnostic Tests (RDTs) have become increasingly popular in the laboratory diagnosis of malaria because they are low technology, require less expertise than blood film examination and performing the test is faster than making, staining and examining blood films. Since RDTs are being used more frequently in clinical laboratories particularly by on-call personnel and as UK NEQAS aims to ensure that participants have access to specimens that are relevant to their current laboratory practice, UK NEQAS Parasitology established an ISO 17043-2010 accredited Scheme for RDTs for malaria. We present findings from the first 7 years' performance of participants. The overall performance for specimens to date is good with a mean of 91% correct results from participants. On an average 4% of participants reported false negative results for specimens containing *Plasmodium* antigens and 2% reported false positive results for specimens containing no malaria antigens. More false negative results were reported when non-*P. falciparum* antigens (11%) were present than when *P. falciparum* antigens (1.6%) were present. The scheme has highlighted problems with the reporting of false positive and false negative results particularly in the detection of non-*P. falciparum* species. We discuss whether these problems could be kit related or operator related. Furthermore, to reduce the chance of antigen degradation potentially encountered by the EQA samples during transit to the participating laboratories, the UK NEQAS team investigated the feasibility of using freeze dried blood as sample matrix. Results of those analyses will also be presented here and will illustrate that freeze dried blood is a suitable matrix as the performance of freeze dried blood is at par with liquid blood with respect to performance at various parasitaemia levels, identifying different malaria species and suitability of use with various RDT kits.

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EVALUATION OF THE PERFORMANCE OF ULTRA-SENSITIVE RDT COMPARED TO CONVENTIONAL RDT AND ULTRA-SENSITIVE QPCR FOR THE DIAGNOSIS OF MALARIA

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An ultra-sensitive malaria rapid diagnostic test (us-RDT) for the detection of low malaria parasite densities in the field was recently developed, but its usefulness to aid in elimination efforts is disputed. The diagnostic performance of us-RDT was evaluated against conventional RDT (conv-RDT) and ultra-sensitive qPCR (us-qPCR, sensitivity: 0.1 parasites/µl [p/µl]) in 3004 Tanzanian children (age 2-59 months) and 515 adults (age 18-80 years) presenting with fever (≥37.5 °C) to outpatient clinics. Venous blood samples were retrospectively examined with these different diagnostics. For us-qPCR, infections with parasite densities below 10 p/µl were scored positive only if detected in two replicate DNA extracts. conv-RDT detected *P. falciparum* in 232/3004 children (8%) and 35/515 adults (7%). us-RDT detected an additional 10 children (0.3%) and 2 adults (0.4%). us-qPCR

detected an additional 81 children (3%) and 11 adults (2%). Diagnostic sensitivity of conv-RDT and us-RDT against us-qPCR was 73% and 74%. Diagnostic specificity of both RDTs was above 99%. Half of us-RDT positive/conv-RDT negative samples (6/12) were also us-qPCR negative. Geometric mean parasite densities by us-qPCR were higher in children compared to adults (3'108 p/µl vs 1'101 p/µl). Particularly in children, the distribution of parasite densities was skewed towards high densities (children: median 45'132 p/µl, interquartile range [IQR] 12-376'759 p/µl; adults: median 1781 p/µl, IQR 27-8'7812 p/µl). Both, conv-RDT and us-RDT, detected practically all infections with densities above 100 p/µl (conv-RDT: 98% positive, 236/242; us-RDT: 98%, 237/242). In infections with densities below 100 p/µl the proportion of RDT-detectable infections increased from 19% (25/130) using conv-RDT to 23% (30/130) using us-RDT. In conclusion, the majority of *P. falciparum* infections in febrile children and adults are readily detectable by conv-RDT and the gain achievable by applying us-RDT is low. Additional us-RDT positive results may represent residual circulating antigen rather than active PCR-detectable *Plasmodium falciparum* infections.

1019

DIAGNOSTIC PERFORMANCE OF THE XN-30 HEMATOLOGY ANALYZER FOR DETECTION OF *PLASMODIUM* PARASITEMIA IS COMPARABLE TO BLOODSMEAR MICROSCOPY: VALIDATION FROM A FIELD STUDY IN NANORO, BURKINA FASO

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There is a lack of easy-to-use, affordable and accurate diagnostic tools that directly detect *Plasmodium* in endemic areas. We previously demonstrated that the novel Sysmex hematology analyzer (XN-30) can reliably detect and quantify parasitized erythrocytes in-vitro and in a controlled human malaria infection trial by using blue laser technology and flow cytometry (Limit of Detection 20 parasites(p)/µl). Next, a phase-3 diagnostic trial was performed at the district hospital of Nanoro between March 2016 - July 2017, to validate the XN-30 for detection of *Plasmodium* parasitemia in a malaria endemic setting. Patients with acute febrile illness aged > 3 months were included. XN-30 and thick bloodsmear microscopy (BS) were performed at inclusion. *Plasmodium* qPCR was performed in batch on stored whole-blood after trial conclusion. Researchers were blinded until all results were obtained. BS was used as reference standard, XN-30 results were also compared to qPCR. In total 916 patients were included. *Plasmodium* was detected in BS in 244 cases: n=229 *P. falciparum*, one *P. malariae*, two *P. ovale* and 12 mixed infections. Parasite density ranged from 24 - 491,802 p/µl (IQR 745 - 77,055). Compared to BS, sensitivity and specificity of XN-30 was 98.8% and 99.0% respectively, with positive- and negative predictive value of 97.9% and 99.4% (AUC 0.99). An additional 165 cases were detected by qPCR, 20 with parasitemia of >10 p/µl. XN-30 detected *Plasmodium* in 30 cases missed by BS. Three BS positive cases were missed by XN-30, two with low parasite density (<20 p/µl in PCR) and one with high parasite density. The latter was most likely a sample mix-up. Correlation in parasite density between smear and XN-30 was 0.81. Costs of XN-30 will be comparable to rapid diagnostic tests. In conclusion, the diagnostic performance of XN-30 to detect *Plasmodium* parasitemia in a malaria endemic setting is comparable to BS but with higher sensitivity to low parasite densities. Hematology analyzers are used globally. Implementation of the XN-30 in malaria endemic areas will provide basic hemocytometry data as well as an effective and affordable tool to detect and follow-up malaria.

1020

PLATFORM FOR PROSPECTIVE MULTIPLEX SEROSURVEILLANCE MONITORING ESTABLISHED IN SENEGAL

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Malaria control interventions have resulted in a dramatic decline in malaria In Senegal during the past decade, with parasite prevalence declining from 6% in 2008 to less than 1% in 2016 in nationwide surveys. However, reported incidence has not declined appreciably since the roll out of rapid diagnostic tests (RDTs) in 2008 (26 per 1000 residents in both 2010 and 2017), as data completeness, care seeking, and testing rates improved. Given the low parasite prevalence and challenges interpreting health facility surveillance data, other methods are needed to monitor changes in malaria transmission. In 2017, the National Malaria Control Program began collection of dried blood spots on filter paper from patients aged 0-19 years seeking care for febrile illness (and who thus received an RDT) at 24 malaria sentinel sites throughout Senegal. Multiplex serology was used to measure prevalence of long half-life (MSP1) IgG antibodies to all four species of human malaria, as well as short half-life (LSA1) IgG against *P. falciparum,* from 1380 samples collected October 2017 to January 2018. Detection of antibody to vaccine preventable pathogens, relevant parasitic pathogens, and other pathogens of public health interest (dengue) was also performed. RDT positivity in febrile patients nationwide was 9%, ranging from 1% in the low transmission north to 34% in the southeast, and ranging from 5% among children < 5 years to 13% among children 15-19 years. Seroprevalence of antibodies to PfMSP1 was 28% nationwide, ranging from 15% among children under 5 years to 46% among children 15-19 years, and ranging from 18% in the north to 60% in the moderate transmission southeast. Analysis of other antibodies is ongoing. Seroprevalence of antibodies to *Plasmodium falciparum* may provide more information to measure changes in malaria transmission than currently used methodologies. While not nationally representative, this serosurveillance platform, built on a network of sentinel sites from which epidemiological data have been collected for many years, will allow the Ministry of Health to prospectively monitor seroprevalence of antibodies to pathogens of interest.

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MAKING THE CASE FOR REGIONALLY TAILORED MALARIA CONTROL INTERVENTIONS ACROSS THE DIVERSE ISLAND OF MADAGASCAR

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Malaria remains a major burden in Madagascar despite decades of control efforts. Reflecting the island's ecology, malaria epidemiology is highly diverse, encompassing all phases of the control spectrum. Major disparities exist in access to healthcare, frequently impeded by neglected infrastructure. This overall heterogeneity is not sufficiently reflected in current National Malaria Control Programme (NMCP) policy, which remains rooted to a sub-national stratification from the 1990s with policy mainly driven by a dichotomous divide between "high" and

"low" transmission districts. Through collaboration with the NMCP, we have developed an updated stratification reflecting current transmission patterns, and conducted an analysis of routine case data to characterise the sub-national epidemiology according to the new stratification. Complementary to this, high resolution malaria prevalence maps were derived from nationally representative Malaria Indicator Survey datasets to allow quantitative assessments of spatio-temporal trends in infection prevalence, a dataset independent of the biases associated with routinely reported clinical case data. Results from these two analyses indicated recent resurgences in transmission, with prevalence more than doubling between 2011 and 2016 in all parts of the country. High resolution spatio-temporal epidemiological analyses can help identify vulnerable regions and populations, supporting efforts to tailor control interventions to local needs. Public health in Madagascar remains precarious, largely externally funded. Nevertheless, the country has stated its objective for malaria pre-elimination status by 2022. The path to elimination differs across the country, driven by complex constellations of factors far more complex than current disease incidence levels which are commonly relied on exclusively. The different threads of this collaboration between the NMCP implementers and the geospatial modelling combine to support epidemiological analyses and policy-setting, with a priority placed on the need for targeted high-resolution programme implementation.

1022

A SYSTEMATIC REVIEW AND META-ANALYSIS ON THE GLOBAL PREVALENCE OF *PLASMODIUM KNOWLESI*, *P. MALARIAE*, AND *P. OVALE*

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The World Health Organization (WHO) recommends the parasitological confirmation of all suspected malaria cases using microscopy or quality-assured rapid diagnostic tests (RDTs) before administering antimalarial treatments. However, misclassification of rare *Plasmodium* species such as *Plasmodium knowlesi (Pk*), *Plasmodium malariae (Pm)*, and *Plasmodium ovale* (*Po*) is common by conventional microscopic examination. Moreover, in a recently published systematic review, we showed that malaria RDTs show suboptimal performance for detecting *Pk*, *Pm*, and *Po* mono-infections in human blood. Therefore, national surveillance systems which rely on identification of *Plasmodium*-positive cases by microscopy or RDTs do not provide reliable data on the contribution of these rare species to the global burden of malaria. Therefore, we are currently performing a systematic review and meta-analysis to estimate the global prevalence of human malaria infections caused by *Pk*, *Pm*, and *Po* as identified using molecular diagnostic methods. A systematic search of MEDLINE, EMBASE, Web of Science and CENTRAL databases was performed to identify published studies dating from 1990 which reported data from community surveys of the prevalence of *Pk*, *Pm*, and *Po* species. A total of 6,227 titles were identified to be screened. At the end of the screening process, data from publications eligible for the inclusion in the review will be extracted and sorted by pre-defined variables by two independent investigators. Prevalence data for each *Plasmodium* species will be pooled for each country, and a random effects model will be used to calculate pooled prevalence estimates. Cochrane's Q test and Higgins' I^2 statistics will be used for the assessment of heterogeneity. Sub-group analysis based on the geographic regions, study time periods, and age groups will be conducted to address sources of heterogeneity. This will be the first study to report on the global prevalence of these rare *Plasmodium* species. The data compiled in this review will enable a better understanding of the global malaria burden imposed by *Pk*, *Pm*, and *Po* and galvanize interest further data collection.

1023

SPATIAL EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE IN THE DEMOCRATIC REPUBLIC OF CONGO

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Drug resistant malaria is a growing concern, particularly in the Democratic Republic of the Congo (DRC) where malaria prevalence is 30%. Molecular markers for resistance allow us to monitor the spread of resistant alleles over space and time, and are a critical tool in halting transmission. Previous studies have indicated that sulfadoxine/pyrimethamine (SP) and chloroquine resistant parasites are spatially clustered in the DRC. This study explores how these spatial patterns have changed over time in order to understand how drug resistant malaria may be spreading within the DRC. We selected 540 children with PCR-detectable *Plasmodium falciparum* infection from the 2013 DRC Demographic and Health Survey (DHS). Using molecular inversion probes, we identified known variants in the *pfdhps* and *pfcrt* genes that are associated with SP and chloroquine resistance, respectively. We compared the proportion of single and multiple mutant parasites to those previously reported from 230 adults selected from the 2007 DHS. We used multi-level logisitic models to determine the association between individual and community level factors and the odds of carrying a resistant allele. Finally, we used Markov chain Monte Carlo to fit a spatio-temporal model to the observed data, providing smooth allele frequency estimates over space and time while taking covariates into account. The prevalence of both *pfdhps* and *pfcrt* resistance mutations increased slightly between 2007 and 2013. Additionally, the proportion of double and triple *dhps* mutants increased significantly (p=0.02). The spatial distribution of these mutations highlights hotspots of resistance, with *pfdhps* concentrated in the northeast and *pfcrt* spread across the middle of the country. The spatio-temporal model suggests that the ranges of these hotspots shifted between 2007 and 2013. This study reveals the changing prevalence and geographic distribution of SP and chloroquine resistant *P. falciparum* within the DRC. Close monitoring of resistance with molecular techniques will be a vital part of designing effective intervention strategies to control the spread of drug resistance.

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VALIDATING NOVEL SEROLOGICAL MARKERS OF MALARIA EXPOSURE: EVALUATING THE EFFECT OF MASS DRUG ADMINISTRATION (MDA) AND SEASONAL MALARIA CHEMOPREVENTION (SMC) ON TRANSMISSION IN RURAL GAMBIA BASED ON POPULATION-LEVEL ANTIBODY RESPONSES

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Recent studies have identified a number of novel serological markers highly correlated with recent malaria infection. However, limited studies have assessed their potential use in surveillance or cluster trial evaluation. Using a multiplex immunoassay (MAGPIX) with a panel of 25 *Plasmodium falciparum* antigens, we quantified IgG antibody responses in an all-age longitudinal cohort of 2,688 individuals in rural Gambia as part of a countrywide mass drug administration (MDA) from 2013-2015. We

estimated antibody decay rates following infection and the association between antibody intensity and time since last infection. Combined antibody responses to several antigens (Etramp5.Ag1, HSP40, GexP18, Rh2.2030 and EBA175) showed strong sensitivity and specificity as markers of patent and sub-patent malaria infection in the last 30-150 days. Receiver Operating Characteristic (ROC) analysis showed that Area Under the Curve (AUC) values for ages 1-5 years and 6-15 years were 0.85 (95%CI 0.72-0.91) and 0.85 (0.77-0.92), respectively, but slightly lower for individuals aged greater than 15 years (AUC 0.72 [0.62-0.82]). Antibody responses in the Upper River Region of Gambia following the 2014 transmission season were lower in villages receiving all-age MDA (*N*=823) compared to villages receiving seasonal malaria chemoprevention (SMC) in children under five (*N*=1,155). Seroprevalence to Etramp5.Ag1 was 4.0% (95%CI 3.6-4.5) in MDA villages compared to 8.8% (8.1-9.5) in SMC villages. Similarly, seroprevalence to EBA175 was 4.1% (3.5-4.5) and 8.3% (7.6-9.0) in MDA and SMC villages respectively, and 4.4% (3.8-4.9) and 7.2% (6.6-7.9) to Rh2.2030. Age-adjusted cumulative antibody intensities to several antigens (Etramp5.Ag1, Rh2.2030, EBA175, MSP1.19 and GLURP) were also lower in MDA villages. Findings suggest these biomarkers could have application in routine surveillance or clinical trials to measure short-term changes in malaria transmission. Future research will need to investigate geographical variation in antibody response, determine optimal seropositivity thresholds by antigen and age, and validate their use in cluster-randomised trials.

1025

EFFECT OF REPEATED BED-NET DISTRIBUTIONS AND IMPROVED TREATMENT ON MALARIA INCIDENCE IN SEVEN SENTINEL SURVEILLANCE SITES IN PAPUA NEW GUINEA

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The diverse social and ecological environments in Papua New Guinea (PNG) are echoed in intricate human host-parasite-vector interactions, resulting in a complex malaria epidemiology. Increased coverage with long-lasting insecticidal nets (LLIN) and improved diagnosis and treatment (artemisinin-based combination therapy or ACT) have reduced malaria prevalence in PNG since 2008. Yet, national trends in malaria incidence are inconclusive and difficult to interpret due to confounding effects of the scale-up of Rapid Diagnostic Tests (RDT), changes in reporting forms, and inconsistencies in routine reporting. We analyzed malaria incidence trends between 2010 and 2014 in seven sentinel surveillance sites to provide a better understanding of the impact of interventions (LLIN and ACT) in epidemiologically distinct settings across PNG. The analysis included over 34,000 fever cases diagnosed by RDT and light microscopy. Intervention effects on malaria incidence were quantified using multivariate regression models. We found that each site showed a distinct incidence pattern over time. Age distribution of positive cases significantly varied between sites and after each LLIN distribution. Repeated LLIN distributions showed cumulative effects further reducing the number of malaria cases with each round in all sites but one. In comparison ACT introduction only explained a small fraction of the observed reduction of malaria cases. *P. falciparum* remained the dominant source of clinical malaria in all sites. In general terms, our findings confirm the impact of LLIN on malaria incidence in PNG. However we also found significant heterogeneity between sites including an apparent reduction of the effect of interventions in a site in which early and outdoor biting of *Anopheles* mosquitoes has been identified as a threat to the effectiveness of LLINs. Additional targeted interventions seem essential for more effective control and acceleration towards malaria elimination in PNG.

1025

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HOST AND PARASITE FACTORS ASSOCIATED WITH *PLASMODIUM FALCIPARUM* INFECTION PERSISTENCE AND GAMETOCYTE PRODUCTION DURING THE DRY SEASON IN THE GAMBIA

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Malaria in The Gambia is seasonal, with virtually all clinical cases occurring during the four month wet season. Some *Plasmodium falciparum* infections may persist the subsequent dry season and constitute the human infectious reservoir at the moment the transmission season starts. It is currently unknown what human and parasite factors are associated with infection persistence and gametocyte production. We established a cohort with 60 *P. falciparum*-positive Gambian participants at the start of the dry season who were bled monthly for 6 months. In collaboration with MalariaGEN, parasite genomes were sequenced to investigate genetic diversity. Gametocyte production and sex ratio was assessed by qRT-PCR targeting male (PfMGET) and female (Pfs25) transcripts and related to total parasite density assessed by varATS and SBP1 qPCR. Naturally

acquired antibody responses to a panel of pre-erythrocyte, blood stage and sexual stage antigens is currently being assessed by luminex and protein microarray. We observed a striking dichotomy in parasite carriage times with 42.5% (17/40) of subjects carrying parasites until the end of dry season (6 months) whilst other subjects typically carried parasites for <2 months. Infections were almost exclusively single clone infections and all infections had detectable gametocytes at one or more occasions. The mean male:female sex ratio was 0.23 (95% confidence interval: 0.17-0.29) and this ratio remained stable during the season and was not associated with gametocyte density. Plasma samples, taken repeatedly, are currently analysed and related to duration of infection. Whilst some infections are cleared rapidly after the transmission season ends, others persist throughout the dry season and continue to produce gametocytes at a sex ratio that is considered suitable for onward transmission. Parasite complexity of infection and host age were not obvious determinants of infection duration; the human antibody profile is current being compared between individuals who rapidly cleared infections and those who retained their infections throughout the dry season.

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THE FORMULA "AVERAGE + 2 X STANDARD DEVIATION" APPLIED TO THE FOLLOW-UP OF MALARIA EPIDEMIC WARNING LEVEL AND COMPARISON WITH THE FORMULA OF THE "SIMPLE AVERAGE" THROUGH 18 SITES SENTINELS OF SURVEILLANCE IN SENEGAL FROM 2016 AND 2017

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To predict and control malaria epidemics, the Senegal National Malaria Control Program (NMCP) developed an epidemiological surveillance system of rapid detection. This malaria sentinel system, set up in 2008, includes 24 sites, 18 of which are distributed in low to moderate transmission zones at risk for epidemics. Weekly, the selected health facilities (sites) report the total patients consulted; suspected malaria cases, patients tested by rapid diagnostic tests (RDT), and confirmed malaria cases. Since 2009, 100% of suspected malaria cases have been tested by RDT, and 100 % of positive cases received artemisinin-based combination therapy. In 2016, the NMCP introduced a method of calculating epidemic threshold using historical data for each site dating back five years. For every site, a curve of the epidemic threshold curve is drawn, using the following formula: weekly average of the confirmed cases for a given epidemiologic week over the previous five years plus two standard deviations. In any site, if the threshold is met or exceeded, a systematic documentation of the reported cases is made and an investigation of cases is triggered. During 2016 and 2017, the reported cases were systematically compared weekly with the epidemic threshold curve and the same epidemiological week from the previous year. This formula proved to be very sensitive, with detection of 99% of potential epidemic situations, in contrast with the direct comparison with the previous year's epidemiological week or even compared to using a threshold based on a formula of simple averages. In zones of moderate or high transmission, specific actions are taken based on the results of this documentation. In zones of low transmission, the threshold is one case, and every case is systematically documented and investigated. Since implementation, four sites reached or surpassed the epidemic threshold more than four times. Investigations showed that 80% of cases were imported, and 20% of autochthonous cases did not sleep under insecticide-treated nets.

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MONITORING IMPORTED MALARIA CASES IN THE STATE OF RORAIMA, BRAZIL

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In Brazil, three states are known to contribute a majority of the malaria infections reported in the country: Amazonas, Para, and Acre. Roraima State is considered low risk for autochthonous *Plasmodium falciparum* transmission with the majority of the infections being *P. vivax*. However, the state has recently seen an increase in the number of reported *P. falciparum* infections believed to be coming from Guyana and Venezuela. The objective of this study was to determine the prevalence of *Plasmodium* species among patients attending health clinics in Boa Vista (state capital), Rorainopolis and Pacaraima (a health post located in the Venezuelan border town) in Roraima State. Information about residence and travel history was collected from all patients. Microscopic diagnosis was made during presentation at the clinic and a blood spot was collected to confirm diagnosis and for species identification using PET-PCR. For PCR, samples were first screened for the presence of *Plasmodium spp; Plasmodium*-positive samples were evaluated for the four human infecting species. A total of 429 patients were enrolled between 2016-2017 in the three sites, Boa Vista (243), Rorainopolis (57), and Pacaraima (129). Twenty six percent reported that they were from Venezuela; 99% of these attended the health post in Pacaraima. Field microscopy identified 161 (38%) *P. vivax* infections, 114 (27%) *P. falciparum* infections and 6 (1%) mixed *P. falciparum* and *P. vivax* infections. Among 286 samples that were *Plasmodium* positive by PCR, 148 (52%) were positive for *P. vivax,* 117 (41%) were positive for *P. falciparum*, and 14 (5%) had mixed infections (*P. falciparum* and *P. vivax*); species could not be identified for 7 samples (2%). Based on residence/travel history, 234 of the 286 (82%) PCR positive patients were thought to have acquired infection in Venezuela or Guyana, including 111 (95%) of the 117 with *P. falciparum* infections. This finding needs to be considered when developing control interventions.

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INCREASING PREVALENCE OF *PLASMODIUM OVALE* INFECTIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO: INSIGHT INTO A NEGLECTED MALARIA SPECIES

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Of the five species of malaria capable of infecting humans, *Plasmodium ovale* has largely evaded the attention of the malaria research community. Consequently, little is known about the distribution and occurrence of ovale malaria in the high endemicity regions of sub-Saharan Africa. As falciparum malaria continues to decline in these regions, non-falciparum parasites, such as *P. ovale*, may become more important sources of malaria infections. We are using real-time PCR on 25,945 dried blood spots from the 2013-14 Demographic Health Survey (DHS) in the Democratic Republic of the Congo (DRC) to investigate the prevalence of *P. ovale* infections, its distribution, and risk factors for infection. Preliminary screening of 7,607 adult samples identified 213 positives, consistent with a prevalence estimate around 3%. This is considerably higher than our prior 0.4% prevalence estimate from the 2007 DHS. After completing the screening of all samples, the prevalence of infection by *P. ovale* and its subspecies, *P. ovale curtisi* and *P. ovale wallikeri,* will be mapped to examine the spatial

distribution of infections. Additional epidemiological analyses to evaluate risk factors for infection, including bed net use and co-infections with *Plasmodium falciparum* and *Plasmodium vivax,* will be performed. Insight into the epidemiology of *P. ovale* in the DRC is an important starting point for improving our understanding of the burden of non-*falciparum* malaria in one of the most malarious regions of the world.

1030

OPTIMIZATION OF THE ARTEMISININ RESISTANCE MAPS FOR COMMUNICATION TO THE POLICY MAKERS IN SOUTHEAST ASIA (UTILIZATION OF END-USER USABILITY ASSESSMENT AS PART OF HUMAN-CENTERED DESIGN)

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Despite maps having been used for centuries as visual tools for communication, analysis and planning, there are no studies that have shown how to produce efficient audience specific maps. In the current efforts to eliminate malaria in South-East Asia, there has been a significant effort of mapping the burden of artemisinin resistance, none of these maps however have been evaluated to see how they perform as intended. Since various maps are produced from different cartographic techniques, there is a threat of such maps leading to poor planning and hence jeopardise elimination efforts.This study aimed at introducing a technique of how to develop a cartographic framework for guiding such work by integrating the cartographic skills from published information, expert opinion and user characteristics to develop relevant maps to aid malaria elimination plan.As first part of the work, cartographic techniques from relevant articles were summarised and used to develop various maps using the malaria genetic markers data summarised by WorldWide Antimalarial Resistance Network (WWARN). In order to communicate the correct scientific information, expert opinion was sought to review the drafted maps and maps revision was done according to the feedback. Lastly, to incorporate the 'National Malaria Control Programme (NMCPs) personnel's specific features' and evaluate their understanding of the maps, a user-centred qualitative assessment was done to obtain a structured feedback. Despite few respondents were obtained, the analysed feedback was used to optimise maps to produce the final genetic artemisinin resistance maps for communication to the policy makers. This presentation aims at explaining this process, and how the obtained feedback was used to optimise the drafted maps to produce the final maps for the NMCP personnel in South-East Asia.

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NET INTEGRITY DOES NOT PREDICT *PLASMODIUM FALCIPARUM* INFECTION AMONG ITN USERS IN SOUTHERN MALAWI, 2012-2014

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Mass distribution campaigns have increased the use of insecticide treated nets (ITNs) in Malawi, but *Plasmodium falciparum* infection prevalence remains high even among those using ITNs. Previous studies have addressed barriers to ITN use but there is limited information explaining the high prevalence of infection among ITN users. We assessed the impact of ITN integrity on protective efficacy among self-identified ITN users who reported sleeping under a net the night before the interview. From 2012 to 2014, six cross-sectional surveys were conducted in the rainy and dry seasons in southern Malawi. Data were collected on ITN use, number of people sharing each ITN, ITN age, and ITN integrity. ITN integrity was measured based on the number of holes the size of a torch battery in each ITN. Blood samples were obtained from all subjects over 6 months of age, and microscopy and qPCR were used to detect *P. falciparum* infection. Mixed effect logistic regression was used to calculate odds ratios for infection and account for clustering at the household and community level. Overall, there were 9,646 ITN users, of whom 15% tested positive for *P. falciparum* infection. Among net users, 22% were children under 5 years, 29% were children 5 - 15 years, and 49% were 15 years or older. Over half of participants (55%) reported sleeping more than two household members under an ITN, of whom 1,565 (16%) reported sleeping 4 or more people under an ITN. Among net users, preliminary results indicate that after adjusting for sex, age, number of persons sharing a net, survey number, and open eaves, better ITN integrity was not significantly associated with a decreased risk of infection (OR: 1.09, 95% CI: 0.91, 1.31), suggesting that ITN integrity is not a strong predictor of ITN protective efficacy.

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ASYMPTOMATIC CARRIAGE OF *PLASMODIUM FALCIPARUM* AND NON-*P. FALCIPARUM* INFECTIONS IN INDIVIDUALS WITH AND WITHOUT HIV

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Individuals co-infected with HIV and malaria coinfection have have more episodes of clinical malaria and therefore more likely to have higher asymptomatic malaria carriage than non HIV infected individuals. However, while asymptomatic malaria represents the largest reservoir of malaria transmission, many of the of HIV malaria co-infections studies in sub Saharan Africa focus on *Plasmodium falciparum* infections, with little attention being paid to non-falciparum infections. Additionally, diagnosis of the different malaria species has been made difficult by mixed infections with other Plasmodium species and low parasitemia levels. We therefore sought to use sensitive molecular techniques to determine the point prevalence *P. falciparum, P. ovale* and *P. malariae* infections in HIV-infected and HIV-uninfected participants, as well as the overall study population. Using 1100 samples collected in a HIV/malaria co-infection study in a malaria endemic region in western Kenya, the presence of any species of malaria was first determined using an improved assay based on genus-conserved sequences of the Plasmodium 18S ribosomal gene. All infections with ≥1 parasite per 50ul sample were detectable by our assay. Second, if positive for malaria, panels of highly species-specific qPCR assays were developed to determine the presence and quantity of *P .falciparum, P. ovale* and *P. malariae* as single or mixed infections;We will report the point prevalence and magnitude of infection with single and/or multiple species in both HIV-infected and non-HIV-uninfected persons. So far, preliminary results show that our quantitative PCR assays are sensitive and can be used on samples from malaria endemic areas where high prevalence of sub-microscopic parasitaemia have been reported. The relevance of this study is that HIV-malaria co-infected individuals may be more efficient reservoirs of malaria and therefore there is need for higher public health priority of persons at risk for both diseases.

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MALARIA IN PREGNANCY: FROM PRE-CONCEPTION TO EARLY PREGNANCY, A GENOTYPING ANALYSIS

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In Sub-Saharan Africa, preventive strategies against malaria in pregnancy are based on long-lasting insecticide treated bed nets and intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine, which are usually not implemented before the 2nd trimester of pregnancy. Therefore, women remain unprotected or insufficiently protected against malaria during the first months of pregnancy, when malaria has been suggested to be particularly deleterious for the fetus. RECIPAL is a recently completed pregnancy cohort with pre-conceptional recruitment in Benin. From 2014 to 2017, 411 pregnant women were followed from the very first weeks of gestation until delivery, with monthly screening for malaria using microscopy and Polymerase Chain Reaction (PCR). We previously demonstrated that women were two times more likely to have microscopic malaria in early pregnancy than before pregnancy, and that these infections occurred as early as 5-6 weeks of gestation. The risk of malaria infection in the first trimester was highly predicted by microscopic infections occurring before conception, suggesting that malarial infections detected in the first trimester were already present before pregnancy. Our hypothesis is that these pre-pregnancy infections are submicroscopic (ie, detected by PCR but not by microscopy) and they become patent (microscopic) because of changes in women's physiology and immunity during early pregnancy. Using a fragment-analysis method, we are investigating if parasites detected before pregnancy and during the first trimester have the same genotypes. Three highly polymorphic markers in *Plasmodium falciparum* (*msp-1*, *msp-2* and *Glurp*) are used to determine all the genotypes present in each isolate. If this hypothesis is confirmed, our results may argue in favor of starting preventive strategies against malaria as early as the pre-conceptional period.

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HETEROGENEOUS DISTRIBUTION OF *PLASMODIUM VIVAX* AND *FALCIPARUM* CARRIAGE ON THE BORDER AREA BETWEEN BRAZIL AND FRENCH GUIANA

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French Guiana and its Eastern border with Brazil was considered as a low to moderate malaria transmission area. Recent population surveys with PCR analyses in low endemic settings area reported a majority of asymptomatic carriage. In the perspective of malaria elimination, characterization of the infectious reservoir is recommended. A cross-sectional survey was conducted between October and December 2017, enrolling inhabitants from a village bordering Brazil. The prevalence of *Plasmodium* spp. carriage was measured with Rapid Diagnostic Tests (RDT) and real-time polymerase chain reaction (PCR). Houses were georeferenced. Demographics, medical history, clinical and findings were reported and analyzed according to Plasmodium spp. carriage. Geographical clustering analysis (SaTScan) as well as multivariate risk factor analysis were also performed. PCR showed that 100 of 1,501 participants carried *Plasmodium* spp. , 90% *P. vivax* (Pv) and 10% *P. falciparum* (Pf). Only 13/1,549 people tested by RDT were positive. The final PCR positivity rate was 6.66% with a large variation between neighborhood from 0 to 29,5%. Seventy four percent of infections were asymptomatic. The risk of malaria carriage was significantly high in three neighborhoods (Trois Palétuviers: RR=7.16, p<0.01, Adimo: RR=12.43, p=0.046 and Blondin2: RR=5.15, p=0.002). In the multivariate analysis, an age over 15 years old, living in remote neighborhoods, a previous history of malaria, anemia and low platelets were independently associated with an increased odds of *Plasmodium spp*. Carriage. Bed net use was not associated with a reduced odds of carriage. Malaria carriers often lived in remote areas, close to the forest. The current study adds to available evidence on the wide-scale but heterogeneous spatial distribution of asymptomatic parasitemia in this area. Asymptomatic malaria is associated with anemia and low platelets and could have an impact on health. Further unravelling the epidemiology of malaria with early identification of symptomatic and asymptomatic infection can enable public policy to define interventions likely to accelerate elimination efforts.

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EFFECT OF CONTROL INTERVENTIONS ON THE MALARIA RISK PROFILE IN UGANDA

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Improved malaria control, predominantly including long lasting insecticidal nets (LLIN) and indoor residual spraying, has been associated with reduced malaria burden throughout Africa. However, less is known about how control efforts may modify associations between age and malaria risk. We investigated the possible impact of control efforts on these associations using routine malaria surveillance data in Uganda. We used 10 years of data from five government-run level IV health facilities in five areas with varied transmission intensity. Data included children 11 years or younger, who presented to outpatient departments with suspected malaria, and underwent laboratory testing using microscopy or rapid diagnostic tests. We divided data into three intervention-periods: 'limited intervention', 2007 - 2009; 'pre- 1st mass LLIN distribution', 2010 - 2013/2014; and, 'post- 1st mass LLIN distribution', 2013/2014 - 2017. Logistic regression was used to estimate associations between age and diagnostically confirmed clinical malaria, controlling for site, month, and diagnostic test used, after stratifying by intervention-period. A total of 338,590 children were seen during the study period: 88,362, 138,186 and, 103,829 during the limited intervention, pre- 1st mass LLIN distribution, and post- 1st mass LLIN distribution periods, respectively. Relative to the 0 - 1 year age group, the odds of having clinical malaria were highest among the 2 - 3 year age group (OR= 1.59 95% CI 1.51-1.68, p<0.001) during the limited intervention period, then among the 5 - 6 year age group (OR= 1.89 95% CI 1.80-1.99, p<0.001) during the pre-1st mass LLIN distribution period, and among the 6 - 7 year age group (OR= 2.48 95% CI 2.33-2.64,

p<0.001) during the post- 1st mass LLIN distribution period. These results show that as malaria control activities were scaled up, the age group with the highest risk of clinical malaria increased. Thus, policies and surveillance programs that have historically targeted children under the age of 5 years should consider broadening their scope to older age groups, such as school-age children, following expansion of control interventions.

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USING A DIRICHLET REGRESSION MODEL TO PREDICT THE SPATIAL DISTRIBUTION OF *PLASMODIUM FALCIPARUM* GENETIC CLUSTERS IN PAPUA NEW GUINEA

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Genetically distinct pathogen populations can occupy geographically distinct niches and may exhibit differing transmission dynamics. *Plasmodium falciparum* genetic diversity is indicative of endemicity, with high genetic diversity and limited population structure indicating extensive gene flow and mixing of parasite populations. As transmission decreases, parasite populations exhibit lower diversity due to increased inbreeding. Reduced gene flow results in population structure and emergence of distinct genetic clusters or 'demes'. *P. falciparum* genotype data, however, has not previously been used for spatial prediction of genotype clusters. This study uses a Dirichlet regression model to examine associations of *P. falciparum* genotype clusters from 27 survey locations across Papua New Guinea with latitude and longitude, elevation, population density and Euclidean distance to the coastline. Genotype clusters were determined based on ancestry co-efficients derived from Bayesian cluster analysis of 708 *P. falciparum* isolates genotyped with 154 single nucleotide polymorphisms (SNPs). The regression model was used to predict the spatial distribution of eight distinct genotype clusters of *P. falciparum* across PNG. Statistically significant associations were found between latitude and longitude and six genetic clusters. Four clusters were associated with distance to the coastline and population density, and two clusters associated with elevation. Genotype clusters were found to occupy distinct geographic niches in PNG including at least two populations on the mainland that are distinct from those of outlying islands. The results identify potential gene flow between neighbouring areas of high transmission connected by population movement. The definition of distinct demes divides the PNG parasite population into regional blocks that could be independently targeted for control and elimination. Defining the spatial distribution of parasite populations is essential for evaluating the efficacy of malaria control programmes and provides essential tools for planning targeted control efforts for malaria elimination.

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PREVALENCE OF PLACENTAL MALARIA AMONG PREGNANT WOMEN IN THE MIDDLE BELT OF GHANA

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Malaria in pregnancy is a major public health concern, causing maternal, foetal and infant morbidity and mortality. Placenta malaria (PM) is a complication of malaria in pregnancy which leads to adverse outcome in pregnancy such as stillbirth, low birth weight, preterm delivery, abortion and maternal anaemia. The study investigated the prevalence of PM among a cohort of pregnant women in the meddle belt of Ghana. Maternal peripheral blood, cord blood and placenta tissue samples were obtained from 1148 women for histopathological examination to ascertain the presence of malaria parasitization. This was one of the study procedures for the Ghana Randomised Air Pollution and Health Study (GRAPHS) which sought to examine the impact of cleaner cookstoves on child health. Each sample was categorized by a histopathologist as showing no infection, acute infection, chronic infection, or past infection based on a standard classification. The study was conducted in the Kintampo North Municipality and Kintampo South District located in the middle belt of Ghana from September 2013-March 2016. Among the 1,148 placenta tissues collected and analysed using placental histology, 24.3% (279/1148) showed histological evidence of malaria parasitization. Of the 279 PM cases, 21.9% (61/279) showed acute infection, 20.4% (57/279) showed chronic infection, while 63.8% (178/279) showed past infection. There is the need for the Ministry of Health and the Ghana Health Service to institute policies that improve the uptake of sulfadoxine-pyrimethamine during pregnancy to prevent PM. In conclusion, this study showed that the prevalence of PM in the middle belt of Ghana is still high especially for women with past PM infection.

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DEVELOPMENT OF SEROLOGICAL MARKERS FOR DETECTING RECENT EXPOSURE TO *PLASMODIUM VIVAX* MALARIA

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The future transition from malaria control to elimination will bring a number of challenges, one being the dominance of *Plasmodium vivax* in many regions with decreasing transmission. *P. vivax* cases are often asymptomatic with low-density infections, so escape detection via traditional surveillance methods. *P. vivax* hypnozoites (arrested liver-stages) cannot be directly detected using even sensitive molecular tools. Instead, it is proposed that hypnozoite carriers may be indirectly identified by measuring antibody responses to past blood-stage infections. A panel of over 300 serological markers was screened for ability to induce IgG responses in individuals exposed to *P. vivax* in Thailand and Brazil. The kinetic profile of these antibody responses over 9 months in the absence of recurrent infections was characterised. Candidate serological markers were down-selected to a panel of 60 based on high immunogenicity at the time of infection, and similar estimated antibody longevity between the two geographic sites. The down-selected panel of 60 proteins was further validated in three independent observational cohorts conducted in Thailand, Brazil and the Solomon Islands plus three panels of negative controls, constituting observations from more than 3,000 individuals. By measuring antibody responses at the last visit of these cohorts, and relating them to the time since last confirmed *P. vivax* infection, a strong association between antibody titer and time since exposure was confirmed. Classification algorithms were used for a final down-selection to a panel of 8 *P. vivax* proteins that can accurately classify whether an individual has had a *P. vivax* infection within the last 9 months or not. Cross-validated classification algorithms were developed that can identify individuals infected within the last 9 months with greater than 80% sensitivity and specificity. This technology can be used as the basis of a serological screening and treatment strategy allowing primaquine treatment for elimination of hypnozoites to be optimally targeted at only those individuals who need it.

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SUBMICROSCOPIC MALARIA INFECTIONS IN ASYMPTOMATIC INDIVIDUALS AT MILITARY HEALTH FACILITIES IN CENTRAL VIETNAM

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In Vietnam, malaria cases markedly decreased between 2000 and 2015 from about 210,000 to 13,000 cases (i.e., 94% reduction). The Vietnamese Ministry of Health has set a goal for the country to be free from malaria by 2030. To understand true infection rates, the prevalence of submicroscopic infections in asymptomatic individuals should be considered, so that malaria interventions programs such as mass drug administration can be evaluated. In assisting this process MIPM in collaboration with AAMI and NMRC-A conducted a cross-sectional malaria survey in people living in Gia Lai Province between December 2016 and January 2017. Training of MIPM personnel in molecular methods was conducted at AAMI to detect submicroscopic parasitemia. Asymptomatic individuals presenting at 20 military health facilities in malaria endemic areas near the border of Vietnam and Cambodia were invited to participate in the study and those who gave consent were given a questionnaire to obtain demographics data and information on the use of malaria prevention methods. Finger prick blood samples were collected on filter paper to determine the incidence of submicroscopic parasitemia for the five human *Plasmodium* species by PCR. The DNA extraction and multiplex PCR methods were optimized and the criterion for acceptance of the PCR results was a detection threshold of at least 2.5 parasites/μL of blood. PCR screening of blood spots from 3,298 asymptomatic participants by MIPM revealed 7.7% positive samples with 3.9%, 2.1% and 1.0% for *P. falciparum, P. vivax and P. malariae,* respectively, with the remaining 0.7% as mixed infections. This incidence is likely to be an overestimation and the results will be confirmed if at least 2 out 3 positive PCRs are achieved. The molecular markers of drug resistance in *P. falciparum* samples will be characterized and the final results on submicroscopic malaria prevalence and malaria exposure will be presented. Sub-clinical malaria represents a disease reservoir that should be considered when determining the malaria risk for naïve personnel, such as deploying military working in areas with low clinical, but higher sub-clinical malaria rates.

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PREVALENCE AND RISK FACTORS ASSOCIATED WITH MALARIA INFECTIONS IN AREAS WITH PERSISTENT TRANSMISSION FROM NORTHWESTERN AND SOUTHERN REGIONS OF TANZANIA

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Despite a recent decline of malaria in Tanzania, there is still high burden in the southern and nothwestern regions; despite high coverage of long-lasting insecticidal nets (LLINs) (ownership >80 and use >66%). This study asessed the prevalence and risk factors associated with persistence of malaria parasite burden in selected regions of Tanzania. A cross-section study was conducted in 8 districts from 4 regions of Kigoma, Geita, Mtwara and Ruvuma, between September and November 2017. Sixteen villages (2 in each district) were selected based on the burden of malaria reported in health facility records, and 120 households (HHs) were selected in each village for parasitological evaluation. All individuals from these HHs were assessed clinically and finger prick samples were collected for detection of parasites by malaria rapid test (mRDTs). Thin and thick smears were prepared for microscopy and demographic as well as socio-economic status (SES) data were also collected. A total of 7,313 individuals (from 2,527 HHs) with median age of 13.0 years were examined. The positivity rate (PR) by mRDTs was 33.3% (ranged from 21.9 to 41.1%) while the PR was 20.6% microscopy (varied from 27.3% to 8.0%); with the highest PR (by both mRDTs and microscopy) in children aged 5-14years (49.0% and 32.8%, respectively). After adjusting for age, sex, anaemia, family size, bed-net use, district and SES, the risk of carrying malaria parasites was lower in females compared to males (AOR= 0.55, 95% CI:= 0.48 – 0.64, p<0.001) but higher among individuals without bed-nets (AOR = 1.22, 95% CI:=1.09 – 1.38, p=0.001), and children aged 5-14yrs (AOR=2.26, 95% CI:=1.96 – 2.61, p<0.001) compared to 0-4yrs. The risk was lower among individuals from households with middle (AOR=0.89, 95% CI:=0.77 – 1.03,p=0.138) and high SES (AOR=0.81, 95% CI:=0.70–0.931,p=0.005).) Although the study was conducted during the low malaria transmission season, high prevalence was reported indicating that malaria is still a problem in the study areas. Targeted interventions are required in these and other areas with persistently high malaria transmission to reach the pre-elimination targets by 2020.

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USING ROUTINE ANTENATAL-CARE BASED RDT TESTING TO MEASURE POPULATION TRANSMISSION: THE KEY ROLE OF PREGNANCY-SPECIFIC PATTERNS OF SUSCEPTIBILITY AND IMMUNITY

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Increasingly, malaria endemic countries are routinely testing pregnant women for malaria using Rapid Diagnostic Tests (RDTs) at antenatal care (ANC). These tests are likely to be well distributed over time and space: ANC is available year-long and over 90% of pregnant women in these countries attend ANC. If routine RDT testing at ANC can be used to estimate general population prevalence, they would provide an invaluable resource to measure malaria prevalence at much higher spatio-temporal resolution than population-based surveys and more reliably interpret observed temporal trends within case-reporting systems. However, as with all sentinel surveillance approaches, it is necessary to consider and account for potential biases. We evaluated the key role of pregnancy-specific immunology in determining RDT performance and prevalence within ANC and explored how this relates to transmission and population prevalence by fitting a model of the transmission dynamics of malaria to RDT performance data outside of pregnancy and from trials of Intermittent Screening and Treatment in pregnancy. We estimate that, compared to parasite detection using RDTs in non-pregnant women, the odds of detecting a PCR-positive infection at enrollment in primigravidae using an RDT were 16.6 (14.3-19.2 95% C.I) times higher

but that pregnancy-associated boosting of sensitivity declines rapidly as a function of a woman's exposure to malaria in previous pregnancies, and when women are re-tested later in pregnancy. Through simulation we show that this effect is likely to explain, at least partially, why RDT prevalence in pregnancy matches or exceeds that in children in areas of low transmission but progressively becomes an underestimate in areas of higher transmission. Due to this boosting effect, we anticipate ANC prevalence trends will lag behind changes in transmission to a greater degree than those in the general population, an effect which appears in data comparing continuous surveys in Western Kenya. Future ANC-based surveillance approaches will need to account for these effects in order to provide reliable estimates of population-based transmission trends.

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PERSISTENT *PLASMODIUM FALCIPARUM* INFECTION IN NON PREGNANT WOMEN IS A RISK FACTOR FOR PREGNANCY-ASSOCIATED MALARIA

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Pregnant women are more susceptible to *Plasmodium falciparum* than before pregnancy, and infection has consequences for both mother and offspring. WHO recommends that pregnant woman in areas of transmission receive intermittent preventive treatment starting in the second trimester. Consequently, women are not protected during the first trimester, although *P. falciparum* infections are both frequent and harmful. A cohort of nulligravidae women was followed during subsequent pregnancy. Malaria was diagnosed by microscopy and PCR. Parasites were genotyped at polymorphic loci. Among 275 nulligravidae enrolled, 68 women became pregnant and were followed during pregnancy. Before pregnancy, *P. falciparum* prevalence rates were 15% by microscopy and 66% by PCR. Microscopic infections increased to 29% until IPT administration and their density has been increased by 20 fold on average. Conversely submicroscopic infections decreased in favor of more microscopic infections. Following IPT administration, all types of infections decreased, but increased again late in pregnancy. The risk of infection during pregnancy was higher in women with a microscopic (OR = 2.2, p = 0.001) or submicroscopic (OR = 1.52, p = 0.0001) infection before pregnancy. Most infections during pregnancy were persistent infections acquired before pregnancy. Using a transcription profiling analysis approach, we are investigating the molecular basis of *in vivo* switching of var genes expression. All of these results will be discussed during the congress with their involvement in the prevention strategies of placental malaria.

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RAPID INCREASE AND NORTHWARD SPREAD OF ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM* IN LAOS

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The emergence and transnational spread of artemisinin resistance in *Plasmodium falciparum* in the Greater Mekong Subregion (GMS) is a serious threat to malaria elimination in the region and could present a threat to malaria control in Africa. Recently, the Lao government adopted the ambitious goal of malaria elimination by 2030, for which monitoring of artemisinin resistant malaria within the country is indispensable. The objective of present study was to assess the distribution of K13 mutations in Laos, which are associated with artemisinin resistance. We collected 1,151 *P. falciparum* isolates from five southern provinces in Laos from 2015 through 2016, and one isolate from the northern-most province bordering China in 2017. We analyzed polymorphisms of the K13 gene and two flanking regions. Overall, 55.5% possessed non-synonymous K13 mutations (C580Y, P574L, R539T, Y493H). The frequencies of the K13 mutations were heterogeneous in the five southern provinces, but with a clear tendency showing the highest frequency in the south (72.5%) and to a lower degree when moving northward (28.0%). The one isolate from the Lao-Chinese border also possessed the C580Y mutation. Analysis of the flanking loci demonstrated that this isolate from the Lao-Chinese border was genetically very close to the resistant lineage originating from western Cambodia, where the first case of artemisinin resistance was reported. Artemisinin resistance was observed to be rapidly increasing and spreading northwards through Laos and has now reached the Chinese border. The Lao and Chinese governments, as well as the international community, should make dedicated efforts to contain the spread of K13 mutations within Laos and in the GMS to avoid its spreading to Africa.

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PRIORITIZING THE SCALE UP OF INTERVENTIONS FOR MALARIA CONTROL AND ELIMINATION

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The World Health Organization recommends a core set of intervention and treatment options for use against *Plasmodium falciparum* malaria. These consist of treatment, long lasting insecticide treated bed nets, indoor residual spraying and a number of chemoprevention approaches. Given the resource limitations many malaria endemic countries face the introduction and scale-up of intervention and treatment options must be prioritised. We combined estimates of the cost of different interventions with a mathematical model of intervention impact to estimate the most cost-effective prioritisation of interventions in different epidemiological settings. Starting from zero coverage, each intervention was assessed at fixed steps in coverage and the most cost-effective (as determined by the incremental cost effectiveness ratio, ICER) chosen. Across all settings, vector control was selected first as the most cost-effective initial intervention with treatment included once moderate levels of vector control were achieved. Prevention through vector control reduces the incidence of malaria, thereby allowing treatment to be implemented

more cost-effectively as well as reducing the stress on the health system. Chemopreventive measures were selected alongside treatment scale-up. It is vital that investments in the available tools be effectively prioritised to maximise the impact for a given investment. The cornerstones of malaria control: vector control and treatment, remain key to efforts, but questions of when to scale and when to introduce other interventions must be rigorously assessed. Strong surveillance is key to informing such prioritisation.

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AGE-RELATED RISK CLINICAL MALARIA OVER TIME IN A HIGHLAND AREA OF KENYA DURING A PERIOD OF DECREASING MALARIA TRANSMISSION FOLLOWED BY AN EPIDEMIC

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Continued progress towards global elimination of malaria requires additional knowledge into how to achieve zero transmission and maintain malaria-free regions within countries that have ongoing transmission in neighboring areas. We have conducted longitudinal surveillance of clinical and asymptomatic malaria in the Kenyan highland areas of Kipsamoite and Kapsisiywa, areas of of unstable transmission, since 2002. To investigate potential changes in clinical immunity over this time, we assessed age specific incidence of clinical malaria in this community from 2002-2017. Malaria incidence decreased after 2005, and an epidemic occurred in 2017. In 2002-2005, malaria incidence was highest in individuals <15 years old: malaria episodes/1000 person-years (incidence rate ratio [95% confidence interval]) compared to <5 year old group; <5 y, 59.0 (1); 5-14 y, 61.2 (1.03 [0.89, 1.20]); ≥15 y, 40.0 (0.71 [0.53, 0.96]). From 2007-2016, malaria incidence decreased markedly in all age groups after malaria indoor insecticide residual spraying campaigns, and was highest in individuals 5-14 years old: <5y, 4.9 (1), 5-14 y, 7.9 (1.63 [1.20, 2,23]), ≥15y, 4.8 (1.01 [0.74, 1.37]). During the epidemic in 2017, incidence was again highest in the 5-14 year olds and was higher in the 5-14 year olds and ≥15 year olds than the <5 year olds: <5y, 2.7 (1); 5-14 y, 21.0 (7.71 [2.51, 38.52]); ≥15y, 13.0 (4.78 [1.58, 23.65]). The study data suggest that in areas with a prolonged decrease in malaria transmission, delayed acquisition of immunity and increased exposure in older children and adults may lead to high malaria incidence in these groups during epidemics.

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THE EFFECT OF LAND USE CHANGE IN THE ABUNDANCE OF MALARIA CASES IN NORTHERN ARGENTINA

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Malaria is a parasitic disease widely distributed in tropical and subtropical areas of the world. The appearance of malaria cases is determined by multiple factors including changes in land use, and climatic and environmental variables, which are key factors contributing to the prevalence and transmission of the disease. Remote sensing allows for the integrated analysis of vector-borne diseases for the purposes of disease management and control. The aim of the present research was to determine whether there is a relationship between the emergence and abundance of malaria cases and changes in land use, and, in climatic/environmental variables provided by remote sensing in San Ramón de la Nueva Orán (northwestern Salta, Argentina). Random Effect Poisson Regression was used to analyze the relationships. The emergence of malaria cases was related to decreases in piedmont rainforest of the Yungas Ecoregion, and increases both in Maximum Normalized Difference Vegetation Index (NDVI) and in mean minimum temperature and relative humidity. These results were expected since environmental change derived from agriculture-driven deforestation together with climate change are likely factors driving transmission of this disease by affecting the biology and ecology of both vectors and malaria parasites.

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CONTRIBUTION OF ASYMPTOMATIC CARRIAGE AND IMPORTED MALARIA TO SUSTAINED RESIDUAL TRANSMISSION IN KWAZULU-NATAL, SOUTH AFRICA: A PROVINCE ON THE BRINK OF ELIMINATING MALARIA

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KwaZulu-Natal (KZN), one of South Africa's three malaria endemic provinces, has made impressive progress in reducing local transmission since the country's last major malaria epidemic in 1999. With only 33 locally-acquired malaria cases reported during the 2016/2017 season, KZN appears to be on the brink of halting local transmission. In an attempt to accelerate the province towards malaria elimination a community-based malaria prevalence survey was undertaken to identify factors contributing to sustained residual malaria transmission. The two primary research questions addressed by the survey were determining the (1) contribution of a potential asymptomatic/subclinical reservoir and (2) dynamics of malaria importation by travelers or mobile and migrant populations to the sustained transmission. The survey consisted to two parts: a) an assessment of malaria prevalence, malaria knowledge and recent travel among 3 000 individuals at 1 400 randomly selected, GPS-located households within areas identified as *Foci* of transmission, as well as 1 000 individuals visiting marketplaces along the border between KZN and Mozambique; and b) determining the prevalence and distribution of malaria vectors within the identified *Foci* of transmission. All consenting participants were tested for malaria by standard falciparum-specific rapid diagnostic test (RDT, First Response®) and highly-sensitive falciparum-specific RDT (Alere®), with filter-paper dried blood spots collected for downstream molecular analysis. Specific sources, sinks and trends in importation were evaluated by analysis of the participant travel history. Of the population surveyed, less than 2% (n=69) were found to be malaria positive by RDT. Interestingly no discordant results between the two different RDT brands being assessed were observed. All RDT-malaria-positive individuals were subclinical and reported recent travel to Mozambique. Nearly all (n=65) of the malaria-positive individuals detected were diagnosed at border markets. Data from this survey suggests local transmission in KZN is being seeded by malaria infections, primarily imported from Mozambique.

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TRANSCRIPTOME ANALYSIS BASED DETECTION OF *PLASMODIUM FALCIPARUM* DEVELOPMENT IN *ANOPHELES STEPHENSI* MOSQUITOES

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The *Plasmodium* life cycle within the *Anopheles* mosquito includes the gamete, zygote, motile ookinete, and the oocyst stage that supports sporogony and sporozoite formation. Currently, circumsporozoite protein (CSP) is the only marker used for detection of *P. falciparum* infection in mosquitoes. However, CSP is detectable only after day 7 post-infection. To identify novel *Plasmodium* biomarkers that can be used to develop a detection assay for assessment of *P. falciparum* infection in mosquitoes throughout the development cycle of the parasite within the mosquito, we mapped the *P. falciparum* transcriptome as the parasite progresses through the oocyst stage of development on days 2, 4, 6, and 8 post-*P. falciparum* infectious blood meal. Using this genomics approach, we identified 212 novel transmission stage biomarkers including genes that are developmentally expressed at a single time point and genes that are pan-developmentally expressed at all four time points in *P. falciparum* oocysts. Validation of a small subset of genes at the transcriptional and translational level resulted in identification of a signature of proteins that can detect parasites within the mosquito as early as day 2 post-infectious blood meal and can be used to distinguish early versus late stage *P. falciparum* oocyst development in the mosquito. Our results open the prospect to develop a non-CSP based Western blot assay for detection of *P. falciparum* infection in mosquitoes and evaluate the effect of intervention measures on malaria transmission in an endemic setting.

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RELATIONSHIP BETWEEN GENOTYPE COMBINATIONS OF *NFKB1* AND *NFKBIA* PROMOTER POLYMORPHISMS AND CHILDHOOD *PLASMODIUM FALCIPARUM* SEVERE MALARIAL ANEMIA

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The pathophysiological basis of *Plasmodium falciparum*-induced severe malarial anemia [(SMA, hemoglobin (Hb)<5.0 gdL^{-1} with any density parasitemia)] is multigenic - both host and parasite genetic factors contribute to the pathogenic spectrum. Transcription factors regulate cellular processes, including immunity. Nuclear factor kappa B (NFκB) and its inhibitor (IκB) are important in infectious and autoimmune diseases through their control over cellular processes. However, the effects of promoter polymorphisms within *NFκB1* and *NFκBIA* (an IκB gene) on susceptibility to SMA remain unreported. We tested the hypothesis that promoter polymorphisms in *NFκB1* and *NFκBIA* alter susceptibility to SMA in pediatric populations in holoendemic *P. falciparum* transmission regions. Genotyping was performed using TaqMan® assays, while gene expression analysis measured using qPCR. Logistic regression analyses (controlling for potential confounders) were used to determine the relationship between promoter polymorphisms in *NFκB1* (rs747559, G-8079A and rs980455, C-3297T) and *NFκBIA* (rs2233406, G-826A and rs2233409 G-310A), and susceptibility to SMA in children with malaria children (n=1,026; age=6-36 months). Results demonstrate that combined genotypes: *NFκB1*-8079AA/*NFκBIA*-826GA (OR=2.31, 95%CI: 1.30-4.08, *P*=0.004), *NFκB1*-3279TT/*NFκBIA*-826GA (OR=2.77, 95%CI: 1.10-6.97, *P*=0.031), and *NFκB1*-3297TT/*NFκBIA*-310GG (OR=2.10, 95%CI: 1.32-4.10, *P*=0.002) were associated with increased susceptibility to SMA. In addition, *NFκB1*-3279CC/*NFκBIA*-826GG (OR=0.69, 95%CI: 0.48-0.96, *P*=0.033) and *NFκB1*-3297CC/*NFκBIA*-310GG (OR=0.64, 95%CI: 0.44-0.92, *P*=0.016) combinations were associated with protection against SMA. *NFκBIA* expression levels were 1.6-fold higher in SMA; [mean (SEM)]; [3.37 (± 0.34), n=28] vs. non-SMA; [2.18 (± 0.46) n=28, *P*=0.045]. Results presented here demonstrate that promoter polymorphisms within NFκB/IκB gene pathways influence multigenic diseases, such as childhood SMA.

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USING AMPLICON DEEP-SEQUENCING TO DETERMINE THE ETIOLOGY OF RESIDUAL ASYMPTOMATIC PARASITEMIA FOLLOWING HIGHLY EFFECTIVE INDOOR RESIDUAL SPRAYING IN A PREVIOUSLY HIGH TRANSMISSION SETTING IN UGANDA

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Currently there is limited understanding of to what degree asymptomatic infections with malaria parasites represent persistent infections of long duration vs. new infections from ongoing low-level transmission or importation. Characterizing the reservoir of asymptomatic parasitemia that persists following effective indoor residual spraying (IRS) is imperative to inform additional interventions needed for durable malaria control and eventual elimination. Data and samples come from a cohort of children and adults followed in Nagongera, Uganda from Oct 2011 to June 2017. Three rounds of IRS were conducted during this time, starting in Dec 2014. Active surveillance for parasitemia was conducted using both smear microscopy and loop mediated isothermal amplification (LAMP) to assess for submicroscopic parasitemia on all participants every 3 months from Aug 2011 to June 2017. In this study, we will extract DNA from RBC pellets collected from all participants with microscopic or sub-microscopic parasitemia, starting 6 months before IRS started (May 2014) and continuing through June 2017. This will include approximately 2000 samples from 94 patients that have at least 2 episodes of parasitemia recorded after the third round of IRS. We will then utilize an amplicon-based deep sequencing method targeting a highly variable (He=0.94) region of *P. falciparum* apical membrane antigen 1 (AMA-1) to genotype parasite clones present in these individuals at every time point. Persistent infection will be defined as a distinct clone being present in that participant for at least 6 months (i.e., detection of the same haplotype for at least 6 months), whereas new infections will be defined as those present for less than 6 months. Each infection detected will be defined as a binary variable (persistent vs. new), and we will determine what proportion of infections are persistent and how this changes over time, starting in Dec 2014. We will use generalized estimating equations to account for repeated measures within each participant and hypothesize that after 3 rounds of IRS, the majority of the parasite reservoir consists of persistent infections.

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AFRICAN POPULATIONS OF *PLASMODIUM FALCIPARUM* UNDERGO FLUCTUATIONS IN ALLELE FREQUENCIES ACROSS THE GENOME OVER TIME

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Plasmodium falciparum populations can differ greatly in genetic composition and extent of genetic diversity, resulting in significant population structure between and within geographic regions. While diversity between geographic regions is well documented, few studies have examined how parasite populations change over time in a given location. Characterization of these temporal dynamics might inform predictions or measures of the success of interventions, including antimalarial drug use and vaccines. To address this knowledge gap, 164 samples from a single village in Mali (collected in 2002 and 2010) and from southern Malawi (collected in 2007-2008 and 2016) underwent whole-genome sequencing, and the evolution of parasite populations was investigated using biallelic SNPs and indels. Regions of the genome with significant change in allele frequencies across timepoints were identified, with patterns congruent with positive selective pressure. Loci associated with resistance to antimalarial drugs, such as chloroquine and sulfadoxine-pyrimethamine, showed distinct signs of positive selection at one or more timepoints, highlighting the ability of population genetic analyses of field samples to detect the impact of public health interventions (such as changes in drug policy). Cross-population extended haplotype homozygosity tests identified genomic regions in both Mali and Malawi with evidence of selective sweeps; some of these selection signals were common to both geographic regions, and some have previously been identified in genome-wide association studies of artemisinin resistance in Southeast Asia. A region on chromosome 14 with signs of a selective sweep was also near a window with high F_{ST} containing a protein kinase. Finally, while many genes encoding antigens were under strong balancing selection, there was little evidence that allele frequencies at these loci (including leading pre-erythrocytic vaccines candidates) changed during the study period. These data provide a baseline of allele frequency changes against which the selective effects of public health interventions can be measured as they are deployed.

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POPULATION GENOMICS OF *PLASMODIUM VIVAX* IN PANAMA FROM SELECTIVE WHOLE GENOME AMPLIFICATION OF CLINICAL SAMPLES

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Malaria incidence in Panama has increased in recent years (~500 cases in 2010 to ~900 cases in 2016), despite elimination efforts, with the majority of cases caused by *Plasmodium vivax*. Notwithstanding the increase in cases, overall prevalence remains low (less than 1 case per 1000 persons). Genome sequencing of *P. vivax* parasites can inform epidemiology, population structure,and patterns of drug resistance. However, it can be difficult to sequence *Plasmodium* genomic DNA from patient blood samples due to the predominance of host DNA. Selective whole genome sequencing (SWGA) mitigates this problem by priming a highly processive polymerase from motifs that are common in the target genome and rare in the background gist genome. We used SWGA to sequence 60 *P. vivax* samples from Panama to gain insight into the population structure and transmission dynamics of the parasite, and 39 yielded usable sequencing data. Samples having a parasitemia greater than 120 parasites/ul exhibited at least 3X mean coverage at variant sites. There was variance in coverage across the genome, with the subtelomeres exhibiting low coverage across all samples. All of the successfully sequenced samples appeared monoclonal on the basis of low heterozygosity and allele balance. We observed low differentiation of parasites among districts via identity by state-based clustering and principal component analysis, and samples did not exhibit structure with respect to their position north or south of the Panama canal. More than half of the samples exhibited high genetic similarity to each other, with a small cluster of distinct samples in the Chepigana district. This suggests overall low genetic diversity, with distinct subpopulations driven by clonal transmission. We also used identity by descent (IBD) to explore patterns of recent common ancestry among the samples. This analysis confirmed the low haplotypic diversity in this parasite population and clarified spatial and temporal patterns of parasite relatedness resulting from transmission history. This work illustrates how SWGA data and IBD analysis can inform malaria epidemiology in low transmission settings.

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GENOME-WIDE DIFFERENCES IN *PLASMODIUM FALCIPARUM* PARASITES IN MALAWIAN CHILDREN AND ADULTS

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After repeated *P. falciparum* infections, individuals in highly endemic regions acquire clinical immunity to malaria. This immunity is associated with increasing exposure and age. However, which loci or combination of loci are important in determining acquired immunity is not completely understood. Here we take a genomic approach to identify loci that may be involved in acquired immunity to malaria by examining parasite genomes collected from children and adults participating in a longitudinal cohort study in southern Malawi. Assuming adults have acquired immunity to most common antigenic variants in the parasite population, we hypothesize that parasites in adults will be distinct from parasites in children at loci related to acquired immunity. We also hypothesize that parasites in individuals' consecutive clinical infections will be more different from each other, compared to random infections from different individuals, at loci contributing to acquired immunity. We performed whole-genome sequencing on 212 parasite isolates from 95 individuals, after enriching for parasite DNA using selective whole genome amplification. F_{ST} was used to estimate differentiation between infecting parasites from children and adults and statistical significance was assessed by permutations. Preliminary results indicate several significant differences in parasites that cause infections in adults compared to children. These highly differentiated genes include pre-erythrocytic and erythrocytic vaccine target antigens such as RH2b, CLAG8 and SIAP2 (p-value < 0.001). Some of these genes have been previously shown to be under balancing selection. We will also present results on differences between parasites that cause consecutive symptomatic infections compared to differences within parasites in the population, as well as variants associated with symptomatic infection in adults. This whole-genome approach in the context of a longitudinal

study of different age groups may identify loci for further investigation as potential vaccine candidates and provide insights into mechanisms of acquired immunity and antigenic escape.

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POLYIBD: INFERRING IDENTITY BY DESCENT FROM COMPLEX, POLYCLONAL INFECTIONS

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Unraveling the genetic relatedness between malaria subpopulations can be leveraged to better understand how drug-resistant malaria parasites evolve and spread through time and space. Traditionally, genetic relatedness and population structure measures have been determined using a combination of F-statistics (i.e. Fst) or grade-of-membership models, such as STRUCTURE. However, these measures often do not account for multiplicity of infection (MOI), where a single individual can be infected with several distinct malaria genotypes. Recent evidence has suggested that models accounting for MOI and patterns of identity by descent (IBD) may provide additional power to resolve genetic relatedness and population structure at a fine-scale. Here, we present polyIBD which extends two recent programs: hmmIBD and isoRelate, and calculates the extent of IBD between isolates of any MOI. An extension of the existing software was needed, as in high-transmission areas, MOI estimates are frequently greater than two. polyIBD is a first-order, multilevel hidden Markov Model that uses a Markov chain Monte Carlo approach to infer parameters of genetic relatedness, recombination rate, and MOI. In addition, it is currently being extended to account for within sample relatedness (F_{ws}). Preliminary data indicates that we can reliably detect genetic relatedness in samples with MOIs of three over a full range (0.0-1.0) of IBD sharing (Pearson's Correlation, $r = 0.90$, $p < 0.01$). Currently, we are validating the model with the MalariaGen genetic cross and Pf3K datasets. polyIBD may help us better understand malaria population structure in high transmission countries within Sub-Saharan Africa and elsewhere.

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DRUG RESISTANCE AND POPULATION STRUCTURE OF *PLASMODIUM FALCIPARUM* ACROSS THE DEMOCRATIC REPUBLIC OF CONGO USING HIGHLY MULTIPLEXED PANEL OF THOUSANDS OF MOLECULAR INVERSION PROBES

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Understanding the drivers of the spread of malaria parasites and drug resistance across space and time is needed. These drivers can be elucidated using genetic tools. Here, we report novel molecular inversion probes (MIPs) allowing the rapid and cost-effective targeting of all major drug resistance mutations and 1800 moderate-frequency SNPs including a subset selected for high Fst values between countries in Africa. MIPs were used to genotype *Plasmodium falciparum* infections of over 1000 individuals from the 2013-14 Demographic and Health Survey conducted in the Democratic Republic of the Congo (DRC). Analysis of spatial structure suggests that parasites within the DRC form a single population with major geographic differentiation occurring from north to south suggesting decreased gene flow. A second component of spatial structure correlated with SNPs adjacent to drug resistance loci. Among drug resistance mutations, pyrimethamine resistance mutations in pfdhfr-ts are ubiquitous across the country while sulfadoxine resistance markers in dihydropteroate synthase (pfdhps) show the most marked spatial structure with apparent entry and spread from Eastern borders for both the double mutation and likely more recently the triple mutation. Pfcrt mutations show high prevalence in east and west while remaining low in north and south DRC. Pfcrt mutations also show an apparent selective disadvantage where their frequency within overall parasite population (30%) is markedly lower than their prevalence (51%) in infections. Overall, these findings suggest that parasites in DRC, while relatively panmictic, show identifiable patterns of variation that can define geographic origin. Drug resistance mutations appear to be a major perturbation in the population structure. Moreover, highly-multiplexed targeted sequencing using MIPs emerges as a cost effective method for elucidating pathogen genetics and drug resistance across entire countries and regions.

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PLASMODIUM VIVAX INFECTION IN A GOLD-MINING AREA SITUATED IN THE BRAZILIAN AMAZON REGION: IMMUNOLOGICAL AND GENETICS CLUES

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The endemic areas of malaria in Brazil are virtually restricted to the Amazon Region, but the distribution of the disease is not uniform. Among the Amazonian municipalities, Itaituba, in Para state, a gold-mining area in the Brazilian Amazon Region, reported the largest number of malaria cases in 2016 caused by *P. vivax*. We assessed the immunological aspects, genetic alterations related to CNVs that could lead to phenotypic alterations, as well the presence of polymorphisms in cytokine genes and their association with the infection. From a total of 279 individuals, the individual selections were performed. Six SNPs in four IL-6, IL-10, TNF-α and IFN-γ genes were determined; blood cell count was conducted on automatic analyzer; plasmatic cytokines were quantified by flow cytometry; density parasite was estimated by thick blood films with confirmation by nested-PCR; the CNVs was estimated by aCGH and association between copy number and parasite load, mean number of clinical infections and gender was assessed. Significant increase in the IL-6 and IL-10 levels in both malaria groups; the primary malaria patients displayed the highest significant IFN-γ levels; recurrent malaria patients displayed the highest significant TNF-α; malaria infection demonstrated correlation between parasite density and TNF-α, IL-6 and IL-10 levels; production of IL-10 was higher in the presence of *IL10*GCC/GCC haplotype; IFN-γ levels were correlated with previous malaria episodes; a total of 112 amplified genes and 12 deleted genes were observed and the CNVs found no included any gene related to receptors or vivax malaria resistance factors; there were no statistically significant correlations among clinical and pathological data and the presence of CNVs in the patients studied. This study provides preliminary data on Plasmodium-host interactions in an endemic area of gold-mining area and describes the quantitative changes and SNPs in the human genome in *P. vivax* infection.

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INVESTIGATING THE SELECTION OF ARTEMISININ RESISTANCE AS THE BACKGROUND OF EVOLVING PFS47 LOCUS

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Plasmodium falciparum (Pf) gene Pfs47 mediates evasion of the mosquito complement-like immune system, enabling parasite survival and transmission. Adaptation to local mosquito populations or different species has led to genetic differentiation at the Pfs47 locus among Pf populations. Pfs47 is encoded in chromosome 13, ~160 Kb downstream from kelch13 (K13), primary determinant of resistance to artemisinin (ART). Given the strength of selective pressure on both phenotypes, and the physical linkage between the two loci, it has been hypothesized that the fitness of this genomic region is determined by both loci, which might be in linkage disequilibrium with each other. We analyzed ~400 samples from five malaria-endemic regions, Peru, Brazil, Mali, Malawi and Cambodia. These regions differ in malaria transmission (highest in Africa), in the frequency of ART-resistance (high in Cambodia and low elsewhere), and in the number of mosquito species that transmit Pf (lowest in Africa). As expected, there is a high degree of genetic differentiation at the Pfs47 locus among geographic locations, with FST>0.3 in all pairwise comparisons except between E and W Africa. FST at Pfs47 is an order of magnitude higher than in flanking regions, consistent with a direct role of Pfs47 to population differentiation. Genetic diversity in Pfs47, as measured by nucleotide and haplotype diversity, is highest in Africa, and reflects ancient, neutral polymorphism. In contrast, the Pfs47 locus in Cambodia shows signs of recent positive selection, and significantly lower genetic diversity than seen in Africa, congruent with recent selective sweep(s). We will discuss the distribution of Pfs47 haplotypes among Cambodian Pf subpopulations, potential linkage between K13 and Pfs47 haplotypes, and their relationship to the genetic diversity in Pfs47 prior to the emergence of ART resistance, to determine the extent to which genetic diversity in one locus impacts the other. Most importantly, the low genetic diversity at the Pfs47 locus in Cambodia may limit the spread of ART-resistant parasites to continents with evolutionary distant anopheline species.

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THE GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN GHANA

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In malaria-endemic regions, many individuals experience asymptomatic *Plasmodium* infections, particularly older children due to their level of immunity. Transmission from humans to mosquitoes requires blood meals containing gametocytes. Gametocytes often occur at submicroscopic densities, challenging measurement in human populations. The population structure of the causative agents of human malaria, *Plasmodium* species including the most prevalent *Plasmodium falciparum*, depends on the local epidemiological and demographic situations, such as the incidence of infected people, the vector transmission intensity and migration of inhabitants. In this regard, genotyping malaria parasites to evaluate the diversity in different transmission settings is essential to understand the distribution and dynamics of parasite populations. The aim of this study is to determine the *Plasmodium falciparum* genetic diversity and population structure in regions of varying patterns of malaria transmission in Ghana. Understanding the *P. falciparum* population structure variations across the country provide new insights to guide malaria control strategies. A cross sectional study was conducted in five endemic sites. These sites were picked based on the different ecological zones of Ghana. Finger prick blood was collected from 1153 school children aged between 4 years and 12 years during the dry and rainy seasons of 2017. The school children were from five study sites in Ghana with different transmission settings. Malaria parasites were detected by light microscopy (LM) and nested polymerase chain reaction (nPCR). Characterization of *P. falciparum* was employed using 12 microsatellite markers. Microscopy results estimated that 16.65% (192/1153) of the population carried gametocytes. Findings from this study will attain data on the genetic diversity of *P. falciparum* in Ghana. The study will also determine the prevalence of asymptomatic malaria infections in the population. In addition, this study will also determine the proportion of sub microscopic infection carriage among asymptomatic people in Ghana.

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NOVEL *IN SILICO* READ CAPTURE APPROACH OF WGS DATA FROM CLINICAL SAMPLES REVEALS TWO VAR2CSA HAPLOTYPE CLASSES RESPONSIBLE FOR MOST ANTIGENIC DIVERSITY

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Pregnancy-associated malaria (PAM) has tremendous negative consequences for both fetuses and mothers in malaria-endemic settings. Sequestration of *Plasmodium falciparum* in the placenta, the hallmark of PAM pathophysiology, is mediated by VAR2CSA, a member *of P. falciparum* erythrocyte membrane protein-1 family. VAR2CSA induces protective immunity following successive malaria-exposed pregnancies, supporting its choice as a vaccine candidate against placental malaria. However, development of a VAR2CSA-based vaccine has been hampered by extensive polymorphism of the gene encoding this protein. Because immunity to placental malaria is known to develop rapidly after the first pregnancy, we hypothesize that at most a few major antigenic variant classes of VAR2CSA exist. Here, we developed a novel approach to identify *var2csa* sequence reads from any whole genome sequence dataset, using as a reference a collection of 30 highly diverse, full-length *var2csa* publicly available sequences and others we have generated ourselves from a geographically diverse collection of *P. falciparum* strains. Using this approach, we reconstructed full-length *var2csa* from approximately 200 clinical samples. As expected, we observe high genetic diversity across samples with average amino acid sequence similarity ~75%. However, our analysis reveals that despite the extensive diversity of global *var2csa* sequences, sequences can be clustered into two main haplotype groups, each characterized by a unique 32 amino acid residue signature and two additional minor clusters. Our results suggest that a broadly protective vaccine to prevent placental malaria could include a limited number of representative strains.

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B CELL MEDIATED IMMUNE RESPONSES AGAINST *PLASMODIUM* INFECTIONS ARE MODULATED BY THE T-BET TRANSCRIPTION FACTOR

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Malaria is a global health concern which affects over 200 million individuals worldwide. Although immune system rapidly responds to *Plasmodium* infection with specific antibodies, natural antibodies fail to

establish long term protection often leading to repetitive infections and chronicity. Recent studies showed that in chronic malaria setting both B cells and T cells induce T-bet transcription factor expression yet it is unclear whether this is a host defense strategy or a consequence of disease. Here we show in T-bet zsgreen reporter mice that, infection with *Plasmodium chabaudi* leads to gradual increases in T-bet expression of various B cell subsets including very high levels in germinal center B cells. Furthermore, parallel infection of WT and T-bet KO mice showed that T-bet deficiency increased IgM and decreased IgG response to *Plasmodium chabaudi* which, in turn, led to the failure of KO mice to clear the infection. In line with this observation, using bone marrow chimeric mice reconstituted with 1:1 ratio of T-bet KO and WT bone marrow cells, we showed that while the reconstitution is normal for both cells in steady state, upon infection Plasma cell compartment is dominated with WT cells. Altogether we conclude that T-bet expression in germinal center B cells is critical in inducing isotype switching and plasma cell generation in *Plasmodium* infection.

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EXPRESSION AND REGULATION OF ACTIVATION-INDUCED CYTIDINE DEAMINASE AID MRNA IN SYMPTOMATIC AND ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTED CHILDREN

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Activation-Induced cytidine Deaminase, (AID), initiates somatic hypermutation and class switch recombination in B-lymphocytes. Despite this essential physiological activity of the protein, it mutates other genes in addition to the Ig gene when it is aberrantly expressed. Recently, AID has been postulated to be the molecular link between infection with *Plasmodium falciparum* the occurrence of the endemic form of Burkitt's lymphoma. Although *P. falciparum* is reported to induce AID expression, it is not known how the expression of the protein is regulated in response to *P. falciparum* infection. The aim of this work was to investigate how *P. falciparum* infection affects the expression levels of AID mRNA transcripts. We therefore measured the levels of AID mRNA transcripts in PBMCs isolated from the blood of asymptomatic and symptomatic *P. falciparum* infected children. We also used nested PCR to determine the splice variants of AID mRNA expressed in these PBMCs. We observed significantly high levels of AID mRNA in *P. falciparum* infected children with clinical symptoms compared to asymptomatic infected children. Splice variant nested PCR also revealed more splice variants of AID mRNA transcripts in the PBMCs of symptomatic children as compared to asymptomatic and non-infected children. AID mRNA levels did not vary significantly between healthy un-infected and *P. falciparum* infected asymptomatic children. In addition, infection with EBV did not affect the expression of AID mRNA in healthy un-infected and infected asymptomatic children. The findings so far suggest that a factor in symptomatic *P. falciparum* infected children may be involved in the upregulation of AID mRNA expression.

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ACUTE EPSTEIN BARR VIRUS INFECTION IS A RISK FACTOR FOR THE DEVELOPMENT OF NON-CEREBRAL SEVERE MALARIA

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Infants in malaria-endemic areas are often disproportionately affected by the symptoms of malaria with many ending up hospitalized with severe symptoms of this disease. It is during the first few years of life that these infants also become co-infected with Epstein Barr Virus. It has long been appreciated that interactions occur between EBV and *Plasmodium falciparum* infections that can lead to the loss of immune mechanisms preserving viral latency and the development of an EBV-related malignancy known as endemic Burkitt's lymphoma (eBL). However, very little is known regarding the potential impact of primary EBV infection on the severity of *P. falciparum* infections. Although acute EBV infection is generally asymptomatic in young children, virus-induced humoral immune deficiencies have however been observed towards unrelated antigens. The immunosuppressive effects of acute lytic EBV infection have been reported to last for at least 4 weeks. We tested the hypothesis that infants with acute EBV infection had reduced anti-malarial antibody titres compared to their uninfected or latently-infected peers. We also tested the hypothesis that children with acute EBV infection were more likely to develop severe malarial symptoms. We conducted serological analysis to classify the EBV infection status of 240 infants (four age groups (0-12, 12-18, 18-24 months) in Yaounde, Cameroon. The total incidence of EBV-infected infants was 57.5% with acute or latent EBV infection in older infants. However there was no difference in the average age of those with uninfected with malaria compared with those experiencing uncomplicated or severe malaria. We found that children with acute EBV infection were more likely to have severe malaria compared to those with no EBV infection or latent EBV infection, irrespective of age. We then quantified the breadth of the antibody response against *P. falciparum* antigens using a protein microarray. Contrary to our hypothesis we found that antibody reactivity within the acute EBV group was higher than those who were uninfected with EBV or latently infected with EBV. The implications of these results will be discussed.

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LONG-LIVED MEMORY B CELL RESPONSE TO *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN IN MALARIA LOW TRANSMISSION AREA

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Very little known about the durability of antigen-specific memory B cells and antibody responses to malaria. The aim of this study was to analyze the longevity of antibody and memory B cell (MBC) response of promising vaccine candidate of blood-stage, Duffy Binding Protein region II (DBPII), in *P. vivax*-exposed individuals living in a malaria endemic area in southern Thailand. We found that anti-DBPII antibody increase during acute infection, but a majority of these responses were significantly decreased at 9 months post-infection. In sharp contrast, circulating DBPII-specific MBCs were stably maintained at least 3 years post-infection without re-exposure to infection. Phenotyping of MBCs subset showed the expansion of activated and atypical MBCs occur during *P. vivax* infection. Interestingly, the ratio between activated/atypical MBCs increased during recovery

from infection, increasing from 1:2 at acute phase to 1:3, 1:3 and 1:4 at 3-month, 9-month and 12-month post-infection, respectively. The alteration of activated/atypical MBC ratios appeared to be related to short-lived anti-DBPII antibody responses. To demonstrate which MBC subset that play a role in maintaining or regulating of efficient antibody responses to DBPII in naturally acquired *P. vivax*-infection, we generated fluorescent antigen tetramer for characterization of DBPII-specific MBC on the basis of the antigen specificity of their BCR. A better understanding of the function of activated/atypical MBC and their role in generating protective antibodies is important for developing an effective DBPII-based vaccine.

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MEMORY B CELLS ARE REACTIVATED IN VACCINATED C57/BL6 MICE AFTER CHALLENGE WITH *PLASMODIUM BERGHEI*

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Malaria continues to be a major global health burden, with more than 216 million cases in 2016. One challenge impeding elimination is the lack of understanding of B cell immunity in malaria and, furthermore, the lack of research tools to phenotype rare antigen specific B cells. The aim of this research was to develop a novel method for identifying carbohydrate-specific B cells and, by doing so, determine if a candidate carbohydrate-conjugated vaccine generated functional memory B cells that could reactivate upon challenge with *Plasmodium* parasites. The vaccine used was a synthetic glycosylphosphatidylinositol (GPI) conjugated to keyhole limpet haemocyanin (KLH). GPI is expressed freely on the membrane of the parasite or used as an anchor for important surface proteins. It is conserved across all *Plasmodium* species and lifecycle stages. In the current study, B cell immune kinetics were analysed after vaccinating C57/Bl6 mice subsequent to validating a flow cytometry probe that binds GPI-specific B cells. Mice were rested (10 weeks) and challenged with parasites or GPI-KLH (positive control). Kruskal-Wallis and paired t-tests were used to assess significance. Our innovative probe was able to detect GPI-specific class-switched memory B cells after vaccination. These functional cells reactivated and boosted when challenged with carbohydrate or parasites, albeit to a lesser degree with the latter. Additionally, priming B cell responses to GPI-KLH measured in naïve mice and previously *Plasmodium*-exposed mice did not significantly differ, indicating that prior infection did not have an irreversible detrimental effect on B cells. Our novel findings can contribute to the knowledge gap concerning B cell immunity in malaria, are clinically significant for the potential inclusion of carbohydrate conjugates in the malaria elimination agenda, and the novel cytometric technique is applicable for exploring other relevant epitopes for their role in antigen-specific B cell responses in malaria.

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THE CD73$^+$ B-CELLS THAT ARE PROTECTIVE AGAINST *PLASMODIUM YOELII* (PYNL) ALSO EXPRESS IGM AND GRANZYME B.

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Despite the critical importance of humoral and B cell mediated immunity in protecting against malaria, the biological nature of anti-malaria B cell responses have not been fully delineated. Examining PD-L2, CD80 and CD73 marker expression on splenic B cells by flow cytometry, we previously showed that when compared to naïve mice, the frequencies of CD73$^+$ B cells increased from 6.1±0.3% at day 6 to 28.0 ±0.6% at day 13 post infection. CD73$^+$ B cell-frequencies (52.1±0.9%) continued to increase even when parasitemia levels had cleared at day 22 post infection. By day 55 post-infection, the frequency of CD73$^+$ B cells remained elevated (16.06±0.84) when compared to naïve mice (2.32±0.3). To determine whether CD73$^+$ B-cells from PyNL immune mice play a role in protection, we passively transferred immune CD73$^+$ or CD73$^-$ B cells from malaria recovered mice into naïve mice prior to challenge with PyNL and assessed parasitemia. Parasitemia levels were reduced 96.6% in mice that received immune CD73$^+$ B-cells (0.26±0.14%) compared to mice infected for the first time (12.58±0.14%). Interestingly, CD73$^-$ B cells from immune mice afforded only a modest reduction in parasitemia (8.8 ± 1.49%). Further characterization of immune CD73$^+$ B-cell population indicated that significant proportion of CD73$^+$ B cells were GrB$^+$ at day 13 post primary immunization. There was also an increase in the CD73$^+$GrB$^+$ population after a second infection. Four days after a secondary infection, CD73$^+$GrB$^+$ cells increased 3-fold as compared to 1 month post parasitemia clearance frequency (p= 0.0079), and 7-fold with respect to day 4 post primary PyNL infection (p=0.0079). Interestingly, 33.46% of the CD73$^+$ B cells also expressed IgM. In conclusion, we showed that passive transfer of immune CD73$^+$ B-cells conferred protection against a primary PyNL infection and that a significant fraction of these immune CD73$^+$ B cells expresses GrB and IgM.

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RECOGNITION OF EXCRETED-SECRETED ANTIGENS OF *PLASMODIUM FALCIPARUM* BY IMMUNOBLOTTING

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In vitro cultures of *Plasmodium falciparum* (*Pf*) excrete-secrete into the culture medium a complex mixture of antigenic proteins also known as exoantigens, which provide the parasite with the necessary mechanisms to evade the host's immune system. The purpose of the present study was to partially characterize the analysis of antibody reactivity of malaria positive patients with different parasitic densities. Cultures of *Pf* strain 3D7 were synchronized with 35-65% Percoll and 5% D-sorbitol. The exoantigens were obtained from Albumax free cultures with parasitemias ≥20%, which were separated by SDS-PAGE electrophoresis and evaluated by immunoblotting, facing sera from malarial and non-malarial patients. Ten immunoreactive bands (148, 139, 124, 120, 116, 110, 72, 53, 18 and 12 kDa) were observed in malarial patients. The 72, 18 and 12 kDa proteins were present more frequently but were not related to the increase in parasite load. The 62, 57 and 28 kDa proteins showed reactivity with sera from non-malarial patients and other diseases such as Toxoplasmosis, Leishmaniosis, Herpes and serum from non-endemic area individuals. The recognition of immunoreactive bands opens up the possibility of optimizing the technique and evaluating purified antigens for the serological diagnosis of symptomatic and asymptomatic malaria.

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IMPACT OF PREVIOUS MALARIA EXPOSURE ON HOST RESPONSES TO PLACENTAL MALARIA

Jennifer Howard[1], Robert Morrison[1], Omely Marte[1], Ankur Sharma[2], Lynn Lambert[1], Sachy Orr-Gonzalez[1], Michal Fried[1], Patrick Duffy[1]

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Plasmodium falciparum infected erythrocytes express VAR2CSA and bind chondroitin sulfate A (CSA) to sequester in the intervillous space of the placenta. Poor outcomes in placental malaria (PM) have been associated with the inflammatory response during chronic episodes of PM, which is characterized by a monocyte/macrophage-rich inflammatory infiltrate in the intervillous space. It has previously been postulated that the inflammatory engine of PM has features of a type III hypersensitivity reaction, in which antibody-antigen immune complexes activate the

complement pathway leading to a cytokine storm. We hypothesize that pre-existing immunity to malaria antigens is activated early in infection and contributes to triggering the subsequent inflammation. To assess the impact of the adaptive immune system and immune complexes in early PM immune response *in vivo*, we immuno-profiled placental blood from infected, primigravid Malian women by multi-colour flow cytometry and ELISA. Activated memory B cells, detected by flow cytometry, were found to be significantly increased in chronic (late), but not acute (early) PM infection. However, circulating immune complexes, as detected by ELISA, were already significantly increased in acute PM, suggesting an early role of pre-existing Abs in PM immune responses. The impact of previous non-PM malaria exposure is not feasible to study in human cohort studies, however *Aotus nancymaae* is a new world non-human primate which can be infected with *P. falciparum*. We are developing Aotus as a new model of PM. To address the impact of previous (non-PM) exposure to *P. falciparum* on immune responses during PM; naïve Aotus and Aotus with one previous infection, were allowed to become pregnant and infected with *P. falciparum*. Placental blood mononuclear cells were collected, and the extracted RNA sequenced and quantified. Differences in gene expression relating to immune activation was compared between the naïve and previously exposed groups, and will be presented. This study aims to provide a better understanding of the immune mechanisms which trigger pathological inflammation during PM.

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IMPACT OF PREVIOUS MALARIA EXPOSURE ON HOST RESPONSES TO PLACENTAL MALARIA

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A STRUCTURALLY DEFINED EPITOPE IN *PLASMODIUM VIVAX* PVDBP THAT MAY MEDIATE ANTIBODY CROSS-REACTIVITY TO SIMILAR EPITOPES IN VAR2CSA

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Malaria remains one of the most important parasitic diseases in the world. Adherence to host ligands is a mechanism used by malaria parasites for essential functions such as invasion and evasion of the host immune system. Many of the parasite proteins that mediate adherence contain a structurally conserved Duffy binding-like (DBL) domain. The parasite protein PvDBP, which contains one DBL domain, mediates *P. vivax* merozoite invasion by binding to the Duffy Antigen Receptor for Chemokines (DARC) receptor on the red blood cell (RBC) surface. In pregnant women, *P. falciparum* parasites express VAR2CSA, which contains 6 DBL domains and mediates adherence to chondroitin sulphate A (CSA) in the placenta. We previously observed immunological cross-reactivity between the DBL domains of PvDBP and VAR2CSA in naturally exposed populations in Colombia. We then characterized a monoclonal antibody (mAb) raised against the DBL domain of PvDBP that cross-reacts with VAR2CSA, stains *P. falciparum* iRBCs that express VARCSA and blocks parasite adhesion to CSA *in vitro*. Here, we mapped the minimal epitope in PvDBP that is recognized by the mAb to a 40 amino acid sequence, called 'SD1', consistent with previous work based on site-directed mutagenesis of this region in PvDBP. We further characterized the epitope using synthetic peptides and molecular dynamic simulations. Our data suggest the epitope is likely conformational, containing a small alpha helix stabilized by two pairs of disulphide bonds and salt bridges. Based on homology modeling and published crystal structures, we then identified structurally similar SD1-like epitopes in several of the DBL domains in VAR2CSA. We are now evaluating the immunological dominance of these epitopes using polyclonal antibodies against PvDBP and VAR2CSA. We propose that SD1 in PvDBP is structurally conserved in VAR2CSA and mediates the cross-reactivity between PvDBP and VAR2CSA.

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ACQUISITION OF INTRALEUKOCYTIC HEMOZOIN DOWNREGULATES *LAIR1* EXPRESSION AND LEUKOCYTE INHIBITORY SIGNALLING IN PEDIATRIC SEVERE MALARIAL ANEMIA

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Severe malarial anemia (SMA) [hemoglobin (Hb)<5.0 g/dL, with any density parasitemia] is a multifactorial complication of malaria in children residing in *Plasmodium falciparum* holoendemic areas. To identify molecular pathways involved in SMA pathogenesis, we conducted whole transcriptome profiling in a discrete subset of "polarized" phenotypes: non-SMA and SMA (3-36 months) from Western Kenya. These experiments revealed that leukocyte-associated immunoglobulin like receptor 1 (LAIR1) transcripts were downregulated in SMA (-1.6 fold, P=0.0042). TaqMan® gene expression analysis in non-SMA (n=52) vs. SMA (n=52) confirmed downregulation of *LAIR1* (-3.0 fold, P=0.017) in severe

disease. *LAIR1* transcripts were also negatively correlated with hemozoin (*Pf*Hz) containing monocyte (PCM/μL) titres (r = -0.298, *P*=0.003). To further explore the relationship between LAIR1 transcripts, acquisition of intraleukocytic *Pf*Hz, and the subsequent impact on leukocyte signalling, cultured PBMCs from malaria-naïve donors were treated with physiological concentrations of *Pf*Hz (10 μg/mL) found in SMA. *Pf*Hz treatment suppressed LAIR1 transcript expression (-4.0 fold *P*<0.001) and inhibited LAIR1 signalling, i.e., decreased phosphorylation of LAIR1 (*P*<0.0001) and SH2-domain containing phosphatase-1 (SHP-1) (*P*<0.001). *Pf*Hz also induced NF-κB activation (*P*=0.004), and enhanced production of IL-6, IL-1β, and TNF-α (all *P*<0.0001). These novel findings reveal that LAIR1 expression is downregulated in children with SMA, and that phagocytosis of *Pf*Hz abrogates leukocyte signalling through the LAIR1 pathway.

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ELEVATED LEVELS OF CD4+CD45RA-CD62L+CD11A+ ARE ASSOCIATED WITH MALARIAL ANEMIA DISEASE SEVERITY IN KENYAN CHILDREN

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L-selectin (CD62L) attracts lymphocytes into secondary lymphoid organs. LFA-1 (CD11a) promotes emigration of lymphocytes from the blood and is upregulated on activated T cells. Since CD62L and CD11a are largely unexplored in malarial anemia, CD4+ T-cell populations were characterized in malaria-infected children (n=54; aged 12-36 months) with varying severities of anemia presenting at Siaya County Referral Hospital, a holoendemic *Plasmodium falciparum* transmission region. Complete hematological profiles and parasitemic indices were obtained. Parasitemic children were stratified based on hemoglobin (Hb): uncomplicated malaria (UM; Hb≥11.0 g/dL; n=12), mild malarial anemia (M/MA; Hb≥8.0<11.0 g/dL; n=22), and severe malarial anemia (SMA; Hb<6.0 g/dL; n=20). Peripheral blood was stained with anti-(CD3; CD4; CD45RA; CD11a; and CD62L) antibodies. Cells were then acquired using four-color FACSCalibur™ flow cytometer. The proportion of (CD4+ CD45RA-CD11a+) were comparable across the groups [median (IQR) UM, 98.16% (1.97); M/MA, 97.41% (2.71); SMA, 97.43% (2.69); *P*=0.422]. In addition, the proportions of (CD4+ CD45RA- CD62L+) were comparable across the groups [median (IQR) UM, 72.52% (11.90); M/MA, 76.88% (12.80); SMA, 77.33% (11.90); *P*=0.229]. However, the co-expression of CD11a and CD62L (CD4+ CD45RA- CD11a+ CD62L+) differed significantly across the groups [median (IQR) UM, 67.98% (5.96); M/MA, 75.46% (15.07); SMA, 72.17% (9.89); *P*=0.025]. Further analysis showed that both the M/MA (*P*=0.012) and SMA (*P*=0.040) groups had elevated levels of circulating CD4+ CD45RA- CD62L+ CD11a+ relative to the UM group. These results suggest that CD4+ T cells co-expressing CD11a and CD62L are associated with malaria severity, and may be important in the pathogenesis of SMA.

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PROFILING OF PHENOTYPIC CHARACTERISTICS OF IMMUNE CELLS FOLLOWING MALARIA TREATMENT BY MASS CYTOMETRY

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Plasmodium falciparum malaria infection results in a complex pattern of immune modulation. To understand how the host responds to malaria infection, we utilized mass cytometry (CyTOF , Cytometry by Time of Flight) to perform a comprehensive immune phenotypic analysis. Mass cytometry is a multiparameter analytical platform that enables simultaneous evaluation of up 42 cell markers per single-cell, thus providing in-depth analysis of immune cellular heterogeneity. We applied this platform together with computational algorithms and clinical data to comprehensively explore the phenotypic immune cellular heterogeneity in children with acute malaria and post-recovery (four weeks' post treatment with Artemether-Lumefantrine) samples. Whole blood samples from children with malaria infection were prospectively collected at acute and post recovery timepoints, and processed following RBC lysis, fixation and cryopreservation. The samples were then thawed, barcoded, pooled and stained using a cocktail of antibodies specific to 26 cell surface markers and 14 cytokines conjugated to elemental metal isotopes and analyzed on a Helios mass cytometer. The data was then uploaded to Cytobank where the software program viSNE was used to visualize the high dimensional single cell data. Unsupervised multivariate clustering analysis was also performed on the data using Cytofkit. Unsupervised gating strategy revealed a dramatic expansion of distinct monocyte cell populations during acute malaria as compared to recovery. Further analyses are ongoing to identify the cytokine patterns in the cell subsets present during acute malaria and following recovery. Our data demonstrate that CyTOF together with unsupervised clustering algorithms can comprehensively characterized multiple cell populations thus enabling extensive profiling of cellular subsets. Acute malaria causes a complex pattern of lymphocyte subset distribution. Novel cellular populations identified could drive hypothesis generation for further experiments.

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A SELECTIVE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR SIMULTANEOUS QUANTIFICATION OF ARTEMETHER, LUMEFANTRINE AND THEIR PRINCIPLE METABOLITES IN HUMAN PLASMA

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Dosage regimens for antimalarials are often extrapolated from the non-pregnant population despite the fact that the pharmacokinetics (PK) of antimalarials are changed in pregnancy and even more so in pregnant women with malaria. Currently there is insufficient PK data of artemisinin based combination therapies in pregnancy to recommend any changes in the dosage regimen. Lower drug concentrations in pregnancy compared with non-pregnant populations increases the risks of therapeutic failure and sub-optimal concentrations can lead to selection of resistant parasites. A quantitative assessment of these antimalarials in plasma in the course of artemether-lumefantine treatment is essential in order to evaluate the bioavailability and pharmacokinetics of these co-formulated compounds and how their potent metabolites influence treatment outcome. We have developed and validated a sensitive, selective and reproducible reversed-phase high-performance liquid chromatography method coupled with electrospray ionization mass spectrometry for the simultaneous quantification of artemether (ART), dihydroartemisinin (DHA), lumefantrine (LUM) and desbutyl-lumefantrine (DBL) in human plasma. Mefloquine was used as an internal standard (IS). The analytes were extracted by protein precipitation procedure and separated on a reversed-phase Zorbax SB-Ciano column with a mobile phase composed of acetonitrile and 20mM aqueous ammonium formate containing 0.5% (v/v) formic acid. The assay response were linear ($r^2 \geq 0.9992$) over the range of 5-1500 ng/mL for ART/ DHA and 5-5,000 ng/mL for LUM/DBL. The lower limit of quantification was 10 ng/mL ART/ DHA and 5 ng/mL for LUM/ DBL. The mean intra-run precision were < 4.7% and the inter-run precision were < 4.4% for all the analytes. The mean percentage recovery values were >93.2%. No matrix effect was detected for all the analytes and the IS.

The validated method was successfully applied to determine the plasma concentrations of ART, DHA, LUM and DBL in pregnant and non-pregnant women volunteers in a multiple-dose pharmacokinetics study over the course of 336 hours.

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MODELING HOST-PARASITE INTERACTIONS IN MALARIA BLOOD-STAGE INFECTIONS IN RHESUS MACAQUES

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Malaria is globally the most deadly parasitic disease in humans, and the long-time coexistence with malaria has left indelible marks in the human genome that are the causes of a variety of genetic disorders. Anemia is the most common and severe complication of malaria, yet the root causes and mechanisms involved in malarial anemia are unclear and very difficult to study in humans. Non-human primate model systems enable the study and quantification of underlying, causative factors of malarial anemia, and particularly the onset of severe anemia. A discrete recursive model was developed to simulate host-parasite interactions during the blood stage infection; it accounts for reticulocytes, red blood cells (RBCs), and infected RBCs. The parameters of this mechanistic model were optimized against the readouts of individual macaque data, which had been obtained in the course of *Plasmodium coatneyi* and *P. cynomolgi* infections of cohorts of malaria-naïve rhesus macaques (*Macaca mulatta*). The model allowed detailed estimations of the levels of erythropoietic output, reticulocyte lifespan, RBC removal, and the immune response against the parasite in each macaque. The results showed that rhesus macaques have a response to a *P. cynomolgi* infection that is difficult to understand: As expected, the infection resulted in anemia, yet 60% of the RBCs were lost by bystander effect a mechanism other than parasite invasion. To compensate for the severe anemia, the host released younger reticulocytes and increased the erythropoietic output. These responses, however, appeared to be poorly coordinated, as the release of younger reticulocytes occurred too early, while anemia had not yet set in, thereby probably aiding the parasite more than the host. Increased production of RBCs was only detected after treatment that lowered the parasitemia. The model also showed that, similarly to humans, reticulocytes in rhesus macaques circulate for about 24h before becoming mature RBCs. Anemia, as a sequela of malaria, was due in 60% to bystander destruction of RBCs, and by an inability of the host to up-regulate erythropoiesis before suppression of parasitemia.

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GLOBAL ECONOMIC COSTS DUE TO *PLASMODIUM VIVAX* MALARIA TREATMENT AND THE COST-BENEFIT OF RADICAL CURE

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The global cost burden related to the treatment of vivax malaria has not been assessed. Here we estimated the current global economic cost of illness to healthcare providers and patient households and to explore the potential cost-benefit of implementing radical cure following a normal G6PD test result. Estimates of the cost of vivax malaria treatment for healthcare providers and patient households were collated from a literature review and primary costing studies, and combined with case estimates generated by the Malaria Atlas Project. A cost-benefit analysis of implementing effective global radical cure compared the cost of G6PD screening and supervised radical cure with the cost savings from the prevention of relapses. It was assumed that when adhered to, the 14-day primaquine regimen was 85% effective and required 3.5 healthcare worker days to supervise daily therapy (valued at one gross domestic product per capita per day). The estimate of the global cost of *P. vivax* was US$330 million (range US$205-459 million). Adopting a policy of screening for G6PD deficiency and delivery of highly effective radical cure was projected to save US$45 million per year from the societal perspective. Household costs decreased by US$124 million; however, provider costs increased by US$80 million attributable primarily to the inclusion of supervised primaquine therapy. The global societal costs of vivax malaria are substantial, but can be reduced through the use of effective radical cure following screening for G6PD deficiency. Novel, less costly interventions for ensuring adherence to primaquine regimens for effective radical cure are needed to reduce healthcare provider costs and provide a pathway to malaria elimination.

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MULTIPLICITY OF INFECTION - HOW TO ESTIMATE IT AND HOW TO DESIGN YOUR STUDY

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Multiplicity of infection (MOI), i.e., the number of super-infections, is a potent measure of transmission intensities in malaria, which can be incorporated into metrics for monitoring disease-control interventions. It can be readily estimated from molecular data gained from parasite-positive blood samples using different methods, some of which use ad hoc approximations while others are based on statistical models. While the focus has been on applications, the statistical properties of such methods and their impact on study designs have received less attention. Targeted to theoreticians and applied researchers, we present the statistical properties of a maximum-likelihood (ML) method to estimate MOI along with allele frequencies at molecular markers and compare them with some ad hoc methods. Particularly, we report on the asymptotic and finite-sample properties. While the ML estimate has the typical desirable asymptotic properties (asymptotic unbiasedness, consistency, efficiency) and adequate finite-sample properties, alternative ad-hoc methods might be severely biased. Particularly, ad hoc methods are not asymptotically unbiased, leading to misinferences even in large samples. Additionally, the ML estimate is relatively robust against model violations, as confirmed by a simulation study. Furthermore, the method is easy to compute and available as an R script. Moreover, it is possible to adapt the statistical model for study design purposes in order to ensure precision and accuracy goals when estimation MOI and allele frequencies. We apply illustrate the methods on a data set from Venezuela and exemplify sample-design issues.

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CAUSAL DISCOVERY WITH KERNEL INDEPENDENCE TESTS FOR UNDERSTANDING AND MODELLING SEASONALITY OF MALARIA TRANSMISSION

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Seasonality in malaria transmission is observed widely around the world and varies greatly between areas, even varying from village to village in the same region and between different years. Many environmental factors have been identified as possible drivers of seasonality, including temperature, rainfall and vegetation. Many studies have also considered these covariates with various time-lags. Other data summaries may also be considered, such as mean monthly rainfall or total rainfall over a set time interval, as well as non-environmental factors, resulting in a large number of candidate drivers of seasonality. Due to the many possible covariates and highly variable nature of malaria seasonality around the world, standard regularisation techniques in machine learning may have trouble correctly identifying important covariates. We performed causal inference

to identify the most significant covariates for the temporal prediction of malaria transmission. Recent developments of kernel-based independence tests have led to a number of potential non-parametric independence and conditional independence tests which allow us to identify non-linear causal relationships. We used the Hilbert Schmidt Independence Criterion as our independence test and applied the pc-algorithm for causal discovery to a dataset of temporal and spatially-indexed malaria case data from health facilities in Mozambique along with satellite-based covariate data, including ecological covariates such as vegetation indices, land surface temperature and rainfall, all at multiple time-lags. We used the resulting causal relationships to cluster related covariates and identify significant covariates for modelling seasonality of malaria transmission.

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IMPACT OF GPS DISPLACEMENT AND SAMPLE SIZE ON LOCAL RISK MAPS OF MALARIA

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Maps of disease risk have proven to be useful tools for strategic planning of malaria prevention and control activities at the national level and often are built using data from demographic health surveys (DHS) with GPS information. For local-mapping efforts though, demographic health data lacks the fine-grain resolution required to infer spatial heterogeneity and suffers from GPS displacement to protect the confidentiality of survey respondents. However, conducting prevalence-based surveillance is frequently expensive and infeasible to sustain especially if extensively done. But, if sampling efforts were minimized to the least amount required to power mapping efforts it could be sustained for longer periods. The aim of this work is to 1) determine the impact GPS displacement has for local mapping efforts that rely on precise spatial covariates for accurate model predictions and 2) illustrate the minimum sample size required to make local-level fine-scale maps without compromising the underlying spatial trends. Using a geostatistical model in a Bayesian framework, we demonstrate modelling techniques for micro-scale spatial heterogeneity of malaria in northern Ghana between 2010 and 2013 and then displace the known coordinate locations up to five kilometers to match standard displacement practices. We also randomly sampled between 10 - 90 % of the original survey at increments of 10% to determine how the model would predict at lower sample sizes. The maps created show a unique north-east to south-west trend in malaria prevalence. Interestingly, differences greater than 10% in predicted prevalence estimates were observed with and without GPS displacement, which might have important implications for local operational malaria prevention and control activities. Maps using At least 30% of the original sample size were able to demonstrate a similar spatial trend, without compromising the uncertainty in the model predictions. The high variability in malaria prevalence over a small region coupled with the strong impact of GPS displacement indicate important short-comings of country level spatial modelling for programmatic purposes.

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SUPPORTING DECISION-MAKING FOR MALARIA ELIMINATION IN SOUTH AFRICA: A MATHEMATICAL MODELLING APPROACH

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Having made substantial progress in controlling malaria and reducing deaths from the disease since 2000, South Africa is attempting to achieve malaria elimination by 2020. With heterogeneous incidence across provinces and a high proportion of imported cases, districts within provinces are faced with their own unique challenges and conditions, and solutions need to be adapted as necessary. There is no "one size fits all" intervention for malaria elimination due to the range of available interventions acting at different stages of the parasite life cycle and the diverse transmission landscape. Mathematical models have in the past provided a valuable framework for analysing the dynamics of malaria transmission to predict the impact of interventions in the design of optimal strategies. An epidemiological-economic model was developed to predict the path to elimination by 2020 for the three endemic provinces in South Africa. The model included several features such as details on the environmental conditions and biology of malaria transmission, infection and disease in the geographical region under study. The biological mechanisms were layered over a population distribution and behaviour sub-model that would reflect the way individuals live and move as well as the key features of their treatment-seeking behaviour. Developed alongside available health system data, the model was calibrated to mimic trends and patterns observed in the data. Simulated interventions included scaling up of Indoor Residual Spraying, Active Case Detection, mobile clinics and mass screening activities. These interventions were costed to inform the development of an investment case to support malaria financing and budgeting and determine a cost-effective path to achieving malaria elimination by 2020.

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THE ROLE OF HUMAN AND VECTOR MIGRATION, SEASONAL HETEROGENEITY, AND OPTIMIZED TARGETING OF LIMITED ANTI-MALARIAL INTERVENTIONS IN ACHIEVING MALARIA ELIMINATION IN THE SAHEL

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Although malaria transmission has decreased in many parts of the world, the Sahel in Africa still faces high burden. In this highly endemic setting, malaria is very seasonal, resulting in extreme highs and lows of transmission through the year. This extreme seasonality may offer short but high impact windows of opportunity for the deployment of anti-malarial strategies. Using the entomological data collected during the Garki project of the 1970's, we develop a spatial mathematical model to explore how small pockets of perennial vector habitat can sustain transmission through low periods, how vector migration topologies affect the reseeding of transmission after the dry season and impact the success of localized vector control, and how optimally timed and targeted vector control strategies can effectively suppress transmission. This modeling framework is used to simulate the interventions deployed during the Garki Project and understand why and how malaria returned after the program was terminated, and further used as a test bed for predicting what interventions would be needed to efficiently reduce burden and eventually achieve elimination in Sahelian settings.

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THE IMPACT OF SEASONAL VARIATION IN THE DETECTION OF CLINICALLY RELEVANT *PLASMODIUM FALCIPARUM HRP2* GENE DELETIONS: A MODELLING STUDY

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The introduction of rapid diagnostic tests (RDTs) has enhanced the diagnosis of malaria, with the most widely used RDTs detecting *P. falciparum* histidine-rich protein 2 (PfHRP2). However, false-negative RDTs due to *pfhrp2/3* gene deletions have now been reported in multiple locations across Africa. In February 2018, the WHO issued guidance advising national malaria control programme (NMCP) managers to investigate suspected false-negative RDT results. This included a protocol for estimating the prevalence of *pfhrp2/3* deletions by sampling from symptomatic *P. falciparum* patients seeking treatment at public health facilities, with sampling to be completed within an 8-week interval. The specific interval chosen, however, could lead to bias in the observed prevalence of *pfhrp2/3* deletions. Here we present an extension to a published individual-based model of malaria transmission to characterise the impact of seasonality on *pfhrp2/3* deletions. Our modelling predicts that the observed prevalence of *pfhrp2/3* deletions is higher when monoclonal infections are more prevalent, with the highest prevalence observed when sampling from younger children at the start of the rainy season. As the rainy season progresses, individuals are more likely to be superinfected and acquire wild type parasites, resulting in positive RDT results and a decrease in observed *pfhrp2/3* deletions. Our predictions are consistent with data from the Democratic Republic of Congo where children were more likely to be monoclonally infected with *pfhrp2* deleted parasites if the samples were collected during periods of lower relative transmission intensity (MW p-value = $1.92e^{-7}$) and if the individuals were younger (MW p-value = $9.47e^{-4}$). Decisions made concerning whether to switch from HRP2-based RDTs should thus be made in the context of both the age of patients and time in the transmission season that they were sampled. We have mapped which regions in Africa have the greatest potential for bias in the observed prevalence of *pfhrp2/3* deletions, and identify the least biased sampling intervals for each level 1 administrative region to help guide NMCP surveillance strategies.

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MODELLING THE POTENTIAL IMPACT ON SPREAD OF ARTEMISININ AND PARTNER-DRUG RESISTANCE OF INTERMITTENT PREVENTIVE THERAPY OF MALARIA IN PREGNANCY

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Intermittent preventive treatment of malaria in pregnancy (IPTp) in high or moderate malaria-endemic settings significantly reduces maternal malaria episodes, maternal and foetal anaemia, placental parasitaemia, low birth weight and neonatal mortality. Development of sulfadoxine pyrimethamine resistance has reduced the efficacy of IPTp in some areas of Africa, prompting the World Health Organisation to consider switching to dihydroartemisinin-piperaquine (DHA-PQP). We explore if widespread use of IPTp-DHA-PQP could accelerate the spread of artemisinin and piperaquine resistance and reduce DHA-PQP benefit to the wider population. We developed a 4-strain malaria transmission model, which includes mosquito dynamics, heterogeneous biting preference, immunity, case management of clinical infections, parasite survival rates using data from Cambodia, recombination, pregnancy rates, infectiousness in pregnancy, and IPTp frequency. Percentage resistance, computed as prevalence of low or high resistant parasites in a population of all parasites including wild-types, is used to explore the chance and rapidity of resistance spreading. We show that IPTp-DHA-PQP can promote the spread of resistance but its effect is much smaller compared to effect of case management in the general population. Moreover, if fitness cost of resistance is assumed via increased resistance parasite clearance rate, resistance cannot spread under certain IPTp frequency and coverage. We also show that resistance will spread faster with higher pregnancy rates, higher infectiousness of pregnant women, and more frequent IPTp. We have demonstrated that IPTp can promote the spread of DHA-PQP resistance only if the proportion of women in IPTp exceeds a threshold where resistant parasites have a sufficient advantage over sensitive parasites.

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MODELLING COUNTRY-LEVEL MALARIA PREVALENCE USING DHS DATA: COMPARISON OF MODEL APPROACHES

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Geospatial statistical models are now widely used in malaria epidemiology to identify risk factors, assessing efficacy of intervention program, and to produce reliable and comprehensive malaria risk maps. Many modelling approaches have been used in the literatures, e.g. Gaussian point process model, commonly implemented using Bayesian MCMC method (also known as Bayesian geospatial model, BGM) or stochastic partial differential equation (SPDE) approximation method, generalized linear model (GLM) and P-splines regression. The abundance of methods calls for comparison study as it can inform the researchers on selecting the right model. However, such studies are limited and their findings may not be applicable as the geographical scope changes. In this study, we compared the predictive performance of various techniques in modelling malaria prevalence in six African countries (Burkina Faso, D.R. Congo, Ghana, Nigeria, Togo and Uganda) separately, using their latest DHS data. These techniques are BGM, SPDE, stepwise GLM, elastic net regression, generalized additive model (GAM), random forest and gradient boosting regression tree. We found that the Gaussian process methods, BGM and SPDE, are generally reliable for country-level malaria modelling. However, GAM approach can be on par or even outperform the Gaussian process methods in some countries. Tree-based models, which are increasingly prominent methods in ecology and epidemiology models, perform poorly in this exercise. Given that fitting GAM takes only a small fraction of time required to run BGM or even SPDE approximation, we think that GAM is a good alternative and should be considered in malaria models.

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SUBSTANTIAL REDUCTION IN THE TRANSMISSION OF *PLASMODIUM FALCIPARUM* LOW-DENSITY INFECTIONS ASSOCIATES WITH TRANSMISSION-BLOCKING VACCINES *IN SILICO*

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The continued transmission of low-density *Plasmodium falciparum* infections from humans to mosquitoes may pose challenges for malaria control and elimination efforts as they cause insufficient symptoms to trigger anti-malarial treatment for parasite clearance. While high-density infections are observed to induce transmission-blocking antibodies, low-density infections may not. A recent field study indicated that low-density infections can remain infectious over an extended period, contributing

to onward transmission--likely because functional transmission-blocking antibodies are insufficient and short-lived. Malaria transmission-blocking vaccines (TBVs) against sexual-stage surface antigens of *P. falciparum* are potentially effective tools for the reduction of malaria transmission from asymptomatically infected individuals who do not seek anti-malarial treatment. Here, we estimate the impact of the next generation of TBVs on malaria transmission and their relevance for elimination and eradication. Novel understandings of immune dynamics and antibody functionality against high- and low-gametocyte infections were used to design relevant properties of future TBVs, including potency and immunogenicity. The temporal and spatial impact of potential TBVs on malaria transmission is simulated across different transmission settings using an agent-based mechanistic model of within-host effects coupled to a vector model.

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INVECTS (INDOOR VECTOR CONTROL TESTING SIMULATOR): A 3D COMPUTER SIMULATION MODEL OF INDOOR MOSQUITO BEHAVIOR FOR RAPID EVALUATION OF VECTOR CONTROL TOOLS

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Increasing insecticide resistance threatens recent advances in control of malaria in Africa; emerging arboviruses are linked with urbanization, human and vector behavior change; sustainable integrated control of multiple diseases will require more cost-effective approaches. Vector control remains under constant pressure to innovate, iterate and improve. Yet developmental pipelines for new tools are high risk, expensive and time-consuming processes, often hampered by results of tests that are poorly linked to the actual behavior of products in field settings. Improving the speed of this decision-making process is essential if the generation of novel, safe and appropriate tools is to be sustained. Indoor environments (the human home) harbor infection risks from endophilic and endophagic malaria vectors but have also provided the best methods to date. Additional opportunities almost certainly exist within the home. Using a unique room-scale video-tracking system, we have gained exceptional insight into the host-seeking behavior of *Anopheles gambiae* at human-occupied LLINs. We have utilized this information to create a novel virtual testing simulation of mosquito-host interactions within the indoor environment. This system enables rapid assessment of a wide range of innovative vector control ideas, both actual and theoretical, at marginal cost. The InVeCTS model is a software package, simulating vector behavior in a virtual mosquito population during interaction with human hosts and responses to specific vector control tools in a complex indoor spatio-temporal environment. The model operates at a level of granularity that reflects the complex nature of mosquito-host interactions not captured by coarser scale models. Our results show the model can explore mechanisms underlying the observed response of a mosquito population to insecticides on bed nets and the relative performances and benefits of particular bed net barrier designs. InVeCTS is an effective tool in response to the immediate challenge from the growth of resistance. Future model development will include increased spatial scales and multiple-host interactions.

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IDENTIFYING KEY FACTORS OF THE TRANSMISSION DYNAMICS OF DRUG-RESISTANT MALARIA

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Development of resistance to malaria treatments remains a great threat to malaria control and continued transmission reduction. Understanding the key factors that increase the spread of drug resistance or emergence can guide intervention strategies. In particular, to inform risk versus benefits assessments in regards to mass treatment strategies and their potential impact on resistance in different settings. Mathematical modelling provides a framework to understand factors which contribute to the spread of malaria, and importantly can simulate specific drugs and different treatment strategies. The simplicity and relevance of the Ross-MacDonald has ensured that it continues to be a strong basis for a broader theory of mosquito-borne disease transmission and control. We present a model that builds upon these well-established foundations to understand drivers of resistance. Our model includes three infection classes: sensitive, partial-resistance and fully-resistant. These classes are flexible and could represent a single infection with an intermediary step to full-resistance, or each infected host carries two infections where one may be sensitive and the other resistant. Via sensitivity analysis of the model we find that the most effective way to control the spread of resistant infections is by interrupting the rate that more resistant infections replace less resistant infections, and as such this mechanism should be the focus of further research. Importantly, our results imply that reducing treatment has a comparatively small impact, since the most effective way to prevent resistance spreading throughout a population is by controlling the within-host mechanisms of resistance via pharamacokinetics. Our model serves as an introductory model that can act as the foundation for further studies of resistance drivers in a population, and compliment and inform within-host models of resistance.

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ASSESSING THE IMPACT OF IMPERFECT ADHERENCE TO ARTEMETHER-LUMEFANTRINE ON ONWARD TRANSMISSION OF THE *PLASMODIUM FALCIPARUM* PARASITE USING WITHIN-HOST MODELLING

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Artemether-lumefantrine (AL) is the most widely recommended and prescribed antimalarial worldwide. Clinical trials have clearly demonstrated its safety and efficacy. Timely access to quality-assured antimalarials is vitally important, both to ensure good clinical outcomes and minimise onwards transmission of the parasite. In recently published work, we developed a within-host model of falciparum malaria to assess the performance of AL in routine healthcare settings, where adherence to treatment may be imperfect. To do this, we utilised individual-level data on patient adherence. Here we focused on the parasitological outcomes for the patient: we now develop our investigation to quantify the impact that poor adherence has on malaria transmission. With the aid of malaria therapy data, we extend our model to include gametocyte production, explicitly modelling both the immature (sequestered) and mature parasites. With the aid of likelihood-free inference methods, we used individual-level data from clinical trials to calibrate a pharmacodynamic model, quantifying the drug action against sexual-stage parasites. Using our model, we highlight the importance of timely access to treatment by showing how delay to treatment leads to an increase in circulating gametocytes post treatment. In addition, we use adherence data for 482 patients taking a course of AL, collected in Tanzania in 2012, to assess the contribution that sub-optimal adherence makes to transmission of the malaria parasite.

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IN VITRO CULTURE OF A MADAGASCAR *PLASMODIUM VIVAX* ISOLATE IN HUMAN WHOLE BONE MARROW

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Long-term cultivation of *Plasmodium vivax* (Pv) is challenging. Recently, we achieved success in maintaining Madagascar Pv isolates in *Saimiri boliviensis* (Sb) leukocyte-depleted whole blood for 36-233 days at parasitemia 0.1-0.4%. Reports of the occurrence of Pv in bone marrow (BM) have appeared from time to time in the literature. It is suggested that as with *P. falciparum*, the BM could be a niche for gametocyte production and maturation and/or a reservoir in Pv infections. Here, we attempted to culture a Madagascar Pv isolate in human leukocyte-depleted whole BM. A culture of cryopreserved patient isolate Ext3020 was initiated in complete AIM V medium containing 4% Sb blood. On day 30 post-culture, this culture was divided into 4 1-ml cultures: one culture each in Sb blood and 4% fresh BM (BM1, North American donor, Duffy genotype FyA+/B+) was maintained in AIM V medium under 10% CO_2 environment, whereas the other same set of two cultures was maintained in IMDM medium under 5% CO_2 environment. After 7 days, the cultures in Sb blood were further split to initiate new cultures in another fresh BM (BM2, North American donor, Duffy genotype FyA+/B–). These cultures were fed with Sb blood (obtained every 2 weeks) or BM1 or BM2 (both original), stored at 4° C, every 96-120 h, and their medium was changed daily. Imaging flow cytometry using Hoechst or a polyclonal *Plasmodium* MTIP antibody was used to assess parasitemia based on >200,000 cells. Standard Giemsa-stained slides confirmed culture of Pv, showing rings, late trophozoites/early schizonts, and possible gametocytes, with parasitemia 0.1-0.2%. The Pv isolate culture in BM1 maintained in IMDM medium under 5% CO_2 environment was ended on day 48 because of bacterial contamination, whereas all other cultures are being continuously maintained (BM1 [53+ days] and BM2 [46+ days] in AIM V 10% CO_2; BM2 [46+ days] in IMDM 5% CO_2). These results suggest that long-term culture of Pv isolates is possible in human whole BM.

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ASSESSING ORGANIZATIONAL CAPACITY TO DELIVER QUALITY MALARIA SERVICES IN RURAL LIBERIA

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Malaria prevalence in Liberia in children under five years is 45% nationally, and higher in rural counties. Since 2015, Maternal and Child Survival Program (MCSP) has worked with the Liberia Ministry of Health to rebuild and strengthen the health system. Organizational capacity of local public health agencies has been linked to service delivery performance. As part of an expansion of program support to additional high burden and neglected counties, MCSP assessed county health teams' (CHTs) organizational capacity to identify ways to improve quality of malaria health services. MCSP assessed five rural (of 15 total) CHTs' capacity using a modified organizational capacity assessment (OCA) tool. Areas examined were delivery of essential health services (service delivery), health information system (HIS), health workforce (HWF), and leadership/governance with a focus on malaria. A participatory approach was used allowing CHTs to self-evaluate with guidance from the assessment team. Probing techniques and item verification were used to assure validity of responses. The average score of the five CHTs was 83% (range: 67-100%) in service delivery, 65% for HIS (range: 50-100%), 78% for HWF (range: 50-100%), and 70% for leadership/governance (range: 50-100%). Findings show a range of reasonable organizational capacities to manage, monitor, and implement malaria services. Results will be used by MCSP to further strengthen and optimize CHTs' capacities to provide quality malaria services, with the goal of improving malaria prevention in pregnancy and case management at facility and community levels in five high-burden counties in Liberia. While there is reasonable capacity for malaria management in the counties assessed, MCSP will further optimize this capacity to ensure every malaria case receives quality care. This OCA methodology is applicable to other programs aiming to improve the quality of malaria service delivery.

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SAFETY AND FEASIBILITY OF APHERESIS TO HARVEST AND CONCENTRATE PARASITES IN SUBJECTS WITH INDUCED BLOOD STAGE *PLASMODIUM VIVAX* INFECTION

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In the absence of a method to culture *Plasmodium vivax (P. vivax),* the only way to source parasites is *ex vivo*. This hampers many aspects of research on *P. vivax*, including evaluation of candidate hypnozoiticidal drugs. Currently, sporozoites can only be sourced using expensive, logistically complex and unreliable processes requiring transportation of *P. vivax* infected mosquitoes from endemic areas. Apheresis is the removal of a specific component of blood with the remainder being returned to the individual. We aim to assess a) safety of apheresis following inoculation of healthy subjects with *P. vivax, and b)* feasibility of apheresis to extract and concentrate all stages of *P. vivax*. One non-immune healthy human subject was inoculated with blood stage HMPBS13 *P. vivax* parasites and subjected to apheresis 10 days later. Blood samples from haematocrit (HCT) levels; 1%, 2%, 3%, 5% and 7% were tested by qPCR for the presence and concentration of asexual and sexual stages of *P. vivax*. Standard safety assessments were performed. Mosquito membrane feeding assays used percoll enriched blood taken pre-apheresis and apheresis samples taken from 1%, 2% and 3% HCT layers. Parasitemia was quantified by a validated qPCR method targeting the 18S rRNA gene of *P. vivax*, and gametocytes by qRT PCR targeting *pvs25* mRNA, a female gametocyte transcript. There were no serious adverse events and no significant safety concerns. Asexual and sexual parasites were successfully harvested using apheresis with highest concentrations in the 7% HCT and 5% HCT layers. Apheresis demonstrated a 335 fold increase in concentration of parasite genomes, and 3.7 fold increase in the *pvs25* mRNA transcript compared to a whole blood sample taken pre-apheresis. Downstream experiments including membrane feeding assays were inhibited by clotting of post-apheresis samples. Apheresis was safely carried out in a human volunteer experimentally infected with *P. vivax*, and can be used to extract asexual and sexual *P. vivax* parasites. Studies are underway to prevent apheresis sample clotting and improve laboratory processing in order to enhance parasite yields and downstream mosquito feeds.

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INTERMITTENT PREVENTIVE TREATMENT WITH DIHYDROARTEMISININ PIPERAQUINE IN YOUNG UGANDAN CHILDREN IN THE SETTING OF INDOOR RESIDUAL SPRAYING OF INSECTICIDE

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Intermittent preventive treatment of malaria in infants (IPTi) is a promising strategy for the prevention of malaria in young children living in Africa. Dihydroartemisinin-piperaquine (DP) is safe and highly efficacious when used for the treatment of malaria and an excellent candidate for IPTi because of its prolonged post-treatment prophylaxis. However, the optimal dosing strategy for DP as IPTi is unclear. We conducted a double-blinded randomized controlled trial of two different IPTi with DP dosing strategies in a historically high transmission area of Uganda where the burden of malaria has been dramatically reduced following the implementation of indoor residual spraying of insecticide (IRS). A birth cohort of 183 children were randomized in a 1:1 ratio to receive IPTi with DP every 4 weeks or every 12 weeks from 8 weeks to 2 years of age followed by 1 additional year of follow-up after stopping IPTi. The incidence of malaria was measured using passive surveillance and the prevalence of microscopic or sub-microscopic parasitemia measured routinely every 4 weeks. Between 8 weeks and 2 years of age, children receiving DP every 4 weeks had a significantly lower incidence of malaria (0.02 vs 0.39 episodes PPY, p<0.001) and parasite prevalence (0.6% vs 3.8%, p<0.001) compared to children receiving DP every 12 weeks. Follow-up after stopping IPTi will be completed in May 2018, but preliminary results show that children who previously received DP every 4 weeks had a lower incidence of malaria compared to children who previously received DP every 12 weeks (0.79 vs 1.15 episodes PPY, p=0.04). Both treatment arms were safe and well tolerated. In summary, IPT with DP given every 4 weeks was superior to DP given every 12 weeks for the prevention of malaria during infancy and preliminary results indicate that this protection was extended for up to one year after stopping IPT. Indeed, the combination of IRS and IPTi with DP every 4 weeks virtually eliminated malaria during infancy in an area where children suffered ~ 5 episodes of malaria per year prior to the implementation of IRS in the absence of IPTi.

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COMPARISON OF TWO QUANTITATIVE ASSAYS TO MEASURE G6PD ACTIVITY IN A MALARIA RISK POPULATION IN WESTERN CAMBODIA

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Primaquine (PQ) is the only drug currently available for the radical cure and relapse prevention of *P. vivax*, but its widespread use is hampered by the risk of hemolysis in G6PD deficiency (G6PDd). Therefore accurate diagnosis of G6PD status is required for safe use of PQ and other 8-aminoquinolines such as tafenoquine. Pointe Scientific (PS) (Canton, Michigan) is currently one of the quantitative tests available on the market after discontinuation of the Trinity Biotech (TB) (Bray, Ireland) product. We assessed the performance of the PS and TB kits in a Cambodian malaria risk population. After calculating separate population medians using both kits from 414 healthy males, an adjusted median was then calculated, excluding values ≤ 10% of median. Patient values were categorized as either <60% or <30% of the adjusted median; those falling below 30% of the adjusted median were categorized as deficient. Simple linear regression analysis of our volunteer population yielded a slope of 0.93, an intercept of 0.64 and Pearson correlation coefficient is 0.946. The range of quantitation was 0.06 to 20.99 U/g Hgb for TB and 0.23 to 22.05 U/g Hgb for PS, yielding adjusted medians of 7.84 U/g Hgb (IQR 5.11-10.57) for TB and 8.11 U/g Hgb (5.67-10.55) for PS. 78/414 (18.8%) and 79/414 (19.1%) fell below the 60% G6PDd thresholds of 4.70 U/g Hgb and 4.87 U/g Hgb for TB and PS, respectively. Both kits classified 77/414 (18.6%) subjects as deficient using 30% G6PDd diagnostic thresholds of 2.35 U/g Hgb (TB) and 2.43 U/g Hgb (PS). These data show that the two kits yield comparable results and can be used interchangeably in our study population.

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CONTRIBUTION OF THE IMPROVING MALARIA CARE (IMC) PROJECT IN TRANSFORMING THE FACE OF MALARIA CONTROL FOR VULNERABLE POPULATIONS IN BURKINA FASO

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In Burkina Faso, malaria seriously affects vulnerable populations (children under 5 and pregnant women). The 6-year (2013-2019) Improving Malaria Care Project (IMC) funded by PMI aims to improve the quality of malaria prevention, diagnosis and treatment to reduce malaria morbidity and mortality by 50% from baseline figures. IMC has also focused on clinical and health information capacity-building and national-level system change. From 2014-2016, 1,819 providers from 1,349 health facilities in 53 (76%) districts and 185 trainers were trained on new malaria treatment guidelines developed in partnership with the NMCP. 1,300 health workers were trained to use monthly report forms, and providers received post-training supervision. 897 providers from referral hospitals were oriented on severe malaria case management. 38 managers were trained to roll out malaria quality improvement systems. IMC is also supporting the government to change national systems for malaria control, data collection, and supervision and improve data quality. From 2013 to 2016, the number of total malaria deaths decreased by 37% (6,294 to 3,974) and the overall fatality rate decreased by 40% (from 1.5% to 0.9%). Malaria deaths amongst pregnant women decreased by 75% (151 to 38) and the malaria fatality rate declined by 83% (0.6% to 0.1%). For children <5, malaria deaths decreased by 43% (4,761 to 2,730) and the fatality rate declined by 44% (2.7% to 1.5%). IMC-supported sites also distributed 6,347,503 courses of ACTs, 1,220,537 2nd doses of IPTp, and 1,146,185 LLINs between Jan. 2014 and Sep. 2017, possibly averting an estimated 32,435 deaths and 2,758,327 DALYs (calculated using the PSI Impact Calculator). To achieve this, IMC's work was associated with positive impacts. IMC will continue to pursue a comprehensive, multilevel approach, including capacity building, updating the malaria prevention and treatment guidelines according to WHO recommendations, aligning training packages, and improving the malaria data system, and work towards the goal of reducing malaria morbidity and mortality.

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MALARIA RESPONSE PLAN IN TIMES OF HIGH TRANSMISSION: AN APPROACH TO IMPROVING THE QUALITY OF HOSPITAL MALARIA MANAGEMENT

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Malaria is a major public health problem in Burkina Faso. According to the 2016 HMIS statistical yearbook, it is the main cause of consultation (45%), hospitalization (23%) and deaths (18%) in health facilities. During the rainy season (between June and September), there is an upsurge of

malaria cases exceeding the capacity of care in hospitals and causing a high number of deaths especially in children under five years. To help reduce the severity of malaria cases and malaria-related deaths in referral hospitals, the Improving Malaria Care Project (IMC) funded by PMI provided support to the National Malaria Control Program to develop and implement Malaria Preparedness and Response plans in 11 out of 16 regional Hospitals from March to October 2017. The main purpose of these plans was to provide guidance and coordinated action at hospital-level to prepare for and properly treat cases of severe malaria and reduce malaria-related deaths during the period of high transmission, which runs from July to October. Each hospital developed its plan according to a defined outline which included the following strategies: i) capacity building of providers on malaria treatment protocols; ii) the security of a stock of commodities including blood for transfusions in cases of anemia; iii) redeployment / reinforcement of staff in care units; iv) communication; and v) data collection. The implementation of these plans took place from July to October 2017. Follow up visits were organized from October 24 to November 8, 2017. Review of the hospital data revealed that despite the increase in number of severe malaria cases, from 6800 to 9100, malaria-related deaths decreased from 5% to 4.3% in the general population and from 6.2% to 5.4% among children under 5 in these hospitals between 2016 and 2017. Response plans may provide a way to reduce malaria mortality, which needs to be further studied, and which each hospital may want to consider incorporating into its annual work plan.

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WARD LEVEL APPROACH TO DATA VALIDATION: A PANACEA TO DATA QUALITY IMPROVEMENT IN KANO STATE, NIGERIA

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Poor data quality is a major problem in Nigeria's routine health information system. Prior methods of data validation (DV) activities occurred monthly at local government area (LGA) level comprising LGA staff, Heads of health facilities (HoHF), record officers (ROs) and partners before entry of data into the District Health Information System-version 2 (DHIS-2) platform by the Monitoring and Evaluation (M&E) officer. This method is time consuming taking a whole day or more and the payment of per diem for participants. The cost involved was borne by a partner and therefore not sustainable. In June 2017, the US CDC-funded Malaria Frontline Project (MFP) implemented in 20 out of 44 LGAs in Kano State initiated ward-level data validation. A ward-level DV meeting was piloted in Dawakin-Tofa LGA for 3 months and scaled-up to all 20 LGAs. Averagely, an LGA is made of 8 to 13 wards with 3 to 5 health facilities (HF) in each ward. A ward technical officer at the apex HF in each ward convenes a monthly DV meeting of HoHF and ROs in the ward to validate data under the supervision of LGA level senior staff. The aggregated data is sent to LGA where M&E officer collates all ward validated data for entry into the DHIS-2 platform. Due to the closeness of facilities within a ward and less time spent on validation, no per diem is required. The ward level DV meeting has consistently improved data completeness from 97.5% in Q2 to 98.6% in Q4 of 2017 in the LGAs; timeliness has also increased by 7.2% from Q2 to Q4. Due to more HFs reporting, there is an increase in uptake of first dose of Intermittent preventive treatment of malaria in pregnancy indicator among antenatal care attendees from an average of 90% to 95% in DT, and from 77% in Q3 to 80% in Q4 2017 in other 19 LGAs. DVMs helped identify 27% gaps with bed nets requirement for children under five and pregnant women in DT in Q4. The ward-level DV is sustainable, non-monetary dependent and health care system-driven. It has built capacity of health care workers in ensuring quality data. We recommend state and nation wide up-scaling of this approach.

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CHANGES IN CONFIRMED AND CLINICAL MALARIA CASES REPORTED THROUGH THE DHIS 2 SOFTWARE PLATFORM IN KENYA, 2011-2015

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Health facility-based data are valuable for monitoring trends in malaria morbidity and evaluating the impacts of malaria interventions. To examine the relationship between malaria testing and the quality of surveillance data, we conducted a 5-year retrospective, longitudinal analysis of reported outpatient malaria cases from Kenya's DHIS 2 software platform, stratified by age (<5 and ≥5 years), from January 2011 to December 2015. The change in number of confirmed and clinical malaria cases reported between 2011 and 2015 was determined. Testing rates were determined from 2013 onward, because data on testing were not available for the preceding years. Confirmed malaria cases reported in children <5 years increased by 79.2% (992,362 to 1,777,959) and clinical malaria cases decreased by 77.2% (3,083,595 to 702,248). Confirmed malaria cases reported in patients ≥5 years increased by 121.2% (1,680,574 to 3,717,092) while clinical malaria cases decreased by 70.1% (4,928,535 to 1,475,610). In 2013, only 0.1% (5,308 of 8,753,287) of reported cases (<5 and ≥5) were tested for malaria by microscopy or Rapid Diagnostic Tests (RDTs). In 2015, 61.2% (4,697,027/7,672,909) of reported cases (<5 and ≥5) were tested for malaria by microscopy or RDTs. Scale-up of RDTs since 2013 and continued emphasis on parasitological confirmation before treatment may be responsible for the observed increase in the number of reported cases tested. This implies improvements in case-detection practices, supported by the decrease in presumptive cases. Improvements in reporting of malaria cases through DHIS 2 and the revision of data capture tools to record numbers tested and malaria-positives detected may also have supported reporting of confirmed cases. These findings indicate that the quality of malaria surveillance in Kenya has improved. Efforts must continue, to sustain that achievement.

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COST-EFFECTIVENESS ANALYSIS OF MEDICINE QUALITY SCREENING DEVICES IN LAOS

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Poor quality antimalarials are a common problem in many malaria-endemic areas, leading to devastating consequences including increased morbidity, mortality and economic losses. Field detection devices to screen the quality of medicines are increasingly available, however, their cost-effectiveness is not known. To evaluate the cost-effectiveness of handheld devices for medicine quality screening during drug inspections in pharmacies in Laos, conservatively focusing on their benefit in detecting substandard or falsified antimalarial artemisinin-based combination therapies (ACTs). We use a decision tree model to simulate the deployment of devices in inspections at the pharmacy level. Seven devices were evaluated including Minilab, Truscan, MicroPHAZIR, 4500a FTIR, NIRScan, Progeny and Paper Analytical Devices (PADs). We performed two scenario analyses of the prevalence of poor quality medicines. In Scenario 1, 20% of ACTs were assumed to be substandard and 20% falsified, while in Scenario 2, 10% were assumed to be substandard and 5% falsified. We evaluated different sampling strategies using 1, 2, or 3 sample(s) for each brand of ACT. Analyses were carried out for each device against a baseline of visual

inspections, and in a multiway head-to-head comparison of all devices and sampling strategies. In Scenario 1 all but one devices were cost-effective with a 1-sample strategy. In Scenario 2 only three devices, the Minilab, NIRScan, and PADs were cost-effective with a 1-sample strategy. In the multi-way comparative analysis of all devices, in both scenarios the Minilab with a 1-sample is the most cost-effective option, followed by the Minilab with a 2-sample strategy, and the NIRScan with a 2-sample strategy. Routine inspection for poor quality ACTs with field detection devices is cost-effective in Laos where malaria is endemic. This information can aid policy-makers or regulators considering investment in handheld screening devices to improve medicine quality and reduce the undesired health and economic burden associated with poor quality medicines.

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DEVELOPING A DATA STANDARD FOR MALARIA

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Until recently there was no international standard to guide data capture for antimalarial drug efficacy and safety, resulting in a large heterogeneity among studies. The WorldWide Antimalarial Resistance Network (WWARN) partnered with Clinical Data Interchange Standards Consortium (CDISC) and the Critical Path Institute (C-Path) to develop these standards for malaria building on WWARN's experience in establishing a global data platform for malaria studies. WWARN's proposal to CDISC to develop a data standard was accepted in December 2014. Key stakeholders from the malaria community were invited to join a working group, to share protocols, case record forms (CRFs), data dictionaries and to review draft standards. The scoping and planning of the project was followed by identifying key concepts which guided the development of the draft standard. These draft standards were sent to the wider malaria research community for open review, comments resolved then submitted to CDISC for internal review followed by public review and publication. The malaria data standard was published in 2016. This was followed by the development of a training course and assessment, currently under peer review. The standard applies primarily to uncomplicated malaria (P *falciparum* and P *vivax*), and consists of a Malaria Therapeutic Area User Guide (TAUG-Malaria), which includes concept maps, required metadata, Standard Data Tabulation Model (SDTM) examples, an analysis considerations section, controlled terminology and an updated WWARN Clinical Data Acquisition Standards Harmonization (CDASH)-compliant CRF. The development of a data standard for malaria has been an unprecedented collaborative effort to overcome large heterogeneity which have prevailed in antimalarial drug efficacy trial to date. The documents will guide prospective data collection, reduce heterogeneity, facilitate future re-use of data including individual patient meta-analyses. This achievement paves the way of providing data standards for other poverty related diseases as it has recently been done for Ebola in collaboration with the Infectious Diseases Data Observatory (IDDO).

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PREVALENCE OF HIV, HEPATITIS B AND HEPATITIS C INFECTION AMONG POTENTIAL PARTICIPANTS TO EXPERIMENTAL VACCINES TRIALS IN A RURAL AREA OF BURKINA FASO

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In Burkina Faso, there are few studies that reported the prevalence of HBV and HCV in the general population. During early clinical development phase of vaccines, volunteers with these conditions are often excluded from participating to the trials due to safety concerns and the need to minimize any interference with the outcomes measures. This study aimed to evaluate the prevalence of hepatitis B (HBV), hepatitis C (HCV) and the Human Immunodeficiency Virus (HIV) infections in potential trial participants in the context of vaccines study sites characterization. A voluntary testing was opened to anyone interested. These testing were held at the research clinics of Saponé and Banfora. Rapid diagnosis tests to detect the surface antigen HBs, the anti-HCV antibodies and HIV were carried out on apparently healthy persons who voluntarily answered a range of questions before the venous blood sample collection. A total of 450 individuals were screened of whom 247 (54.89%) were women and 203 (45.11%) were male. The age ranged from 15 to 70 years (with a mean of 30.54 ± 10.01 years). The prevalence of HBV, HCV and HIV was 6.20% (95% CI: 3.59-9.88%), 2.71% (95% CI 1.10-5.51), and 3.78% (95% CI 2.29-6.10), respectively. The most affected group were subjects aged 30-40 years for HCV (3.88%), and 20-40 years for HBV (13.28%) and HIV (8.44%). There was no gender difference for any of the infections tested. No case of co-infection was reported. Overall the prevalence of HBV and HCV were relatively low. However, the prevalence of HIV was higher than in previous country's reports. These findings should be considered when planning for vaccine trials in our context.

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WE'VE GOT STOCK: IMPACT OF IMPROVED SUPPLY CHAIN MANAGEMENT ON MALARIA COMMODITY STOCK-OUT RATES AT THE COMMUNITY LEVEL IN MADAGASCAR

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Community health volunteers (CHV) in Madagascar play an integral role in diagnosing and treating malaria cases in children under five (CU5) in populations living more than five kilometers from a health facility. To offer these services, CHVs need a continuous, adequate supply of essential malaria commodities including rapid diagnostic tests (RDT) and artemisinin-based combination therapy (ACT). However, the lack of a standardized community logistics management system compromised not only the availability of, but also the quality and correct use of products by CHVs. To improve the supply of essential health products for community-based primary health care, the USAID Mikolo Project designed and implemented a commodity logistics management system for CHVs working in eight of Madagascar's 22 regions. The project developed standards, procedures, and tools for selecting, quantifying, and managing commodities and integrated them into a training program on stock management for CHVs, health center staff supporting the CHVs, district and regional health managers, and the national directorate of essential medicines. Following the training, the project provided on-site supportive supervision to monitor and further strengthen the CHVs' skills in stock management and to maximize compliance with the standards. The project also supported monthly meetings between CHVs and health center supervisors to review stock usage data to ensure adequate distribution based on need. With the implementation of this community-based logistics system, the USAID Mikolo Project has witnessed great reductions in ACT and RDT stock-outs: across the eight regions, ACT stock-outs decreased from 20% in 2014 to 7% in 2017 and RDT stock-outs decreased from 13% in 2014 to 6% in 2017. The rate of fevers tested with RDTs by CHVs increased from

61% in 2014 to 86% in 2017, and RDT-confirmed malaria cases treated with ACTs by CHVs increased from 35% in 2013 to 89% in 2017. The greater availability of RDTs and ACTs contributed to better malaria case management practices among CHVs. A scale-up of these monitoring tools could improve stock management and malaria care in other communities.

1100

LESSONS LEARNED FROM DEPLOYMENT OF DHIS2-BASED HEALTH NETWORK QUALITY IMPROVEMENT SYSTEM (HNQIS) OVER 4,000 PRIVATE SECTOR MALARIA PROVIDERS IN MYANMAR

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Private sector health care providers are frequently the only source of healthcare in the most remote communities, where malaria is highest and the reach of public and formal health services is most stretched. In November 2015, Population Services International (PSI), in collaboration with the National malaria control program (NMCP), started the roll out of malaria RDTs among thousands of private sector providers in Myanmar. These providers were trained and equipped to conduct RDTs, and to treat malaria cases in accordance with national guidelines, and to report the results. In 2017, PSI launched the Health Network Quality Improvement System (HNQIS), a DHIS2-based android app to assess provider quality of malaria case management, including RDT testing. In 2017, 4,100 providers from 3,052 villages in 15 regions were assessed with HNQIS. The assessment used a comprehensive checklist, covering diagnosis, treatment, counselling, reporting, stock, etc. Following an assessment, HNQIS automatically calculated a weighted quality score and the provider was classified into one of three different classes: class A (score >80), class B (50-80), and class C (<50). Among the providers, 2622 (63.9%) fell into class A, 1125 (27.4%) into class B, and 353 (8.6%) into class C. In 2017, these providers had conducted 354,632 malaria RDTs, and identified 3915 positive cases (1.1% positivity). Among them, 243,728 (68.7%) were by class A providers, 87,468 (24.7%) by class B, and 23,436 (6.6%) by class C. Class A providers had highest average number of tests (93), and highest positivity rates (1.16%); class B were in middle with an average of 78 tests and 0.7% positivity; and class C at the lowest with an average 66 tests and 0.2% positivity. Differences in quality scores among different provider types and different geographic regions were also observed. Using HNQIS, PSI was able to assess the quality of private sector providers in remote regions spanned across the country and to compare the quality scores against reported testing data. In doing so, PSI could identify the areas for further improvement and allocate the resources more efficiently.

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EXPANSION OF MALARIA CONTROL INTERVENTIONS ASSOCIATED WITH THE DECLINE OF ALL-CAUSE CHILD MORTALITY IN THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC) FROM 2005 TO 2015

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In the Democratic Republic of the Congo (DRC), key malaria control interventions—especially insecticide-treated nets (ITNs)—have expanded in the past decade. Measuring the impact of this increased coverage will inform future strategies of the National Malaria Control Program and its partners. We conducted a plausibility assessment of the potential impact of malaria control interventions. We analyzed trends in all-cause childhood mortality (ACCM), coverage of malaria interventions, and other contextual factors affecting child survival. We used Cox proportional hazard models to examine the effect of ITN ownership on child survival. The analyses used data from the Demographic and Health Surveys (DHS) in 2007 and 2013. National-level household ownership of at least one ITN increased from 9% in 2007 to 70% in 2013. All provinces experienced similar patterns. ITN use increased between 2007 and 2013 among children under five years of age (6% to 55%) and pregnant women (7% to 60%). Owing to limited data, we could not assess trends in parasite prevalence, but the prevalence of severe anemia (<8g/dl) among children ages 6-59 months decreased significantly, from 11% (95% CI: 9%-13%) in 2007 to 6% (95% CI: 5%-7%) in 2013. ACCM declined from 148 to 104 deaths per 1,000 live births between 2007 and 2013. The decline in all-cause mortality was greater among children ages 6-23 months (relative reduction of 36%)—the age group at highest risk for malaria mortality in high endemicity areas—than among children ages 24-59 months (relative reduction of 12%). Cox regression showed that household ownership of at least one ITN was associated with a 24% decrease in the risk of mortality among children under five years of age during the 24-month period before the survey (risk ratio = 0.76, 95% CI: 0.64-0.90). During the evaluation period, indicators of socioeconomic conditions and coverage of some health interventions also improved. However, these improvements alone cannot account for the observed 30% decline in ACCM. Thus, we conclude that malaria control interventions have partially contributed to the decrease in ACCM in the DRC from 2005 to 2015.

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METHOD DEVELOPMENT FOR *PLASMODIUM FALCIPARUM* SPOROZOITES PROTEOME INTERROGATION

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Malaria is a life-threatening disease that affects millions of people around the world, with *Plasmodium falciparum* causing the highest mortality rate. The US warfighter faces the highest risk when being deployed to malaria endemic regions, compromising the welfare of the soldier, as well as the mission. We have a novel approach to malaria parasite protein discovery with optimized sample preparation, and Mass Spectrometry (MS) analysis. Current pharmaceuticals are becoming less effective, especially with individuals who are G6PD deficient, and there is not an efficacious vaccine for malaria that is FDA approved. In using a MS-based proteomics approach we able to analyze the complete proteomic profile of *Plasmodium* parasites, assessing proteins that are involved in almost every biological pathway. Characterizing the proteomic profile will be advantageous in the deciphering, discovery, and development of new targets for therapeutic intervention. By applying proteomic principles we have optimized a parasite sample prep methodology that gives us a high yield of proteins, in order to discover candidates. We have observed changes in proteomic profile when comparing sporozoites at zero and four hour time points. The comparison of the parasite in various stages and environments reveals unique proteins depending on the protein expression levels. Using PEAKS Studio 8.5 and Proteome Discoverer 2.2 software, we are able to identify families of proteins present during different time points of development in conjunction with changes in abundance. This will allow for the understanding of proteins present, post-translational modifications (PTMs) that have occurred, and find potential new protein candidates for malaria drug and vaccine development.

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INCREASING THE NUMBER OF HANGING POINTS TO SPREAD A BED NET REDUCED THE RISK OF *PLASMODIUM* INFECTION AMONG CHILDREN IN VILLAGES ALONG LAKE VICTORIA IN WESTERN KENYA

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Long-lasting insecticidal bed nets (LLINs) are effective to prevent malaria transmission. Nevertheless, malaria is still responsible for the death of several children in Africa. One plausible reason is that bed nets are not properly hung when children do not sleep on a bed. Rectangular bed nets would not be easily fixed at four points in each corner when they are used on the floor in particular, because of the distance to the hanging points and obstacles by household goods. There, the net dose not spread enough, and children's body parts may touch the net or extend out of it, which may increase the risk of infection. We directly observed the sleeping condition of children under 15 years through a cross-sectional study in Gembe east, western Kenya. The data collected included the number of points that fixed their bed nets, whether their body parts touched the net, whether their body parts were completely inside, and whether they slept on the bed or not. Then the children were tested for *Plasmodium* infection using polymerase chain reaction (PCR). The data were analyzed using multiple logistic regression analysis. Of 694 children observed, 510 (73.5%) were sleeping under LLINs. The PCR positive prevalence was 47.1% for net users and 51.6% for non-net users (OR: 2.14 for non-users, 95%CI: 1.40-3.30). Bed nets used on the beds were fixed at more points than those on the floor (OR=1.21, 95%CI: 1.11-1.32). Children's body parts were less likely to be outside or touch the net with increasing the number of hanging points (OR: 0.78, 95%CI: 0.69-0.88). Children whose bodies were completely inside a net had significantly lower prevalence than those who were outside or touched a net (OR: 0.66, 95%CI: 0.44-0.99). The risk of infection decreased with increasing the number of hanging points (OR: 0.52, 95%CI: 0.31-0.88). These results suggest that LLINs should be properly fixed and spread for children to sleep completely inside and prevent infection, and a bed net should be used with a bed.

1104

IMPACT OF INDOOR RESIDUAL SPRAYING (IRS) INTERVENTIONS ON MALARIA INCIDENCE TRENDS IN THE EAST COAST OF MADAGASCAR

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Between 2014 and 2017, The President's Malaria Initiative (PMI) Africa Indoor Residual Spray (AIRS) Program conducted annual IRS campaigns, using pirimiphos-methyl CS, in three high endemic districts of the eastern regions of Madagascar, to reduce malaria transmission. The overall spray coverage among the eligible structures was above 90% for all campaigns from 2014-2017, with the total number of eligible structures sprayed per district of 53,074 in Brickaville, 83,536 in Fenerive East, and 66,652 in Tamatave II for the most recent IRS campaign in 2017. The AIRS Madagascar team assessed the impact of IRS on the incidence of malaria in these districts by comparing epidemiological data from three sprayed districts with two non-sprayed districts, which present similar malaria transmission patterns. Routine data from community health agents, basic health centers and district health centers were collected from January 2013 through August 2017 and analyzed to assess the impact of IRS. Multiple regression analysis was used to isolate the effect of IRS on malaria incidence using primary data provided by the National Malaria Control Program controlling for variables that influence malaria transmission, such as near surface humidity, rainfall, elevation, temperature, and long-lasting insecticide-treated nets (LLINs) coverage. Malaria incidence in IRS districts was 7.2/1,000 people among all ages in 2013 and 6.0/1,000 in 2016. However, in non-sprayed districts it was 2.6 and 3.2 per 1,000 people in 2013 and 2016, respectively. While the malaria incidence has decreased in the intervention areas in 2016 compared to 2013, data from the control areas showed an increase in incidence in 2016 compared to 2013 pre-spray data. Our assessment showed that IRS alone contributed to a decrease of 10% in malaria incidence among all age groups, which is significant at the 0.001 level. Combining IRS with LLINs contributed to a greater reduction, 30% decrease in malaria incidence. In conclusion, our results suggest that IRS alone or combined with LLINs can continue to be used as key malaria control interventions in high transmission districts of Madagascar.

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IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON MALARIA TRANSMISSION ON TWO SITES OF THERAPEUTIC EFFICACY STUDY IN MALI

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Seasonal Malaria Chemoprevention (SMC) consists of giving a treatment dose of SP/Amodiaquine for 4 consecutive months. We have selected 2 sites, Sélingué in the south of the Sudanese and Missira in the north sudanese regions. SMC consisted of administering the drugs door to door on designated days in August, September, October and November 2017. The first dose of a dispersible co-blister of SP/AQ which was given each month to children between 3 and 59 months. The child's caregiver then provided the AQ tablets on the subsequent 2 days. The objective was to test the efficacy of AL and ASAQ in children <5 years of age in the context of SMC. In order to enroll the 480 study participants, we have followed 5363 children between 3 and 59 months of age targeted for the 4 rounds of SMC. Children with *P. falciparum* uncomplicated malaria with parasitemia ≥1,000 during the 4 rounds of SMC were assigned randomly into the AL and ASAQ arms. We collected from each study participant 2 ml of blood at the times of recruitment and failure. Slides and filter paper blots were made during the 3 days of the drug administration and the follow-up at days 7, 14, 21, 28, 35 and 42. In case of recurrent parasitemia, quinine was used to treat the patient. We have compared the number of screened children for parasitemia and the number of enrolled children in 2016 and 2017 in order to examine the efficacy of ACTs in Mali. Baseline data was collected in 2016 from Sélingué prior to implementation of SMC. Thus in 2017, we enrolled 240 children between August and November. After the completion of SMC, only 75 children out of the remaining 240 were enrolled after screening 6141 children (between December 2017 and March 2018). In contrast, in 2016 we have enrolled 480 children by screening only 3208 in the absence of SMC in Sélingué (between July and December 2016). The Chi square test for trend was performed using GraphPad Prism showed that the impact was marked

on the malaria transmission when the SMC is performed (P< 0.0001). The decrease of malaria cases was similar in both sites where SMC was performed in 2017 (P= 0.7456). This study suggests that SMC may have had a great impact on the malaria morbidity.

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QUALITATIVE ASSESSMENT OF LONG-LASTING INSECTICIDAL NETS TO PREVENT MALARIA ON BIOKO ISLAND, EQUATORIAL GUINEA

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This qualitative study sought to examine incentives and barriers to LLIN use, care, and upkeep to inform social and behavior change communication strategies for integrated malaria control. What happened to Permanet 3.0 LLINs supplied via mass distributions, schools, and antenatal clinics? Why did household LLIN ownership decline rapidly after universal distributions? What factors influenced LLIN use, and what caused low rates of use of available LLINs? Restricted randomization was applied to households to find individuals who met inclusion criteria for gender, geography, reported LLIN use or non-use, and pregnancy status. Written informed consent was obtained, and focus groups were recorded. Trained Equatoguinean facilitators used a discussion guide in Spanish to facilitate 7 focus groups designed to maximize diversity across groups. Community-based participatory analysis was used to highlight new themes that emerged each day. Transcripts were prepared and discussion in local languages translated into Spanish, and the authors performed thematic analysis to identify barriers and incentives to LLIN use. Knowledge of LLINs was good and had little impact on use. For the 70% of participants who used LLINs, perceived benefits of LLIN use such as protection against malaria and other vector-borne diseases outweighed barriers. Risk perception was the main factor influencing use: malaria was perceived as a severe illness for pregnant women and children, but not for men. Despite having knowledge of malaria's severity, some participants did not internalize the risk to which they were exposed. Common barriers to use included dissatisfaction with the LLIN product due to design or color, heat, bad odor, pruritus from contact with the net, dirtiness, and discomfort. Non-users considered the relative benefit of LLIN use limited in the context of indoor residual spraying of insecticide and outdoor malaria transmission. Participants recommended strengthening community processes, especially having neighbors promote and reinforce acceptance, use, and proper care of LLINs, and providing regular follow-up of households that received LLINs.

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COST EFFECTIVENESS OF PIPERONYL BUTOXIDE (PBO) BED NETS VERSUS PYRETHROID-ONLY NETS IN PREVENTING MALARIA: A FIELD STUDY IN NIGERIA

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Long lasting insecticidal nets (LLINs), being safe and inexpensive, have been the major tool towards halving malaria's burden since 2000. By 2016, 81% of malaria endemic countries with monitoring data reported resistance to pyrethroids. Growing insecticide resistance threatens their ongoing value. In 2017, the World Health Organization concluded that LLINs with a synergist, piperonyl butoxide (PBO), provided additional public health benefit over conventional LLINs alone in areas of moderate insecticide resistance and endorsed them as a new class of vector control products. However, as PBO nets cost 95% more than conventional LLINs ($4.08 vs. $2.09 per net), critics fear that their use could reverse progress towards universal net coverage. This study assesses the cost-effectiveness of a PBO-LLINt compared to pyrethroid-only LLINs in an area with confirmed pyrethroid resistance. Data come from a randomized two-village trial in Nigeria with epidemiological outcomes. After 18 months of observation with no nets, beginning in 2014 residents in the control village received LLINs and those in the intervention village received PBO nets. Both were followed an additional 32 months. The health center recorded and tested all suspect malaria cases from both villages. Impacts on disability adjusted were modeled. The negative binomial regression analyses found that the PBO LLIN reduced symptomatic malaria cases by 33.4% (p<0.01). The incremental cost per net was US $1.99. The incremental cost-effectiveness ratio was US $16 per DALY averted. Even excluding the offsets in treatment costs and health gains, the added economic benefits of PBO nets over conventional LLINS yielded an extraordinary benefit-cost ratio of 85 to 1. Beyond its public health value, PBO LLINs appear to deliver excellent value for a small additional cost in areas of insecticide resistance.

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USE OF A RAPID EVALUATION TO IMPROVE GEOGRAPHIC COVERAGE OF HOUSEHOLD REGISTRATION DURING BENIN'S 2017 INSECTICIDE-TREATED BED NET DISTRIBUTION CAMPAIGN; LESSONS LEARNED AND RECOMMENDATIONS

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Complete household (HH) registration (HHR) is key to successful insecticide-treated bed net (ITN) distribution campaigns. In Benin, HHR teams go door-to-door for 10 days to give coupons exchanged for ITNs, provide ITN distribution information, and mark HH (D: registered, DA: absent, DA+: absent on re-visit, DR: refused). To measure real-time HHR geographic coverage and assess quality of ITN distribution information provided, Benin piloted a rapid evaluation in September 2017. Three villages per arrondissement were randomly chosen; in each village a random HH was chosen to start and 10 rural HH or 20 urban HH were systematically chosen per a sampling frame based on the total number of houses per village. Unregistered HH were those without 'D,' 'DA+,' or 'DR' or those with 'D' that had not received a coupon. Successful HHR was defined as 0-5 unregistered HH per 30 HH/arrondissement; more information was needed if 6-8 HH were unregistered; and registration was to be performed again if >8 HH were unregistered. Of 11,670 HH evaluated, 10,967 (94%) reported that HHR teams had visited them; 10,746 (98%) of their HH were marked. Of 703 (6%) HH that reported no HHR team visit, 186 (26%) were marked. Among 10,947 registered HH, 10,837 (99%) received coupons and 1,970 (18%) and 6,568 (60%) reported they received information on ITN distribution dates and sites, respectively. Overall, 342 (89%) of 384 arrondissements had successful HHR with <5 unregistered HH. The rapid evaluation allowed evaluators to measure HHR geographic coverage in real-time; however, unclear communication channels prevented evaluators from providing results directly to HHR teams in the field which delayed HHR remediation. Data on the quality of ITN distribution information provided were likely underestimates because evaluators did not often encounter the same HH members who were visited by HHR teams. We recommend rapid HHR evaluations define clear evaluator/HHR team feedback mechanisms, focus on collecting verifiable data such as coupons and marked HH, and consider evaluators' time and resources when including questions about the quality of ITN distribution information provided.

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COMMUNITY FACILITATORS AND BARRIERS TO A SUCCESSFUL IMPLEMENTATION OF MASS DRUG ADMINISTRATION AND INDOOR RESIDUAL SPRAYING FOR MALARIA PREVENTION IN UGANDA

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There is growing interest to add Mass Drug Administration (MDA) to existing malaria prevention strategies, such as Indoor Residual Spraying (IRS), to accelerate malaria reduction. A successful MDA and IRS requires high population-wide coverage, emphasizing the importance of community acceptance. This study's objectives were to identify community level facilitators and barriers during the implementation of both interventions in a community with a high malaria transmission intensity. This qualitative study was conducted in two sub-counties in Katakwi district. Kapujan sub-county residents received two rounds of IRS and MDA while Toroma residents received only two rounds of IRS. Key informant interviews and focus group discussions were conducted with key influential district and sub-county personnel and community members respectively. Data was analysed using thematic analysis.Transcripts and interview notes from the in-depth interviews were analysed using ausing a coding scheme developed from pre-defined topics together with themes emerging from the data. The Nvivo software programme was used to aggregate the data by codes and to assist with findings presentation. Overall, fourteen key informants were interviewed; four from Katakwi district and five each from Kapujan and Toroma sub-counties. Five focus group discussions were conducted; four with community members (men and women), two in each sub-county and one with medical staff of Toroma health center IV. Community acceptance of MDA and IRS can be improved through community sensitization, appropriate timing of implementation during the low activity dry season, involvement of government and local leadership, use of the competent locally composed team, community knowledge of malaria effects and consequences and evidence of malaria reduction from interventions. Potential barriers included; miss-communication regarding interventions, the strong unpleasant smell from IRS' Actellic and inadequate duration for engagement with the community. These findings can be used as a basis for improving community acceptability for MDA and IRS campaigns in such settings.

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KNOWLEDGE, ATTITUDES AND PRACTICES OF MOTHERS CARETAKERS OF CHILDREN AGED 3 TO 120 MONTHS ON OF SEASONAL MALARIA CHEMO PREVENTION IN BOUNKILING HEALTH DISTRICT, SOUTH SENEGAL

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Knowledge, attitudes and practices of mothers and caretakers of children aged 3 to 120 months on of seasonal malaria chemo prevention in Bounkiling health district, south Senegal KALY S. N, NDIAYE Y, FAYE A, BA M. The adequate therapeutic coverage of children aged 3 to 120 months targets for seasonal malaria chemoprevention (SPC) is faced with poor administration of both doses of amodiaquine (AQ) by mothers and caregivers (MaC) in Bounkiling Health district. The overall goal was to study the knowledge, attitudes and practices (KAP) of MAC on SMC in this Health District in 2015. A cross-sectional study was conducted; data were collected through a questionnaire addressed to MaC after an informed consent. Data were entered and analyzed using the EPI INFO software version 3.5.3. Results: A total of 396 MaC were enrolled. Their average age was 30.9 years and the average age of children aged 3 to 120 months was 42.9 months. Adequate therapeutic coverage of children was higher 56.8% (n=54) among polygamous MGEs than 18.1% monogamous (18,1%, n=51) (OR=5,93; IC [3,57 – 9,86]). It went from 52.6% (n=10) among MaC who were unaware of the benefits of SMC to 26.0% for MaC who knew about the benefits, (OR = 3.16, IC [1.24-8.01]). Adequate therapeutic coverage was also linked to the type of adverse drug reactions reported; 30.5% (n= 82) among MaC cited vomiting; 17.6% (n= 12) reported sleepiness and 14.8% (n= 4) reported abdominal pain and diarrhea (Chi2 = 6.71, p = 0.03). Improving the adequate therapeutic coverage of children for SMC is essential to achieve malaria elimination in southa Senegal. It is needed to develop strategies for improving full therapeutic coverage.

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SETTING THE STAGE TO INTRODUCE A GROUND BREAKING APPROACH TO PREVENT MALARIA IN PREGNANCY IN SUB-SAHARAN AFRICA: BASELINE-READINESS ASSESSMENT FINDINGS FROM DEMOCRATIC REPUBLIC OF CONGO, MOZAMBIQUE, MADAGASCAR, AND NIGERIA

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Uptake of intermittent preventive treatment during pregnancy (IPTp) is unacceptably low across most countries eligible for the intervention in sub-Saharan Africa. Delivery solely through antenatal care (ANC) has yielded suboptimal results. A Jhpiego-led consortium, in partnership with local ministries of health, is implementing the *Transforming IPTp for Optimal Pregnancy* (TIPTOP) project, which supports community distribution of quality assured SP (C-IPTp), complementing delivery at antenatal care (ANC). TIPTOP aims to increase IPTp3 coverage from 19% to 50% of eligible pregnant women in project areas in Democratic Republic of Congo (DRC), Madagascar, Mozambique, and Nigeria. The project, operating from 2017 to 2022, will provide SP, promote community awareness, and support supervision and coordination efforts between health facilities and community health workers (CHWs) to achieve the 50% target. In year 1, a baseline assessment examined facility readiness for MiP management, SP stock, ANC provider knowledge, CHW characteristics and health facility linkages, and health information system (HIS) quality. TIPTOP assessed 140 facilities and interviewed 175 ANC providers and 67 CHW supervisors. The assessment found that among ANC providers, 80% correctly reported that at least three doses of IPTp are recommended, with a low of 65% in DRC and a high of 100% in Mozambique. Sixty-four percent correctly responded that SP should be initiated in the 2nd trimester, with a low of 15% in Mozambique and a high of 88% in DRC. CHWs varied in terms of gender ratio, ratio to pregnant women, and country-specific eligibility requirements, but all had formal linkages with health facilities. Over-reporting of ANC contacts and IPTp service provision is a data quality challenge. HIS in Nigeria and Mozambique record IPTp3 provision, but only at the local, not national, level. Results from the baseline assessment are informing efforts to improve data quality and CHW-facility data flow; and to strengthen provider knowledge through TIPTOP supported trainings. Additionally, implementation has taken into account the variation discovered among the CHW cadre.

1112

SEASONAL MALARIA CHEMOPREVENTION SCALING UP IN MALI: COVERAGE AND IMPACT ON MALARIA BURDERN AND MARKERS OF THE RESISTANCE OF *PLASMODIUM FALCIPARUM* TO SULFADOXINE PYRIMETHAMINE AND AMODIAQUINE

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Mali was one of the first countries to introduce Seasonal Malaria Chemoprevention (SMC), starting in one district in 2012, and reaching then nationwide coverage from 2016 in covering all of the 65 districts. From 2014, the (ACCESS-SMC project from 2014-2016 and World Bank from 2016 onwards) were also among the partners of Mali in scaling up the SMC coverage but also was involved in evaluating the impact of SMC in Mali. SMC drugs using Sulfadoxine-pyrimethamine plus Amodiaquine was given to children aged from 3 months to 59 months years old monthly during rainy season up to four months by the community health workers. Up to 2016, SMC drugs distributions were done using fix teams, door-to-door, from 2017 onwards. Cross-sectional surveys were undertaken in 2015, 2016 and 2017 about a month after the 4th and last SMC cycle. A total of 50 clusters were selected with probability proportional to size in 5 districts each year and in each cluster about 20 children aged between 4 months and 7 years were surveyed. To assess the impact of SMC on malaria burden in Mali, nationwide data routine malaria data from 2013 to 2017 were collected. In addition, routine individual malaria data from health center registers as well the birth histories surveys from women were collected in 2016 and in 2018 using tablets PC. The impact of SMC drugs on malaria parasite resistance was assessed using two cross-sectional surveys; in February 2016 before the SMC program starts in the study area and in March 2018, two campaigns/seasons when SMC program started. The proportions of children who received the four cycles of SMC were 38% in 2015, 57% in 2016 and 72% in 2017. The coverage rates were relatively higher in 2017 compared to 2015 and 2016 following the change in distribution method from fixed point distribution to the door-to-door dstribution. Detailed results in SMC distribution coverage and SMC impact on malaria and the impact of SMC drugs on malaria parasite resistance will be available and presented.

1113

IMPACT OF CONSTRUCTION OF SOCIAL HOUSING UNITS ON MALARIA VECTOR ABUNDANCE ON BIOKO ISLAND, EQUATORIAL GUINEA

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Construction of massive infrastructures that are often associated with urbanization has profound implications on malaria epidemiology and vector control in Africa. In 2017, the government of Equatorial Guinea created new districts on Bioko Island and embarked on the construction of social housing units in these districts. This study evaluated the impact of the construction of the social housing units on vector abundance on Bioko Island. Mosquitoes breeding habitats at the construction sites of 13 new districts were monitored during the construction phase on a weekly basis. Data were captured on the physical characteristics of each breeding habitat, as well as the larval and pupal forms of mosquitoes. Out of the 13 constructions sites in each of the 13 districts, nine construction sites were intervened with the application of a microbial larvicide, *Bacillus thuringiensis israelensis (*VectoBac GR). Two sites in two districts served as controls without the application of the larvicide. Adult mosquitoes were collected using human landing catches in the surrounding communities at both the intervention and control communities. The proportion of breeding habitats with mosquito pupae in the intervention sites dropped from 30% at baseline to an average of 3% after six months of treatment while that of the control sites increased from 0% to an average of 25.8%. A total of 1,651 mosquitoes were collected during this period. 66% were *Anopheles spp*, 20% *Culex spp* and 14% *Aedes spp*. *Anopheles* man biting rates reduced from 5 bites per person per night at baseline in the intervention communities to an average of 2 bites per person per night while that of the control communities increased from zero to an average of 5 bites per person per night. The construction of housing units which led to the increase in vector densities at the surrounding communities was significantly reduced by the application of larvicide. Larval source management as a complementary intervention is necessary at construction sites to reduce vector populations and prevent possible malaria outbreak.

1114

SPATIAL ANALYSIS OF THE ASSOCIATION BETWEEN LLIN COVERAGE AND MALARIA PREVALENCE AFTER A LLIN CAMPAIGN IN A COMMUNITY IN NORTHEAST UGANDA

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In 2016, a large-scale malaria intervention trial was implemented in three communities in Uganda. Two communities received several rounds of indoor residual spraying and one community also received several rounds of mass drug administration of antimalarials. All three communities received long-lasting insecticide treated nets (LLINs) after the baseline survey. Since that baseline survey, two additional six-month cross-sectional surveys were conducted, and prevalence was measured using rapid diagnostic tests (RDT) and microscopy. Additional questions were administered at each survey regarding LLIN coverage. In this study, we analyzed the association of LLIN coverage and malaria prevalence rate in the community that received only the LLIN intervention using a geostatistical model to control for spatial heterogeneity. At each survey, approximately 200 randomly chosen households were surveyed between Feb 2016 to Jan 2017. A generalized linear spatial model was fit to household prevalence and LLIN use (measured by nets per person). Malaria prevalence rate decreased by 34% according to RDT and by 18% by microscopy from survey 1 to 3. LLIN use increased 14.3% between survey 1 to 3. On average, a 50% increase in LLIN coverage was associated with a 15.7% (95% CrI: 1.53%-24.6%) reduction in prevalence rate by RDT and 20.3% (95% CrI: 3.11%-37.0%) by microscopy.

1115

THE ASSOCIATION BETWEEN INSECTICIDE-TREATED NETS (ITN) USE AND MALARIA PARASITAEMIA IN CHILDREN UNDER AGE 5 IN THE NORTHERN REGION OF SIERRA LEONE

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Despite many malaria programmatic interventions in the northern region of Sierra Leone malaria parasitaemia remains high. According to the 2016 Sierra Leone Malaria Indicator Survey (SLMIS) malaria parasitaemia in children under age 5 ranges from 21% in the western region to 52% in the northern region. This study set out to determine the association

between the use of insecticide-treated nets (ITN) and malaria parasitaemia among children under five years in the northern region compared to other regions of Sierra Leone. Cross-sectional household survey data from the 2016 SLMIS was used to analyze the results of the malaria microscopy test for children under the age of five years. After controlling for child's age, residence (urban/rural), wealth, and mother's education this study found that children who slept under an ITN the night before the survey were significantly less likely to have malaria as compared to those that did not sleep under an ITN in the other regions (South, Eastern, and Western) of Sierra Leone (OR: 0.70 p-value 0.003). However, in the northern region children who slept under an ITN were less likely to have malaria but the result was not statistically significant (OR: 0.9 p-value 0.5). Based on the findings of this study, further research is needed into the efficacy of ITNs as well as other malaria programmatic interventions in the northern region of Sierra Leone.

1116

MALARIA DEATH SURVEILLANCE IN UGANDA: NOVEL APPROACH TO INITIATE MORTALITY AUDIT IN HIGH PATIENT LOAD FACILITIES

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Uganda is one of the malaria high burden countries in the world. The first of three goals of the Uganda Malaria Reduction Strategy is to reduce annual malaria deaths from the 2013 levels to near zero by 2020. Significant (74%) reduction in malaria mortality has been recorded over the past decade. Malaria surveillance including mortality has been adopted as one of the core interventions in the prevention and control of malaria as part of efforts of the National Malaria control Program to achieve zero death due to malaria in Uganda. The objective is to track areas or groups of people that are mostly affected by malaria, identifying underlying factors, and use information to target resources for maximum impact and establish a system for timely data reporting, analysis and use at all target levels by all sectors reporting for both public and private facilities. On a weekly basis surveillance data is extracted from the national database- the District Health Information Systems-DHIS2 and mTRAC; analysis, calling back in all facilities with reported deaths and developing a line list for the confirmed deaths. Supplementary death audits have been done in Hoima, Kabarole and Kiboga District that have persistently reported < 3 death cases due to malaria on a monthly basis. By January 2016, 18/116 districts had an average of 3 death cases due to malaria per month, more pronounced in children under five. Most deaths were attributed to poor health seeking behavior and delayed referrals or access by refugee communities. Through a strengthened effort of the Malaria Control Program and the key stakeholders, behavioral change communication has been scaled up in the affected communities and improved coordination mechanism at district and health facility levels. Health facilities have been supported in terms of capacity building through vigorous integrated malaria management training, improved supply management, Clinical Audits, and data management for program decision making. Currently Malaria mortality has reduced in selected districts with a range of 1-2 deaths per month.

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THE EFFECT OF CHLOROQUINE DOSE AND PRIMAQUINE ON *PLASMODIUM VIVAX* RECURRENCE: AN INDIVIDUAL PATIENT POOLED ANALYSIS

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Chloroquine remains the mainstay of treatment for *Plasmodium vivax* malaria despite increasing reports of failure. We investigated the effect of chloroquine dose and adding primaquine on the risk of recurrence, in a range of vivax-endemic settings. A systematic review identified prospective *P. vivax* clinical trials published between January 2000 and March 2017. Principal investigators were invited to share individual patient data, which were pooled using standardised methodology. Multivariable Cox regression analyses with random effects for study site investigated the role of chloroquine dose and primaquine use on rate of recurrence between day 7 and 42. In total, 37 studies and 5,240 patients were included. 2,990 patients were treated with chloroquine alone, of whom 1,041 (34·8%) received a dose below the recommended 25 mg/kg. The risk of recurrence was 10·4% (95%CI 9·3-11·6) by day 28 and 32·4% (29·8-35·1) by day 42. After controlling for age, parasitaemia, relapse periodicity and gender, a 5mg/kg higher chloroquine dose reduced the rate of recurrence by 18% (Adjusted Hazard Ratio (AHR) 0·82, 0·69-0·97; p=0·0210). The greatest reduction was in children aged <5 years (AHR 0·59, 0·41-0·86; p=0·0058). Adding primaquine reduced the risk of recurrence to 1·4% (0·9-2·1) at day 28 and 4·9% (3·1-7·7) at day 42, a rate of recurrence 90% lower than those treated with chloroquine alone (AHR 0·10, 0·05-0·17; p<0·0001). In summary, chloroquine is commonly underdosed in the treatment of vivax malaria. Increasing the recommended dose of chloroquine to 30 mg/kg in children <5 years could reduce substantially the risk of early recurrence when primaquine is not given. Radical cure with primaquine was highly effective in preventing early recurrence and may also improve blood schizontocidal efficacy against chloroquine resistant *P. vivax*.

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8-AMINOQUINOLINE ASSOCIATED HEMOLYTIC RISK IN FEMALES HETEROZYGOUS FOR G6PD DEFICIENCY IN *PLASMODIUM VIVAX* ENDEMIC POPULATIONS

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Plasmodium vivax is estimated to be responsible for over 100 million clinical infections annually. Treatment with 8-aminoquinoline drugs, such as primaquine, can completely clear *P.vivax* parasites and block vector-borne transmission. These drugs can also cause severe hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency, an X-linked genetic disorder that affects more than 400 million people worldwide. Females who are heterozygous for G6PD deficiency have two populations of red blood cells: one with low G6PD activity and one with high G6PD activity, resulting in intermediate levels of G6PD activity in the majority of women. The risk of hemolysis among those with intermediate G6PD activity levels represents is poorly understood. SMRU and PATH are conducting a randomized controlled trial to associate intracellular G6PD activity to the likelihood of hemolysis following primaquine treatment. 72 total participants will be assigned to one of two treatment arms; each arm will comprise 12 males with normal G6PD levels, 12 females with normal G6PD levels, and 12 females with intermediate G6PD levels (30-80% of normal activity). Arm 1a will receive primaquine for 14 days and Arm 1b will receive chloroquine for 3 days and concomitant primaquine for 14 days. All participants will be healthy volunteers without severe G6PD deficiency, monitored for two weeks after study drug dosing. Blood samples will be taken at regular intervals for hematologic measures, G6PD quantification, and drug level assays. The trial is expected to be conducted from May to September 2018. We will present demographic data of the study population and descriptive statistics of the primary endpoints: absolute hemoglobin reduction and related changes in intracellular G6PD concentration profiles over the treatment course. Data from this trial will be used to better understand the risk of drug-related hemolysis, particularly in females heterozygous for G6PD deficiency with intermediate G6PD activity levels.

1119

ENHANCED SURVEILLANCE FOR CONTROL AND ELIMINATION OF MALARIA IN THE PHILIPPINES

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A subnational malaria elimination approach is currently implemented in the Philippines, where provinces make use of different strategies depending on the level of malaria endemicity in their area. Despite this, case reporting for surveillance remains to be mainly passive through health facilities, potentially underestimating the country's disease burden. This study aims to develop and establish an integrated surveillance tool for enhanced malaria control and elimination activities. Health facility-based surveys were conducted in Palawan, Occidental Mindoro and Bataan, representing 3 areas of differing disease burden, to assess the magnitude of the proportion of infections missed by routine surveillance. Along with clinical information, electronic mobile-based questionnaires with integrated spatial information on location of residence of patients consulting at health facilities and their companions were also collected. Microscopy and RDT diagnosis were performed on-site, and dried blood spot samples were collected for molecular and serological assays. Current results show that 16% of the malaria cases in Palawan came from companions of health facility attendees (i.e, missed infections), and an additional 6% of cases were detectable only by PCR (i.e. subpatent infections). Molecular characterization suggested that the parasites harbored mostly wild-type alleles in genes associated with drug resistance (96-100% for *pfmdr1, pfcrt* and *K13,* but 0-40% for *pfdhps* and *pfdhfr*). Moreover, mapping the serological prevalence in the 3 sites highlighted spatial patterns of local transmission. Health systems analysis was also conducted to explore if the developed surveillance tool can be integrated and operationalized, and results indicate that the decision will largely depend on the local assessment of its feasibility. The study is continuing to work closely with the National Malaria Program and local government units to discuss how to overcome the barriers to implementation. Enhancing the surveillance using these tools that can be adapted in varying elimination settings could help in achieving a malaria-free Philippines by 2030.

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SMALL UNMANNED AERIAL VEHICLES TO COMBAT MALARIA

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While we have come a long way in reducing the incidence of malaria through the use of long-lasting insecticide treated mosquito nets (LLINs) and indoor residual sprays (IRS), the use of outdoor based interventions, such as larviciding, in addition to the indoor preventative measures, can decrease, and possibly even eliminate, the number of malaria cases worldwide. It is infeasible, however, to continuously survey for standing water locations in expansive remote areas where malaria cases often crop up. Previous studies have shown small unmanned aerial vehicles (sUAVs) are cost effective in surveying large areas. These studies typically used a single sUAV and required ground personnel to treat identified sites. Multiple sUAVs offer flexibility in mission planning and robustness in mission execution. In this proposed work, we use a team of sUAVs to survey an area for standing water, collect larvae samples, and deploy environmentally friendly larvicide, *bacillus thuringiensis* subspecies *israelensis* (BTI). Sophisticated directional, vision and terrain observation technologies are planned to be integrated to penetrate dense canopy, locate previously hidden water larvae breeding sites, and treat them with BTI. When combined with education, sanitation, extensive use of bed-nets and housing improvements, sUAV's can potentially save thousands of additional lives in Malaria endemic areas. The aerial vehicles are developed at the unmanned systems laboratory on the campus of the University of Tennessee at Chattanooga (UTC). Field trials are planned to take place in the swampy rice paddy terrain of southeast Tanzania. It is anticipated that the partnership between UTC researchers and experienced Malaria entomologists at Tanzania's Ifakara Health Association will result in a new powerful tool in dealing with the anopheles gambaie culprit.

1121

A CRITICAL ENQUIRY INTO THE VARIABILITY OF INSECTICIDAL NET USE IN CAMBODIA

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National programs that distribute long-lasting insecticidal nets (LLINs) to people living in malaria-endemic regions are a cornerstone of global malaria control efforts. LLIN programs aim to achieve "universal coverage" of at-risk populations, measured by indicators constructed from questionnaires in Malaria Indicator Surveys. Reductions in malaria incidence that can be achieved through LLINs are usually assumed to have been optimised once universal coverage has been reached. Through an ethnographic exploration of variability in LLIN use in Ratanakiri province in Cambodia, we argue that indicators of universal coverage of LLINs are not sufficiently commensurate with the realities the indicators are intended to measure, and that this limits the suitability of the data to serve policy purposes in a malaria elimination era. We consider this gap between 'net use', in all its permutations in practice, and the numerical representations of such local net use, actually justifies further exploration of strategies to improve LLIN use in subgroups where persisting malaria transmission clusters, rather than diverting resources away from LLIN research in favour of novel technologies to achieve further reductions in malaria transmission. In addition to the commonly used criteria of 'efficacy' and 'effectiveness' when assessing LLINs as a tool for malaria prevention, we propose 'appropriateness' as a third key concept by which to evaluate LLINs. We define 'appropriateness' as the extent to which LLINs as an intervention (referring to their design as well as their distribution) align with the conditions and associated requirements of the contexts in which they are intended to be used. We intend to demonstrate how failure to adapt LLIN interventions to local contexts induces variability in use, which is not measured when intervention impacts are evaluated. We therefore also use the concept of appropriateness as an analytical lens to examine the relation between LLIN interventions and LLIN coverage indicators.

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ASSESSING AND IMPROVING THE PERFORMANCE OF THE MALARIA ELIMINATION PROGRAM AT THE SUBNATIONAL LEVEL IN CAMBODIA

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Cambodia is where drug resistance historically emerges and then spreads. Our study area is in a forest "island" where DHA-piperaquine-resistant

parasites first crossed borders. In Vietnam, we developed methods to rapidly eliminate malaria; we will replicate/extend the methods in Cambodia. An approved research protocol with six objectives is in place; the first three objectives are funded and will be executed April to July 2016. The objectives/brief methods include: 1) Well-characterized malaria patients, malaria transmission *Foci*, reporting and response approaches. Case reporting, investigation and response will be conducted using the current methods compared with a new approach to target people co-exposed in the actual forest transmission *Foci*. 2) Improved case-management for malaria patients. Systems will be developed, tested to document patients completed treatment correctly and were followed-up for late treatment failure. 3) Malaria drug quality monitoring in both public and private sector outlets. Survey and drug testing methods routinely used by the Cambodia Ministry of Health standard operating procedures will be evaluated. For all interventions, a results-based funding model will be tested. Our initial findings based on interviews with provincial health staff, village heath workers and security forces reveal that malaria increased by ~ 2.5 fold in our target area from 2016 to 2017, with a district level annual parasite index (APIs) increasing from ~ 22 to ~ 55. Current usage of insecticide treated nets in forest risk areas appears to be very low. Challenges with antimalarial drug adherence have been identified. Security personnel protecting the forest have been identified as a neglected population, a probable malaria transmission reservoir and a group who can really help malaria elimination personnel understand the situation in high risk areas. We will present study results and present methods to demonstrate malaria, despite very high APIs, can be rapidly eliminated in this area before the current and potentially last drug regimen is lost.

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COMMUNITY PERCEPTIONS ON ASYMPTOMATIC MALARIA CARRIAGE FOR UNDERSTANDING ADHERENCE TO REACTIVE CASE TREATMENT IN THE GAMBIA

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Innovative and cost-effective strategies to identify and treat asymptomatic malaria infections are required but remain a challenge. In the Gambia, a transdisciplinary cluster-randomized trial evaluating reactive treatment with a 3-day course of dihydroartemisinin-piperaquine (DHAP) delivered by village health workers (VHWs) to asymptomatic compound contacts of malaria cases was tested. To understand community attitudes towards the intervention, we explored through an anthropological study broadly the socio-cultural representations of asymptomatic disease and specifically asymptomatic malaria treatment. In the pre-trial questionnaire administered, 70% of respondents mentioned malaria could be hidden in the body without symptom appearance. Whilst this may be interpreted as people's comprehension of asymptomatic malaria, qualitative data indicated that respondents had three different interpretations of asymptomatic disease. It was described as a condition where (i) mild symptoms were present but the person could still carry out their daily activities, (ii) symptoms would appear periodically and disappear over the course of the illness and (iii) disease was hidden until symptoms appeared at a certain time. Respondents further mentioned mosquitoes, hot sun and hard work as the triggers for symptom development but this knowledge was surrounded by doubt and uncertainty. As a result of the uncertainty, taking treatment for an asymptomatic condition was perceived acceptable only when the disease status could be confirmed. However with high treatment adherence (92%) reported for the intervention where there was no screening provided for asymptomatic cases, knowledge on asymptomatic carriage was not seen as a crucial reinforcing factor. Adherence to DHAP treatment was seen to reinforced by the perceived protective effect of the medication, the exemplary role of compound heads and the trust placed in the implementing institution and the VHWs. Adherence to reactive case treatment can be improved by employing a systemic approach and engaging the necessary key stakeholders and community structures.

1124

DEFINING THE BOUNDARIES OF LOCAL MALARIA TRANSMISSION TO INFORM TARGETED RESPONSE STRATEGIES IN THE REMAINING HOTSPOTS IN OKAVANGO, NGAMI AND CHOBE MALARIA ENDEMIC DISTRICTS OF BOTSWANA

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Malaria transmission in Botswana is highly focal, with 78% of the 1532 locally infected cases confirmed in case-based surveillance between 2013 and 2016 occurring in three out of 28 districts. Botswana has reoriented its program towards elimination since 2010, but the surveillance system has gaps that hinder an understanding of the geographic limits and drivers of transmission. This inhibits deployment of targeted and aggressive interventions, which are critical to increasing efficiency of resources and accelerate efforts towards elimination. To address this surveillance gap the National Malaria Programme (NMP) and Clinton Health Access Initiative (CHAI) conducted a household parasite survey during the peak transmission season from February to March 2018 in Okavango, Ngami and Chobe districts. The study area was divided into 5x5km blocks, and a total of 180 out of a possible 563 were randomly selected according to incidence estimates. In each block, intended sample size was 130 people to assert that prevalence was <5% at 95% confidence. In each household, information on demographics, mobility, malaria history, preventative measures and health facility utilization was collected through a structured questionnaire. In addition, two individuals (one >5 years and one ≤5 years of age) were randomly selected to receive a Malaria Rapid Diagnostic Test (RDT), and provide blood spots on filter paper for future molecular analyses (quantitative polymerase chain reaction (qPCR) and serology). A total of 5503 questionnaires were administered. Initial results show 5 of 7459 individuals tested with RDTs were malaria positive. Out of the surveyed households, 59% had received indoor residual spraying (IRS) in the recent season, and 58% had at least one bednet for every 2 household occupants. The laboratory results as well as questionnaire data will continue to be analyzed, and the outcome will inform the development of high-resolution malaria transmission maps and the identification of malaria transmission drivers. These findings will help inform targeted response such as IRS campaigns and bed net distribution in Botswana.

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LIMITED EFFECTIVENESS OF MALARIA CONTROL STRATEGIES IN REMAINING HOTSPOTS IN SOUTH-CENTRAL VIETNAM: AN ANALYSIS OF SOCIAL FACTORS

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Residual malaria transmission remains in specific hotspots in mountainous areas in south-central Vietnam despite the success of standardized control program to reduce and eliminate transmission elsewhere in the country. A sequential exploratory mixed-methods study was conducted in Ninh Thuan province in 2016-2017 to assess why malaria persists amongst Ra-glai ethnic minority. Preliminary hypotheses from the qualitative strand

were quantified in a cross-sectional survey with 1,957 individuals and 410 household heads. Firstly, Ra-glai territoriality and mobility patterns made it difficult for the malaria control program to access this population, while exposing them to *An.dirus* the main malaria vector in this region. 97.6% of surveyed households owned at least one field in the forest; 48.2% had a hut there where they regularly stayed overnight. Although 92.1% reported sleeping under a mosquito net the night before the survey, only 66.0% of them brought a net when they last stayed overnight in the field or forest. Secondly, despite free of charge health service, 33.4% did not attend public facilities for their recent illnesses. 24% of malaria patients reported to stop medication after 2 days when fever subsided. Factors related to communication were prominent; language barriers, lacking of health education materials in Ra-glai language influenced people's access to public health service. Mobility factors and the therapy choice were stemmed from the complex interethnic relations with Kinh, the dominant ethnicity. Mobility patterns related to subsistence farming revealed social boundaries that were at odds with regulations on forest management and sedentary farming. For the therapy choice, the hierarchical doctor-patient relationship, as a manifestation of the ethnic hierarchy in Vietnam, constrained trust in the health system for Raglai people. This study highlights the limitation of standardized malaria elimination strategies for heterogeneous minority settings and the relevance of understanding social factors in addition to WHO's recommendation for national strategies to include epidemiological stratification.

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FEASIBILITY AND ACCEPTABILITY OF A PEER NAVIGATOR-LED MALARIA FOCAL TEST AND TREAT INTERVENTION TARGETING HIGH-RISK POPULATIONS IN SOUTHERN LAO PDR

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Populations at higher risk (HRPs) of malaria infection in southern Lao PDR share similar occupational, behavioral, and social characteristics that increase their exposure to outdoor-biting mosquitoes. Based on a series of formative assessments in Champasak Province, shared characteristics include routine forest- and field-based work, sleeping outdoors, and frequent travel to local worksites. To engage these often hard-to-reach populations and improve their access to care, we designed a novel focal test and treat strategy (FTAT) employing peer navigators (PNs) to actively seek out HRPs in forested areas, rice fields, and other non-village sites for participation in an intervention comprising the following: demographic and malaria risk factor survey, testing using standard and Alere highly-sensitive RDTs, dried blood spot collection, treatment of positive cases, and GPS tracking of HRP movement patterns. The FTAT intervention is part of a larger community-randomized trial to assess the effectiveness of different targeted test and treat activities to reduce malaria transmission among village residents and HRPs in 56 villages across 14 health center catchment areas. A mixed-method design will be used to assess the capacity, feasibility, and acceptability of PNs to implement FTAT over an 8-month period from March through October 2018. Data collection methods include direct observations of PNs conducting FTAT and interviews and focus groups with a diverse set of stakeholders including HRPs, participating PNs, formal health sector staff, and national and local malaria officials. This assessment will be important to understand and document stakeholder acceptability and PN competencies, motivations, and perceived FTAT challenges and facilitators to inform potential scale-up or adoption of the strategy in the future. As more countries progress towards elimination, identifying evidence-based, community-led strategies to target HRPs and improve their access to malaria services is a priority, and the PN FTAT intervention represents an innovative approach to addressing this gap if found to be effective, feasible, and acceptable.

1127

THE USE OF THAILAND'S MALARIA INFORMATION SYSTEM TO IMPROVE ACCESS AND QUALITY OF MALARIA SERVICES FOR VULNERABLE COMMUNITIES

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In 2001, Thailand established malaria posts to provide free community-based malaria testing and treatment services without the need for licensed medical workers. With guidance from the Ministry of Public Health (MOPH), pre-set criteria were used to select malaria post workers to ensure operational consistency across all posts. The MOPH's malaria information system (MIS) was used to help determine where and how long malaria posts should be placed as well as how to monitor the quality of services provided. Individual electronic records of confirmed malaria cases are captured in real-time and active transmission *Foci* are geo-coded on the MIS platform. The system also captures information from case finding and treatment activities, which is used to monitor and evaluate screening activities, including case load, quality of care and compliance with the operational guidelines for each post. Key variables include: date of diagnosis, date of report, parasite species, treatment provided, age, sex, ethnicity, occupation and risk behavior. From 2004–2017, malaria posts tested 828,204 persons and treated 68,966 patients; among these, 8% were children under 5 years of age, 25% were students aged 5-14 years, and 32% were female. About 48% of patients treated by malaria posts were cross-border migrants compared to 20% treated in public hospitals. As the number of confirmed cases decreased from 26,566 in 2004 to 14,664 cases in 2017, the malaria positive rate among migrants seeking treatment also decreased significantly from 12.3% in 2012 to 2.1% in 2017. The expanded role of malaria posts included supervised treatment, behavior change communication, and distribution of long-lasting insecticide-treated bed nets. The Thai MOPH uses the MIS to inform decisions about where to allocate resources for the establishment of malaria posts, which are shown to be critically important in reaching cross-border migrants and other vulnerable populations with malaria testing and treatment services.

1128

IVERMECTIN-BASED MALARIA VECTOR ELIMINATION VIA PERI-DOMESTIC CATTLE TREATMENT IN THE HIGHLANDS OF VIETNAM: EXPLORATORY STUDIES AND VILLAGE-BASED TRIAL

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The Greater Mekong Subregion (GMS) has made important progress towards malaria elimination goals, and Vietnam is currently committed to national malaria elimination by 2030. However, some gaps exist, especially in targeting interventions to vulnerable populations at the fringes of national health systems. Ivermectin, an endectocidal drug, has recently emerged as a potential new tool towards malaria elimination, as humans and animals retain blood concentrations that decrease fecundity and are lethal to feeding anopheline vectors. Zooprophylaxis is using large (generally herd) mammals as bait to 'pull' vectors into traps; these alternative feedings then provide passive protection to human populations. High cattle ownership in many rural communities suggests the utility of combining these strategies in an 'attract and kill' approach. This study utilizes semi-field testing, and a community-scale randomized experimental design. It will assess if sufficient densities of ivermectin-treated cattle can drastically decrease vector densities and alter mosquito population compositions in village settings. In Quy Nhon, Vietnam, colonies of *Anopheles dirus* ss and local cattle will be used in a semi-field study to ensure proper drug dosing, quantify impacts of ivermectin on local vectors, and capture blood levels to assess drug pharmacokinetics in local cattle. Thereafter, six villages in Krong Pa district, Gia Lai province, Vietnam will be randomized as control or treatment sites. In intervention villages, the herds of all consenting households will be treated with a 1% ivermectin (at standard veterinary dosing). At intervention and control sites a combination of cattle-baited trapping, human-landing catches, and CDC light trapping will be used to measure anopheline vectors before & after intervention. Reduction in captures from cattle-baited traps will be the primary endpoint; secondary endpoints will be changes in vector composition. Finally, the feasibility and acceptability of the intervention will be assessed. Data collection will occur in the second half of 2018. Category 1: Malaria - Elimination Category 2: Malaria - Entomology

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PLASMODIUM FALCIPARUM POPULATION GENOMIC DIVERSITY ALONG THE SLOPE OF MOUNT CAMEROON

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Malaria remains a global concern, especially in sub-Saharan Africa, despite significant investments in control interventions. In some endemic settings, information on the genetic diversity of the parasite, which will inform future vaccine and drug trials is limited. This study characterized the genomic diversity of *Plasmodium falciparum* populations from different altitudinal zones, with a view to identifying population substructure due to differences in parasite prevalence levels. *P. falciparum* infected individuals were enrolled through cross-sectional surveys in 2013 and 2015 from localities at varying altitudes along the slope of mount Cameroon. Malaria parasitaemic blood screened by light microscopy and parasite's genotype determined using a barcode containing a panel of 24 SNPs. Of the 301 participants enrolled, 176 (58.5%) had single (monogenomic) infections while 125 were polygenomic, with complex parasite populations. The proportion of polygenomic infections was high overall (41.6%, 122/293) and increased (p=0.052) in 2015 (46.4%, 77/166) when compared to 2013 (35.3%, 48/136). In 2013, polyclonal infections were highest (p=0.038) in participants from low altitude areas (59.1%, 13/22) when compared to their intermediate (30.4%, 28/92) and high (31.8%, 7/22) altitude counterparts. Principal component analysis of individual allele frequencies did not identify any parasite population substructure accruing to varying minor alleles in time and space. The overall pairwise Fst of parasite populations across the three altitudinal zones and between 2013 and 2015 was low, with at most 3.5% of the overall allelic variation, due to differences between the populations. The overall complexity of infection (COI, 1.35) did not vary with altitude (p=0.106) and time (p=0.161), although it was higher (p=0.022) in participants at low compared to those from intermediate altitude in 2013. These results indicate enormous diversity, a broadly mixing population and no evidence for recent bottlenecks due to enhanced interventions. We are currently using whole genome sequencing to gain higher resolution of these populations.

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RISK FACTORS ASSOCIATED WITH MALARIA INFECTION AMONG PATIENTS WHO SEEK HEALTH CARE THROUGH THE PUBLIC AND COMMUNITY SECTORS IN MALARIA ENDEMIC TOWNSHIPS OF AYEYARWADY REGION MYANMAR

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Malaria cases in Ayeyarwady, a delta region in the southwest of Myanmar, have decreased by 86% from 2011 to 2016, but exposure to forested areas may pose an obstacle to continued decline. Out of 26 total townships in the region, Ngaputaw, Pathein, and Thabaung represent 74.4% of the total confirmed cases in 2017(n=2,024). Despite this geographic concentration of cases and perceived link between forest exposure and malaria, limitations of surveillance data prevent a thorough understanding of the risks for contracting malaria in this region. A case control study was conducted to ascertain forest travel patterns and use of preventive methods and to evaluate the influence of these behavioral and occupational patterns on risk of malaria infection. Patients consulting for fever and tested with a rapid diagnostic test (RDT) for malaria were recruited since September 2017 by staff at 17 health facilities and by 24 village health volunteers in the three highest endemic townships. A case was defined as any recruited patient over 2 years old living or working in the three townships with a fever > 38 C and positive malaria RDT. Controls presented to selected points of care with fever but received a negative malaria test. Information was collected including demographics, geolocation of participant home villages, forest travel patterns, and use of preventive methods. A total of 1,227 individuals were sampled with 76 positive cases. The relationship between sleeping in forested areas overnight and confirmed malaria was observed to be a significant risk factor for malaria infection, with an OR of 3.29 (95% CI 1.67, 7.06). Reported ownership of bednets was high but not significantly different between cases and controls, with an OR of 1.03 (95% CI 0.57, 1.93). This study identified critical areas of high malaria burden and provided evidence that extended time in forested areas contributes to malaria risk. Further exploration of risk around forest worksites should include a focus on extended stays, specific occupations, and travel patterns to optimize interventions.

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ESTIMATING THE TREATMENT EFFECT IN PARTICIPANTS ACTIVELY SEEKING TREATMENT IN A CLUSTER RANDOMIZED CONTROLLED TRIAL OF TRAINING TEACHERS TO DIAGNOSE AND TREAT UNCOMPLICATED MALARIA IN THEIR LEARNERS

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In a randomised controlled trial, the effect of an intervention can be estimated by calculating the difference in outcomes between the groups. The 'standard' approach uses an intention to treat analysis (ITT) where all participants are included in the group to which they were assigned, whether or not they received their allocated intervention. An ITT analysis reduces post-randomisation selection bias, and estimates the intervention effect under routine application, its effectiveness. However, an ITT analysis does not estimate efficacy, the effect of an intervention under ideal circumstances. Per-protocol (PP) analysis, which includes only those who receive the treatment they are randomised to, and 'as treated' (AT) analysis according to treatment received, both give biased estimates of treatment effect. One approach, which provides unbiased treatment effect when there is 'non-compliance' is the complier-average causal effect (CACE) which estimates effect among those who comply with the treatment offered. A cluster-randomised trial was conducted in 58 schools in Malawi. The intervention involved training teachers to diagnose and treat uncomplicated malaria in learners using rapid diagnostic tests and artemisinin-based combination therapy as part of a Learner Treatment Kit (LTK). The primary outcome was teacher-recorded school attendance monitored over 50 weeks, estimated using an ITT analysis. Of interest is the treatment effect among pupils who potentially had malaria, and therefore sought at least one LTK consultation during the trial; we consider these pupils to be the compliers. We defined the CACE estimate of treatment as the odds ratio for absenteeism among compliers in the intervention arm relative to potential compliers in the control arm. The ITT analysis provided no evidence of an effect of the intervention on school attendance. However PP and AT analyses suggested that that children who sought LTK consultations were less likely to be absent from school. The CACE estimate suggested a larger effect of the intervention than the ITT analysis and suggested that the intervention might be effective in those who sought treatment.

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TOWARDS MALARIA ELIMINATION: DEVELOPMENT AND ROLL-OUT OF AN INTEGRATED SURVEILLANCE SYSTEM FOR MALARIA ELIMINATION IN SOUTH AFRICA, 2018

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Strengthening the surveillance system has been a fundamental pillar of South Africa's 2012-2018 Malaria Elimination Strategic Plan. South Africa has prioritized upgrading the Malaria Information System (MIS) onto a single web-based platform to consolidate, and replace the legacy assortment of decentralized Access, SQL, and excel databases for disease surveillance and monitoring and evaluation of interventions. In 2016, South Africa began work to upgrade the MIS on a District Health Information System 2 (DHIS2) platform. Improving functional attributes (the upgraded MIS generates required reports and facilitates required analyses for all users) and operational attributes (the upgraded MIS decreases the time spent capturing, cleaning, analyzing and reporting upon data) of the system were critical priorities for design of the platform, together with sustainability and feasibility of implementation at the ground level. The integration of these myriad malaria data streams into a single system will allow for a rapid, routine, and robust picture of malaria at different administrative units. Users will be able to seamlessly analyse and visualize cases and intervention coverage for a given location, as each module shares a common system and framework. In May 2018, roll-out of the upgraded MIS on DHIS2 will begin. The DHIS2-based MIS includes modules for case notification and case investigation, active case detection, *Foci* investigation, and indoor residual spraying, with a vector surveillance module pending. It allows for real-time data capture from Android tablets by users in the field, and real-time analysis of data by users at all levels in the form of tables, charts, and maps. Sharing experiences in the development and identification of key attributes for the new Malaria Information System can provide a model to facilitate best practices for other malaria eliminating countries as well as for case-based surveillance of other diseases.

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STRATEGIES FOR INTERRUPTING FOREST-BASED MALARIA TRANSMISSION

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In many Southeast Asian settings, forest-going is a high-risk behavior for individual malaria infection and community transmission. Interventions applied in villages, such as village malaria workers, bednet distributions, and mass drug campaigns, often fail to reach forest-goers, leading to challenges for elimination programs. Because individuals from multiple villages often visit similar forest areas, forest-going can also connect otherwise distant transmission pools. In this study, we build a mathematical model of a transmission system that includes both villages and forest locations. The model is used to inform how forest transmission and migrant movement can sustain malaria in Southeast Asian settings and to test how likely different intervention scenarios would lead to elimination. We describe the impact of missing forest-goers on the success of standard intervention packages, identify optimal and feasible targeting strategies, and describe what should be done in villages to minimize outbreaks and prevent resurgence given continued importation from forest-goers.

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RISK FACTORS ASSOCIATED WITH MALARIA CASES OBSERVED VIA PASSIVE AND REACTIVE CASE DETECTION AFTER TWO YEARS OF INTERVENTIONS AIMING AT ELIMINATION IN SOUTHERN MOZAMBIQUE

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Since 2015, a malaria elimination pilot program has been undergoing in Magude district. After two years of indoor residual spraying followed by mass drug administration (MDA) of dihydroartemisinin-piperaquine (DHAp), prevalence dropped to 2.6% at the end of the 2016-17 transmission season and a system of reactive case detection (RCD) with focal MDA at the household level was implemented. Using a structured questionnaire, demographic data and data on use of malaria prevention tools was collected from passively-detected cases and their household contacts at the end of the 2017 dry season. Each household contact was tested for malaria by rapid diagnostic test (RDT) and treated with DHAp. Logistic regression models adjusting for household clustering via random effects were used to identify the main risk factors associated with actively or passively detected malaria cases after two years of interventions. Data presented is preliminary. 649 passively-detected cases were followed from August to November 2017. Of their contacts, 2,312 (92.3%) were RDT-negative and 181 (7.3%) were RDT-positive, here defined as actively-detected cases. In comparison with contacts, passively detected cases were more likely to be male (odds ratio=1.62, p=0.02), 5-15 years old (OR=1.31, p<0.01) and to have travelled in the previous 30 days (OR=2.84, p<0.01). They were also more likely to have slept under a net

the night before (OR=1.26, p=0.02) although this may be affected by the fact that the questionnaire was administered 1-3 days after diagnosis. Actively-detected cases were more likely to be 5-15 years old (OR=1.91, p<0.01) and to have travelled in the previous 30 days (OR=2.81, p=0<0.01) compared to RDT-negative contacts. Thus far, travelling and being 5-15 years old seem to be the main risk factors associated with malaria cases after two years of interventions aiming at elimination in the south of Mozambique. Taking into account entomological and climate data may shed new light on further risk factors.

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A RIGOROUS AND REPRODUCIBLE BAYESIAN METHOD FOR DETERMINATION OF THE ORIGINS OF MALARIA INFECTION IN AREAS APPROACHING ELIMINATION

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To maintain political and financial support for malaria elimination it is essential to rigorously evaluate a country's progress. For a country on the cusp of elimination it is crucial to accurately determine the origins of each disease case. Currently there is no clear definition of how NMCPs should do this and classification methods vary between countries. Overestimating the number of imported cases risks hiding pockets of ongoing transmission whilst underestimating the number may waste valuable resources. Here we present a Bayesian method for determining whether malaria was locally acquired or imported from abroad. This approach is applied to case data from a country on the cusp of elimination. The NMCP for the country in question routinely collects travel histories of individuals diagnosed with malaria and classify each case as either: imported, local, or intraported. This classification is investigated in a statistical framework that combines travel histories with epidemiological data from the locations visited, to predict the likely origin of each infection and, importantly, the uncertainty associated with this designation. Our approach indicates that there is considerable uncertainty and possible misclassifications in the origins of infection for many cases in the dataset. This uncertainty is driven by poor understanding of the generation time of malaria and the presence of asymptomatic infections which may have been missed by the local health system. Our analysis highlights the difficulty with determining the origins of infection for countries nearing elimination. Often the patient data routinely collected is insufficient to conclude whether a case is acquired locally or abroad. A benefit of applying the methodology we present here is that this uncertainty can be explicitly expressed and included in summary statistics and future analyses. An additional benefit of this approach would be realised by applying it to data from neighbouring countries approaching elimination. Our approach could then produce real-time fine-scale maps of endemicity to enable targeting of local reservoirs of infection to hasten national elimination.

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A COMPARISON OF MALARIA INDICATOR DATA QUALITY WITHIN ZAMBIA'S MALARIA RAPID REPORTING AND HEALTH MANAGEMENT INFORMATION SYSTEMS

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Recent iterations of Zambia's National Malaria Strategic Plans have increasingly emphasized the importance of timely, accurate malaria surveillance data to inform malaria control and elimination efforts. The Malaria Rapid Reporting (MRR) system was developed to meet the growing need for timely, case-specific malaria surveillance data to supplement data provided by the national Health Management Information System (HMIS). Previous evaluation has described the quality of data submitted by health facilities through the MRR system, generating interest in the comparative reliability of overlapping malaria data available through the HMIS. This analysis examines the consistency between the MRR and HMIS for the same facilities and time periods. Two rounds of routine data quality audits across 14 districts of Southern Province captured malaria indicator data values recorded in health facility paper registers and those submitted to both information systems over 6-month periods of 2014 and Q4 2015/Q1 2016. Using registers for source data verification of system values, preliminary analysis summarized data element accuracy for both systems at each facility over the audited periods and then compared median facility accuracy for each system. In 2014 and 2015, data values reported in the MRR system were closer to those recorded in facility registers (i.e., more accurate) compared with the HMIS [median facility accuracy: 80.2% *vs* 67.9% (2014); 90.3% *vs* 70.6% (2015) for MRR and HMIS systems, respectively]. Overall, both the MRR and HMIS saw improvements to reported data accuracy from 2014 to 2015. Accuracy of MRR and HMIS data improved in most audited districts, though varied widely by facility. Improvements in data accuracy within the MRR and HMIS over the past few years are encouraging, yet differential rates of improvement and lagging quality of HMIS malaria data suggest that further improvements are needed to be on par with the MRR. Continuing analysis will include additional data for Q4 2016/Q1 2017 and explore reporting rates and accuracy within the HMIS and how well health facility characteristics predict similarities in HMIS and MRR data quality.

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STRATIFICATION OF MALARIA TRANSMISSION DYNAMICS AND OPTIMAL INTERVENTION PACKAGES IN ZAMBIA AND MOZAMBIQUE

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Elimination of malaria in most endemic environments requires the coordination of time-limited interventions, such as indoor residual spraying (IRS) and insecticide-treated bed net (ITN) distributions, with longer-term investments in surveillance and case management. Given scarce resources, we are motivated to identify the minimal combination of interventions that can reliably achieve elimination in a given malaria context. The choice of optimal intervention package is not universal: It varies geographically as a function of the factors known to influence the efficacy of campaigns, such as historical transmission intensity, population clustering, and importation rates, among others. Any regional optimization of interventions thus requires stratification, the process by which these factors of influence are identified and sub-regions are classified. With this goal in mind, we have employed detailed 1km gridded simulations of two regions—Lake Kariba in southern Zambia, and Magude district of Mozambique—that have both benefited from IRS and ITN campaigns as well as multiple rounds of mass drug administration (MDA). While both regions have experienced dramatic decreases in the incidence of malaria infections, transmission has not been completely interrupted everywhere. Drawing from survey data, we implement the timing and coverage for MDA, ITN, and IRS on a per-grid cell basis. We also implement data-informed migration, treatment-seeking, and reactive case detection. Relative abundances of An. arabiensis and An. funestus vectors are determined by calibrating to observed parasite prevalence and clinical incidence using entomological data as a prior. We then simulate a set of potential intervention scenarios to identify those packages that yield a high probability of interruption in different risk strata. By carrying out this detailed analysis in two sites with different

entomology, climate, hydrology, etc., we are able to identify more general features of the feasibility and impact of various intervention packages in the sub-Saharan context.

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PAST, PRESENT AND FUTURE STRATEGIES FOR MALARIA CONTROL IN TANZANIA: OLD AND NEW APPROACHES TOWARDS MALARIA ELIMINATION

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In Tanzania, between 2000 and 2017 malaria prevalence has declined from more than 40% to less than 10%. Currently about half of the country is in low endemicity while the rest is in moderate and high endemicity. Malaria heterogeneity has been consistently observed in different geographical and socio-economic strata. People living in rural areas and within the lowest wealth quintile are 5-10 times more affected than those living in urban areas and within the highest quintile. Past: During the past 15 years, evidence based malaria control interventions have been deployed on a massive scale: 71 million ITNs distributed, 6 million households sprayed, 170 million ACT and 100 million mRDT distributed in health facilities. As a result malaria services accessibility and usage increased over the time. Future: the NMCP has identified two major strategic matters: 1) Is the country able to continue charting the way towards malaria elimination with the current interventions? 2) Is the current situation conducive to deploying appropriate intervention packages for specific populations with differing risks: low wealth, low education; hard to reach and resilient to changes areas. At the heart of these two questions is the strategic decision to realistically move towards malaria elimination. At this point the most relevant programmatic matters to be addressed are: universal access to quality, safe, affordable diagnostics and therapeutics; high coverage of preventive services where needed and intensified surveillance and response. Macro and micro-stratification, impact and cost modelling have been developed to support evidence for the country strategic planning towards malaria elimination.

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BCG-INDUCED TRAINED INNATE IMMUNITY DURING CONTROLLED HUMAN MALARIA INFECTION

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Recent evidence suggests that certain vaccines, including Bacillus-Calmette Guérin (BCG), can induce changes in the innate immune system with non-specific memory characteristics, termed 'trained immunity'. We performed a randomized, controlled clinical trial in 20 healthy male and female volunteers to evaluate the induction of immunity and protective efficacy of BCG vaccination against a controlled human malaria infection (CHMI). Earlier and more severe clinical symptoms in BCG vaccinated volunteers were associated with heterologous, memory-like monocyte and (innate) lymphocyte re-activation that correlated with reduced parasitemia at 5 weeks post CHMI. These findings demonstrate for the first time that BCG vaccination induces trained immunity with functional activity against an unrelated human pathogen *in vivo*. It forms a strong impetus to further explore its potential in the clinical development of a rational malaria vaccine strategy.

1140

IDENTIFICATION OF *PLASMODIUM* GAPDH VACCINE ANTIGENS THAT TARGET SPOROZOITE LIVER INVASION

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Malaria sporozoite liver infection is an essential step for parasite development in its mammalian host. Previously, we used a phage display library to identify mimotope peptides that bind to Kupffer cells and competitively inhibit sporozoite-Kupffer cell interaction. These peptides led to the identification of a Kupffer cell receptor - CD68 - and a *Plasmodium* sporozoite ligand - glyceraldehyde-3-phosphate dehydrogenase (GAPDH) – that are required for sporozoite traversal of Kupffer cells and subsequent infection of hepatocytes. Here we report that the C-terminal end of *Plasmodium* GAPDH interacts with the Kupffer CD68 receptor and identify two epitopes within this region as candidate antigens for the development of a pre-erythrocytic malaria vaccine.

1141

USE OF NSAIDS FOR MITIGATION OF MALARIA-ASSOCIATED SYMPTOMS AT HIGH DOSES OF SANARIA PFSPZ CHALLENGE UNDER CHLOROQUINE PROPHYLAXIS

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Immunization with 3 doses of ~$5x10^4$ aseptic, purified, cryopreserved, infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) (Sanaria® PfSPZ Challenge) under chloroquine chemoprophylaxis, a strategy termed PfSPZ-CVac, achieved 100% (9/9) and 80% (4/5) vaccine efficacy (VE) in malaria-naïve volunteers against homologous controlled human malaria infection (CHMI) at 10 weeks after last vaccine dose. Achieving a comparable level of VE against heterologous CHMI, which may be more reflective of field transmission of heterogeneous strains of Pf, may be more difficult. While higher doses of PfSPZ Challenge may improve VE against heterologous CHMI, moderate and severe symptoms associated with Pf infection have been a limiting factor in some trials. Reducing the severity of malaria-associated symptoms would significantly improve the tolerability and therefore the acceptability of PfSPZ-CVac. An ongoing clinical trial (NCT03083847) at the NIH Clinical Center is evaluating whether 3 doses of $2x10^5$ PfSPZ of PfSPZ Challenge confer protection against heterologous CHMI in healthy malaria-naïve adult volunteers taking chemoprophylaxis. A pilot group of 4 volunteers received $2x10^5$ PfSPZ of PfSPZ Challenge, and they subsequently experienced 19 Grade 1 symptoms, 5 Grade 2 symptoms, and 3 Grade 3 symptoms, including fever, chills, and myalgia, primarily 7 and 8 days after administration of PfSPZ Challenge when parasitemia was highest. In an effort to improve tolerability during the main phase of the study, non-steroidal anti-inflammatory drugs (NSAIDs) were given prophylactically to 5 volunteers as scheduled medications, even in the absence of any malaria-associated symptoms, on days 7-8 post-PfSPZ Challenge after first vaccination. During this time, there were 23 Grade 1 symptoms, and no Grade 2 or 3 symptoms reported in the group using prophylactic NSAIDs. These findings suggest that the use of prophylactic NSAIDs may reduce or eliminate moderate and severe malaria-associated symptoms if employed during future PfSPZ-CVac trials, furthering the effort to develop an effective and tolerable malaria vaccine.

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DISTRIBUTION OF PFSPZ VACCINE TO TRAVEL MEDICINE CLINICS

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In preparation for licensure of Sanaria® PfSPZ Vaccine, plans are progressing for its distribution to travel medicine clinics (TMC) in the USA and Europe. PfSPZ Vaccine is a live attenuated, metabolically active, non-replicating *Plasmodium falciparum* (Pf) whole sporozoite (SPZ) vaccine that, because PfSPZ are eukaryotes, is cryopreserved and stored in liquid nitrogen (LN2) vapor phase (LNVP). LN2/LNVP cold chains have been used routinely for decades to distribute veterinary reproductive products and vaccines. The new chimeric antigen receptor T-cell (CAR-T) therapies have accelerated LNVP cold chain growth. There are many scenarios for PfSPZ Vaccine distribution to TMCs, ranging from sporadic as-needed shipments to small TMCs serving occasional travelers, to ongoing, biweekly shipments to medium-sized TMCs serving travelers frequently, to maintaining an in-clinic vaccine stock for large, centralized TMCs serving hundreds of travelers/week. The LNVP cold chain utilizes cryoshippers to transport vaccine and as temporary in-clinic vaccine repositories. In development are new electric LN2-free cryoshipper/storage units suitable for some TMCs and semi-automated dry thawing devices. Vaccine, in 0.8 mL cryovials, is packaged in two formats for different sized cryoshippers. Cryoshippers with static hold times ranging from 10 to 53 days, customized for PfSPZ Vaccine, will distribute vaccine payloads ranging from 36 doses (7-12 regimens) to 1,296 doses (259-432 regimens). From two dedicated PfSPZ Vaccine distribution centers in Europe and the USA, a good distribution practices (GDP) hub-and-spoke shipping system will deliver vaccine direct to TMCs as needed with continuous-access, real-time, GPS-based tracking and monitoring (including payload and external temperatures, pressure, tilt, shock) using preferred logistics carriers. Cryoshippers will be cycled back to the hub for recharging and restocking. Models for the different scenarios developed from already extensive experience serving PfSPZ clinical trials will be presented.

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RADIATION IMPAIRS MOVEMENT OF *PLASMODIUM FALCIPARUM* MALARIA SPOROZOITES IN HUMAN SKIN

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Given the resurgence in global malaria cases and deaths, the need for a highly efficacious malaria vaccine for *Plasmodium falciparum* (Pf) is more pressing than ever. Vaccines based on the administration of live-attenuated sporozoites (spz), have been shown to fully protect malaria-naïve individuals and the radiation-attenuated sporozoite vaccine (Sanaria Inc) is now entering phase 3 clinical development. Because migration to the liver is key to the induction of protection in these vaccines, they need to be administered by mosquito bite or intravenously. To date migration of Pf spz in human skin remains wholly uncharacterized in the context of wild type as well as attenuated parasites. Using confocal microscopy movie material was obtained on spz movement *in vitro* and following syringe-based administrations in *ex vivo* human skin explants. To analyse the full migrational complexity of the spz we have developed custom image analysis software able to quantify key motility parameters of spz migration. We compared the movement of different vaccine formulations by recording the motility of wild-type and radiation-attenuated GFP-expressing Pf spz. Image analysis showed that the spz motility was significantly influenced by the human skin environment and was completely different from *in vitro* movement patterns. In human skin the velocity was lower and related to the movement pattern. Compared to *in vitro*, movement patterns were more complex and showed a higher degree of displacement. The tissue morphology appeared to drive directional migration. A quantitative comparison of wild-type and radiation-attenuated spz displayed indicted radiation increases the percentage of sharp turns, reduces the straightness index, and decreases the velocity. Using our custom software we have been able to show that the formulation of live-attenuated vaccines is crucial for the migration of spz in human skin. This provides a novel tool for future vaccine refinement.

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EFFECT OF CHANGES IN NEUTROPHILS AND MONOCYTES COUNTS ON PREPATENT PERIOD AND *PF*CSP ANTIBODY TITER FOLLOWING A CONTROLLED HUMAN MALARIA INFECTION OF YOUNG ADULT EQUATOGUINEANS

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Malaria continues to be a serious public health problem in most of the developing world. The advanced pre-erythrocytic malaria vaccine candidates produced moderate efficacy in malaria pre-exposed population. Neutrophils and monocytes are important in vaccination as they produce initial cytokines that determine the outcome of the adaptive immune response. Our understanding of the role of human neutrophils and monocytes in *Plasmodium falciparum* (Pf) pathogenesis is limited to epidemiological studies of natural infection. In these studies, establishing the relationship between neutrophils, monocytes and times of malaria infection has been challenging. Controlled human malaria infection (CHMI) provides a platform to study malaria infection and pathology in a well-controlled setting. We have completed a CHMI in healthy Equatoguinean young adults who previously received aseptic, purified, live, radiation-attenuated, cryopreserved Pf-NF54 sporozoites or low doses of non-irradiated, infectious NF54 PfSPZ administered under chloroquine chemoprophylaxis. Whole blood was collected to determine heamatological parameters at baseline, 2, 9, 13, and 28 days post-CHMI. Malaria parasite monitoring was performed by qPCR and thick blood smear (TBS) on a daily basis from day 8 post-CHMI until onset of blood stage parasitemia. Subjects were immediately treated with artemether-lumifantine after infection was confirmed by both qPCR and TBS or at day 28 post-CHMI. Serum for anti-Pf circumsporozoite (CSP) antibodies was also collected on baseline and 28 days post-CHMI. We will present data on potential relationships of neutrophil and monocyte counts measured by automated haematology analyzer with regard to the prepatent period and Pf-CSP antibody titer during CHMI of Equatoguinean young adults. This study will generate an insight on potential role of neutrophils and monocytes in modulating protection during CHMI and how such knowledge could be translated into improved malaria vaccine design and immunotherapy.

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LONG TERM EFFICACY OF A THREE-DOSE REGIMEN OF RADIATION AT TENUATED *PLASMODIUM FALCIPARUM* NF54 SPOROZOITES (PFSPZ VACCINE) IN HEALTHY MALIAN ADULTS

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PfSPZ Vaccine is composed of radiation-attenuated (non-replicating), aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) that are administered by direct venous inoculation (DVI). A double-blind, randomized Phase 1/2 clinical trial of PfSPZ Vaccine was conducted in Mali, West Africa, using a regimen of 1.8 x 10^6 PfSPZ given at 0, 2 and 4 months. This trial demonstrated a similar level of protective efficacy against naturally occurring *P. falciparum* infection (24% by proportional and 51% by time-to-event analysis) to that previously reported (29% by proportional and 52% by time-to-event analysis) with a 5 dose regimen of 2.7 x10^5 PfSPZ Vaccine tested in Malian adults. Here, we explored the duration of the protective activity of the 3-dose regimen in a second year of follow up. We re-enrolled all 44 eligible PfSPZ Vaccinees as well as 44 eligible controls, along with 26 additional controls from the area that matched by group with the PfSPZ Vaccinees according to village, age, and sex and according to the same inclusion criteria as the original vaccine cohort. Baseline characteristics, such as hemoglobin type and parasitemia status (blood smear and PCR), were collected and all subjects were treated with a full treatment course of artemether/lumefantrine at the time of enrollment to clear any existing parasitemia. No investigational vaccine product was given. Study participants were seen actively every 14 days for a total of around 6 months of the malaria transmission season. The protective efficacy results and immune responses measured during this second transmission season will be presented.

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EXPERIENCE IN MANAGING SANARIA®PFSPZ VACCINE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF PFSPZ VACCINE IN MALARIA-EXPERIENCED ADULTS IN BURKINA FASO

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Proper study investigational vaccine product management is critical for clinical trial conduct and for generating valid study results. PfSPZ Vaccine comprised of metabolically active, non-replicating, cryopreserved, *Plasmodium falciparum* (Pf) sporozoites (SPZ), is reported to be safe and highly protective against Pf controlled human malaria infection in malaria-naive individuals. Experiences gathered managing a double-blind Phase 1 clinical trialqre repoprted here. Cryovials containing 45X10^4 PfSPZ were stored in a liquid nitrogen vapor phase dry shipper at CNRFP and transported from Ouagadougou to the study site in Balonghin, 45 minutes by road. 792 cryovials of PfSPZ Vaccine, 35 vials of PBS and 75 vials of NaCl, were received from the manufacturer in three, two, and three shipments, respectively. Shipments averaged of seven days in transit. No temperature excursions were recorded for placebo and diluent (15°C to 25°C) or vaccine in LNVP (below -150°C) during shipping or storage, However, the post last inoculation , one excursion for vaccine occurred due to the simultaneous lack of LN2 at the research site and at both localLN2 suppliers in Ouagadougou. A total of 703 cryovials were used for vaccine preparation of vaccine syringes without loss, and the remaining 89 were returned to the manufacturer. One study pharmacy protocol deviation was reported at the first dose: an incorrect treatment was given to one volunteer that was corrected.. Doses were prepared to standard operating procedures. Mean cryoshipper temperature at opening to retrieve cryovials for dose preparation was-187.43 ± 2.89º C, and ranged from (-145.5 to-197.9 °C) during shipment from the USA to Ouagadougou, Burkina Faso. The mean time for syringe preparation for injection was 8:00± 0.0007minand the mean time from cryovial thawing to completing injection was 20.66 ±0.005min.More than 99.95% of the 240 total vaccine and placebo administrationsrequired only one syringe preparation. This study shows that vaccine storage, handling, preparation and administration, according to rigorous pharmacy and clinic SOPs are possible in a resource-limited setting.

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PROTECTIVE EFFICACY OF THE PFSPZ VACCINE AGAINST ENDEMIC MALARIA AMONG MALARIA-EXPERIENCED ADULTS IN BURKINA FASO

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PfSPZ Vaccine is composed of radiation-attenuated (non-replicating), aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) that are administered by direct venous inoculation (DVI) and have proven in 15 trials in the US, EU and Africa to be safe, immunogenic and protective against both endemic Pf and controlled human malaria infection. Five doses of 2.7 x 10^5 PfSPZ administered over 20 weeks demonstrated 52% vaccine efficacy (VE) by time to event and 29% by proportional analysis against naturally occurring infection in Malian adults. The current trial is testing 3 doses of 2.7 x 10^6 PfSPZ administered at 0, 8 and 16 weeks to Burkinabe adults as a more practical regimen. In the first part of the study, an open label, dose-escalation assessment of safety and immunogenicity, four staggered cohorts of 8 participants received two DVI doses of 4.5x10^5, 9x10^5, 1.8x10^6 or 2.7x10^6 PfSPZ at 12-14 week intervals. All doses were safe and well tolerated, and 2.7x10^6 PfSPZ induced the greatest median ratio of post- to pre-immunization anti-Pf circumsporozoite protein (PfCSP) antibody levels of 6.7 (ratio of OD 1.0 post- to pre-immunization). In the second part of the study, 80 individuals were randomized 1:1 in a double-blinded manner to receive 3 DVI doses

of 2.7 x 10^6 PfSPZ or saline placebo at 8-week intervals, with presumptive clearance of parasitemia before the first and last doses using 7 days of artesunate monotherapy. Participants were then actively and passively followed for 24 weeks during the peak malaria transmission season to assess vaccine efficacy (VE). The vaccine was safe and well tolerated. VE against Pf infection by microscopy was 37.7% (95%CI 6.8-68.7%, p=0.017) by proportional analysis (primary VE estimate) and 47.1% (95%CI -3.8-73.1%, p=0.064) by time to event analysis. PfSPZ Vaccine demonstrated significant protection in malaria-experienced African adults against Pf throughout an entire malaria transmission season. Follow-up for part two continues through the 2018 malaria season to assess the durability of immune responses and protection.

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SAFETY, TOLERABILITY AND PROTECTIVE EFFICACY OF PFSPZ-CVAC AGAINST ENDEMIC MALARIA AMONG MALARIA-EXPERIENCED ADULTS IN MALI

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PfSPZ-CVac is a vaccination strategy for malaria that involves concurrent administration of fully infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) (PfSPZ Challenge) and a partner drug to kill the parasites *in vivo*. When the partner drug is a selective blood schizonticide such as chloroquine (CQ), the PfSPZ infect hepatocytes, replicate many thousand fold, are released into the blood stream but are then rapidly killed. PfSPZ-CVac using weekly CQ administration was tested in Germany in 3 groups of malaria-naïve adults receiving 3 doses by direct venous inoculation (DVI) at 4 week intervals of 3.2x10^3, 12.8x10^3 or 51.2x10^3 PfSPZ, respectively, and 3/9 (33%), 6/9 (67%) and 9/9 (100%) were sterilely protected against homologous controlled human malaria infection 9-10 weeks after immunization. We aimed to assess the safety, immunogenicity and protective efficacy of PfSPZ-CVac against naturally acquired Pf in malaria-experienced Malian adults in a randomized, controlled, double blind clinical trial. Participants (n=62) were randomized to receive 3 doses of 2.048x10^5 SPZ of PfSPZ Challenge (NF54) - a 4-fold higher dose than Germany - or normal saline placebo by DVI at 4 week intervals while taking weekly CQ. A Safety and Monitoring Committee reviewed data collected up to 12 days after first injections before subsequent doses were given. Participants received a seven-day curative oral artesunate monotherapy regimen after last injections and just before the 2017 malaria transmission season start, but did not receive antimalarial therapy before immunizations. Participants were then actively and passively followed for 6 months to assess efficacy against Pf infection. The vaccine strategy demonstrated a favorable tolerability profile, with no safety signal. Analysis of efficacy against Pf infection by microscopy and by PCR in those who were and were not infected at the time of immunizations, and analyses of immunogenicity by anti-PfCSP antibody ELISA will be presented. Follow-up for extended efficacy and safety continues through June 2018 to assess duration of immunogenicity and protection against infection.

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NO SEX, NO DIFFERENCE: *PLASMODIUM FALCIPARUM* INFECTIONS IN VIRGIN AND MATED *ANOPHELES STEPHENSI*

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Sanaria® PfSPZ Vaccine and PfSPZ-CVac have been highly protective in clinical trials in the US, Germany, and Africa. These vaccines are composed of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ), and are manufactured using aseptically grown *Anopheles stephensi* mosquitoes. Even though the production of aseptic *A. stephensi* is highly efficient and reproducible, only females are needed for the production of PfSPZ. Male mosquitoes are considered a byproduct of the process and compete for essential resources; growth medium and nutrients in larval containers and resting space in adult chambers. Virgin females of some *Anopheles spp.* will not freely take a blood meal or will take a smaller blood meal, thus limiting uptake of Pf gametocytes and development of PfSPZ. Before Sanaria begins work towards rearing only female larvae and adult *A. stephensi,* we needed to determine the capacity of virgin females to produce PfSPZ. We provided Pf-infected blood meals to 3-day old virgin and mated *A. stephensi* compared prevalence and intensity of Pf infections at the oocyst and PfSPZ stages. To verify that mosquitoes were either mated or unmated, spermathecae of at least ten mosquitoes from each group were dissected and scored visually for the presence or absence of sperm in the spermathecae. In 3 independent experiments, virgin mosquitoes had a mean intensity of 101 oocysts per mosquito (range 67-129) and prevalence of 99% (range 97-100), while mated mosquitoes had a mean intensity of 79 oocysts per mosquito (range 45-121) and prevalence of 93% (86 -100). The PfSPZ infection intensities were 275,661 PfSPZ/mosquito and 262,786 PfSPZ/mosquito for virgin and mated mosquitoes, respectively. These results unequivocally demonstrate that virgin and mated female *A. stephensi* are capable of producing comparable yields of PfSPZ. Further studies are in progress to compare the longevity of virgin and mated female mosquitoes. These characteristics are desirable for scaled up PfSPZ production under good manufacturing practice (GMP) conditions.

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WHOLE SPOROZOITE CULTURING SYSTEMS TOWARDS MANUFACTURING MALARIA VACCINES

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Live sporozoite (SPZ) vaccines are the only vaccines that provide complete sterilizing protection against *Plasmodium* infection in mice and humans. A remaining hurdle for commercialization of whole SPZ vaccines is scalable manufacturing technology for SPZ production. To solve this problem, an *in vitro* culturing system to produce large quantities of SPZ has been developed. Minimal basal lamina components were determined for oocyst transformation and then five different oocyst medias (A-E) were tested for their ability to support oocyst/SPZ maturation over a twenty-five-day time course. Throughout the developmental studies, immunofluorescence assays (IFA) were performed using anti-PfCap380 to detect oocysts, anti-circumsporozoite protein (CSP), and DAPI to identify nuclear DNA. A separate ten-day oocyst time course was performed to study genome replication within *in vitro* oocysts to study endomitosis. SPZ were tested for gliding motility and hepatocyte invasion/traversal. Of the five oocyst medias (A-E), media C supported greatest oocyst growth and produced oocysts > 30 um, resembling mosquito-derived oocysts. On day 25, media C supported growth for the following numbers of oocysts in parentheses with a given diameter: 5 um (1895); 10 um (251); 15 um (107); 25 um (46); >30 um (34). Percentages of oocysts with a given number of

nuclear focus/*Foci* on Day 10 are as follows - single focus = 12%, 2 *Foci* = 12%, 4 *Foci* = 4%, 8 *Foci* = 72%. SPZ that developed from oocysts were identified in media C conditions by morphology and protein marker expression. In conclusion, the *in vitro* culturing system supports maturation of oocysts, which resemble mosquito-derived oocysts in size (> 30mm). Genome replication occurs within oocysts as nuclear *Foci* double over the time course. SPZ resemble mosquito-derived midgut SPZ and are thus developmentally immature compared to salivary gland SPZ. Current and future studies are focused on SPZ maturation by testing additional media and basal lamina components to mature SPZ for malaria vaccines.

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SAFETY, TOLERABILITY AND IMMUNOGENICITY OF PFSPZ VACCINE IN EQUATOGUINEAN CHILDREN AND OLDER ADULTS

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PfSPZ Vaccine is a candidate pre-erythrocytic malaria vaccine composed of aseptic, purified, live (metabolically active), radiation-attenuated, cryopreserved *Plasmodium falciparum* (Pf) NF54 sporozoites (SPZ). In trials in the U.S. and Africa, PfSPZ Vaccine administered by direct venous inoculation (DVI) to young adults proved safe and well-tolerated, and provided durable protection against homologous and heterogenous populations of Pf for at least 24 to 33 weeks. PfSPZ Vaccine has undergone limited testing in children in Tanzania and an ongoing trial in Kenya, but has not previously been tested in older adults. Since the eventual goal is to use PfSPZ Vaccine in mass vaccination programs for malaria elimination in specified geographical areas, it is necessary to test the vaccine in all age groups. As part of a larger randomized, double blind placebo-controlled trial, we evaluated the safety, tolerability, and immunogenicity of PfSPZ Vaccine in 62 healthy malaria-exposed Equatoguinean children and older adults. We randomized 15 adults age 36-65 years to receive 3 doses of $2.7x10^6$ PfSPZ of PfSPZ Vaccine, and 16 children age 11-17 years, 16 children age 6-10 years, and 15 children age 1-5 years to receive 3 doses of $1.8x10^6$ PfSPZ, or placebo at 0, 8 and 16 weeks. A sentinel group of 3 subjects was vaccinated one day prior to the rest of each age cohort. Age de-escalation was done sequentially in children, with safety data evaluated by an external data and safety monitoring board before progressing to the youngest group. Adverse events (AEs) were solicited for 7 days and surveillance for unsolicited AEs done for 28 days after each vaccination. Monitoring for serious AEs was done throughout. Hematologic, renal and hepatic tests were done at baseline, 2 and 14 days after each vaccine dose. Blood samples for immunologic assays were taken prior to and 14 days after each vaccine dose, and at 4 and 20-24 weeks after the final vaccine dose. The vaccine was well-tolerated in all age groups, and DVI was generally straightforward with only mild pain associated with injection. Safety and immunogenicity data will be presented.

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SAFETY, TOLERABILITY, IMMUNOGENICITY AND EFFICACY OF PFSPZ VACCINE VERSUS PFSPZ-CVAC IN EQUATOGUINEAN YOUNG ADULTS

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PfSPZ Vaccine is a candidate pre-erythrocytic malaria vaccine composed of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) NF54 sporozoites (SPZ). In trials in the U.S. and Africa, PfSPZ Vaccine administered by direct venous inoculation (DVI) provided durable protection against heterologous strains and heterogeneous populations of Pf for at least 24 to 33 wks. A second PfSPZ-based vaccine approach - administration of low doses of non-irradiated, infectious NF54 PfSPZ (PfSPZ Challenge) under chloroquine chemoprophylaxis (PfSPZ-CVac) - protected against homologous strains of Pf in the U.S. and Europe for at least 10 wks, but had not been tested in Africa. We conducted a randomized, double blind placebo-controlled trial comparing tolerability, safety, immunogenicity and efficacy against controlled human malaria infection (CHMI) of PfSPZ Vaccine versus PfSPZ-CVac in healthy malaria-exposed Equatoguinean 18 to 35-year-old men and women. We randomized 26 subjects to receive 3 doses of $2.7x10^6$ PfSPZ (PfSPZ Vaccine) or placebo at 0, 8 and 16 wks, and 24 subjects to receive 3 doses of $1x10^5$ PfSPZ (PfSPZ Challenge) or placebo at 0, 4 and 8 wks after an oral dose of chloroquine (CQ) 600 mg base then CQ 300 mg base weekly (PfSPZ-CVac), followed in both groups by homologous CHMI at 10-13 wks post final vaccine dose. Adverse events (AEs) were solicited for 7 days and surveillance for unsolicited AEs done for 28 days after each vaccination. Monitoring for serious AEs was done throughout. Hematologic, renal and hepatic tests were done at baseline, 2 and 14 days after each vaccination, and prior to CHMI. Blood samples for humoral and cellular immunology were taken at baseline and 14 days after each vaccination. Both vaccine approaches were well-tolerated, and DVI was typically straightforward with only mild pain associated with injection. Safety, immunogenicity and efficacy data will be presented. This comparison of PfSPZ Vaccine and PfSPZ-CVac will provide more information as to which product could be used in mass vaccination programs aimed at regional elimination of malaria.

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PROTECTIVE EFFICACY OF CONDENSED PFSPZ VACCINE IMMUNIZATION REGIMENS AGAINST HETEROLOGOUS CONTROLLED HUMAN MALARIA INFECTION

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Optimized regimens for immunization with *Plasmodium falciparum* (Pf) sporozoites (SPZ) have >75% protective efficacy against homologous and heterologous Pf. Thus, they are the only approach meeting WHO's preferred product characteristics. PfSPZ Vaccine consists of radiation-attenuated PfSPZ to translate whole cell vaccination to prevent malaria into a pharmaceutical product. PfSPZ Vaccine clinical development in malaria-naïve adults has focused on dose optimization and protection against homologous controlled human malaria infection (CHMI). Exploratory studies indicate that heterologous, long-lasting protection can be achieved. We systematically investigated if the current 16-week, 3-dose immunization regimen can be condensed into a maximum of 28 days and assessed vaccine efficacy (VE) by cross-over CHMI using homologous (NF54) and heterologous (7G8) PfSPZ (MAVACHE, ClinicalTrials.gov NCT02704533). In the first part of the trial, vaccination on Days 0, 7 and 28 with $0.9x10^6$ PfSPZ protected 5/5 volunteers against homologous CHMI, whereas vaccination on Days 0 and 7 with $1.35x10^6$ or $2.7x10^6$ PfSPZ was less efficacious (4/6 and 3/6 protected, respectively). To verify VE of the Day 0, 7, 28 regimen, 18 volunteers were allocated to placebo (n=6) or PfSPZ Vaccine (n=12) and underwent repeat CHMI by direct venous inoculation of 3,200 PfSPZ of PfSPZ Challenge (NF54) and PfSPZ Challenge (7G8), pharmaceutical products for standardized CHMI. PfSPZ for CHMI were administered sequentially (NF54-7G8 or 7G8-NF54) 3 and 8 weeks following last vaccination and the endpoint was asexual blood stage parasitemia. Allocation ratio to either sequence was 1:1, nested in the placebo and PfSPZ Vaccine arms. Immunization and CHMI were safe and well tolerated. No serious adverse event occurred. Following first CHMI, 9/18 volunteers developed malaria (3/12 immunized with PfSPZ Vaccine). The second CHMI is ongoing with currently 4/10 volunteers parasitemic (1/7 immunized with PfSPZ Vaccine). The study will be unblinded in July 2018. Final results including immunological investigations will be presented.

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CAN DURABILITY OF LONG-LASTING INSECTICIDAL NETS BE PREDICTED FROM A 'RISK INDEX' COMPOSED OF SELECTED VARIABLES ON NET HANDLING AND USE ENVIRONMENT?

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Recent studies on the physical durability of LLIN have shown that the outcomes - measured as median survival of the nets in serviceable condition - can vary significantly for the same LLIN brand between locations with different net use environments and net care and repair behaviors. This suggests that these factors would be more predictive of durability than the textile-based quality differences between brands. To provide proof of principle a net durability "Risk Index" (RI) was constructed using data from ongoing LLIN monitoring by the VectorWorks project from 12 sites in five countries. The RI was constructed from three composite groups of household or net-based variables. *Net handling* (45% of total score) had five variables: ever storing food in sleeping room, ever cooking in sleeping room, leaving net hanging during the day, drying net on fence or bush, and washing with detergent or bleach; *Net use environment* (10%) with four variables: dwelling walls, rodents in vicinity, firewood as cooking fuel and type of sleeping place; *Behavior and Attitudes* (45%) with three variables: recall of message "care for your net" and "repair net" and composite care and repair attitude score. Weights for each variable were given based on hypothetical importance to result in a maximum RI value of 100. At baseline RI scores at the 12 sites varied between 27 and 72 with a median of 57 and inter-quartile range of 50 to 64. Median survival estimates at 24 months of follow up is currently available for five sites while the other sites will collect this data before September 2018. Median survival varied between 2.7 and 5.6 years and in a regression analysis the baseline RI score was significantly associated with the outcome ($p=0.005$, $R^2=0.93$) suggesting that each 10-point increase of the RI results in a 0.6 year decrease in median LLIN survival. While this data shows that in principle a "Risk Index" is able to predict the survival outcome, more work is needed to optimize the RI construction and then validate the RI using independent data.

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SUPPRESSING *PLASMODIUM* INFECTION AND MIDGUT MICROBIOTA OF *ANOPHELES* USING A TRANSGENIC CONDITIONAL SPONGE MICRORNA APPROACH

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MicroRNAs (miRNA) are small endogenous noncoding RNAs that bind to specific miRNA recognition elements of target RNAs, and have been shown to play a central role in the regulation of gene expression, primarily via RNA decay or translational inhibition. miRNAs have been extensively studied in several diseases transmitting mosquitoes vectors, including the yellow fever mosquitoes *Aedes aegytpi* and the human malaria misquotes *Anopheles gambiae*. Most of these studies were concentrated on identification and annotation of miRNAs using high throughput sequencing and subsequent bioinformatics analysis. However, only a few miRNAs have been functionally studied in *Anopheles* mosquitoes. This is partly due to a paucity of genome-wide resources for assessing miRNA loss of function. Recently, miRNA sponges (SPs) were successfully used to define miRNA functions in multiple species and biological contexts. miRNA SP contains multiple or 'bulged', tandem binding target sites to a microRNA of interest. When SP is integrated to the genome of species using transgenic technique, it is regulated by acquired promoters and sequester target miRNAs, blocking the functions of target miRNA, and thus creating a knockdown of miRNA activities or a miRNA null mutant. We have used a transgenic approach to target two miRNAs, aag-miR-14 and aag-miR-305, through conditional expression of miRNA SPs to investigate their implication in regulating *Plasmodium falciparum* infection and the midgut microbiota. We will discuss four transgenic lines expressing these two miRNAs SPs, under the control of the blood-inducible tissue-specific *carboxypeptidase* (*Cp*), or *vitellogenin* (*Vg*), promoters. Expression of selected SPs significantly reduce the amount of endogenous target miRNAs in midguts or fat bodies of females after ingesting a blood meal, resulting in suppressing parasite oocyst loads and microbiota loads in midguts without obvious fitness costs. Ongoing studies will explore potential target genes that are regulated by each miRNA, and may represent anti-parasitic and antibacterial effectors.

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DETERMINING SPECIES OF FIELD-COLLECTED MOSQUITOES USING NEAR-INFRARED SPECTROSCOPY

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Tracking changes in vector populations—such as species abundance, age distribution and infection—constitutes a direct measure of the efficacy of vector control interventions. Near- infrared spectroscopy (NIRS) is a rapid and non-destructive scanning technique that has been shown to differentiate morphologically identical *Anopheles gambiae* and *An. arabiensis* sibling species. In this study, we evaluate the potential of NIRS to determine the species of laboratory-reared mosquitoes using machine learning (ML) models calibrated using colony mosquitoes. Mosquitoes of different species (*Anopheles arabiensis*, *Anopheles coluzzii*, *Anopheles gambiae*) were collected in three different field locations in Burkina Faso and reared to adulthood under laboratory conditions alongside colony mosquitoes. Following emergence, colony mosquitoes were killed and scanned daily using NIRS. Maternal generation (F0) and offspring generation (F1) reared from field collected mosquitoes were killed and scanned 4 days post-emergence and the species determined by PCR. Multinomial generalised linear models were constructed and cross-validated to predict mosquito species from their spectra. NIRS was able to differentiate the three species of colony mosquitoes of constant age, including the previously untested *An. coluzzii*, with misclassification rates below 7% in all cases. Species could also be determined in colony mosquitoes where the age of the mosquito varied and was unknown though the accuracy was lower. Colony mosquitoes could predict the species of laboratory reared F0 and F1 mosquitoes with some loss of accuracy depending in species, location and generation. The work indicates that NIRS is able to identify the three most epidemiologically important species in the *Anopheles gambiae* complex with relatively high accuracy. The precision of the method diminishes with the increased realism investigated here (location of the population, age of mosquito) though NIRS still appears to a promising tool for mosquito surveillance that needs to be further validated in the field.

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INSECTICIDE SUSCEPTIBILITY STATUS OF *ANOPHELES GAMBIAE* S.L TO PUBLIC HEALTH INSECTICIDES FROM TWELVE SENTINEL SITES IN UGANDA

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Increasing insecticide resistance is a threat to vector control interventions in Uganda. This monitoring investigated possible changes in the resistance status of local vectors to insecticides used for indoor residual spraying (IRS) and on long-lasting insecticidal nets (LLINs) in twelve different sites (districts) in Uganda. Susceptibility levels in *Anopheles gambiae* s.l. to DDT, three pyrethroid, two organophosphate and one carbamate insecticide collected from twelve sentinel surveillance sites across Uganda in 2016 and 2017 were determined using the standard WHO insecticide susceptibility test kit. Clothianidin and chlorfenapyr were tested in one and two sites, respectively. Our results show that *Anopheles gambiae* s.l. were susceptible (99-100% mortality) to pirimiphos-methyl in all study sites, but malathion had a reduced mortality of 89.4% in Soroti. Mortality to carbamates varied between 88% in Arua and 100% in all other study districts tested. Mosquitoes were susceptible to DDT in Apac and Tororo Districts (98-100% mortality), with suspected resistance in Bugiri, Lira and Soroti Districts (90.8-92% mortality), and resistance in Arua, Gulu and Kanungu Districts (15-26% mortality). Resistance to pyrethroids was observed in most sites, except for suspected resistance to deltamethrin (91% mortality) and to permethrin (91% mortality) observed in Lira District in 2017. Mortality to clothianidin in Tororo was 96.6% at 7 days post-exposure, while mortality to chlorfenapyr in Soroti and Tororo was 99.7% and 100% respectively at three days post-exposure. We noted reduced susceptibility to carbamates in Arua, Lira, Kanungu and Tororo Districts, indicating the need for continued annual monitoring for insecticide resistance. Both East (L1014S) and West African (L1014F) *kdr* mutations were present in *An. gambiae* and *An. arabiensis*. Widespread resistance to all IRS insecticides except pirimiphos-methyl, currently used in IRS, imparts urgency for testing new compounds such as clothianidin and chlorfenapyr to manage insecticide resistance through rotation with different modes of action.

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COMMUNITY-BASED LLIN DISTRIBUTION: DESIGN AND IMPLEMENTATION LESSONS FROM ZANZIBAR

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In 2014 Zanzibar adopted continuous distribution (CD) of long-lasting insecticidal nets (LLINs) to maintain high levels of ITN ownership and access. Shehas (heads of the ward) issue coupons to qualifying heads of household, who then redeem them for nets at nearby health facilities. The CD channels are run and managed by the Zanzibar Malaria Elimination Program (ZAMEP) and 289,661 ITNs were delivered through community and ANC/EPI channels in 2014-2016. In collaboration with ZAMEP, VectorWorks led the redesign of the community channel in 2017, with the following objectives: 1) improve coupon security, 2) integrate coupon in the health commodities supply chain, 3) integrate coupons and LLINs issued in the Health Management Information System (HMIS), and 4) develop an automated and transparent coupon tracking system. To date, the community channel has been integrated into the health facility-based documentation process where information about coupons and LLIN recipients is captured in outpatient registers. Storage and delivery of coupons to health facilities has been integrated by Zanzibar Central Medical Stores (CMS), with management and delivery of coupons recorded with other health commodities. In order to improve coupon security, a number of steps have been taken. First, security features such as barcodes, holograms and unique numbers have been added to the coupons, to allow health facility staff to visually assess validity of coupons. Second, a coupon numbering system allows CMS to assign batches to specific Shehas, to easily track fraud by respective Shehas. Third, a coupon management system with a dashboard was developed within DHIS2. The coupon management system will be used by CMS to capture baseline information for all coupons received from suppliers, and track validity of redeemed coupons. Redeemed coupons will be sent to CMS with monthly HMIS reports from health facilities. The presentation will report on the

operational issues encountered during the implementation process, how the community channel and coupon management system works, and other programmatic lessons.

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ENTOMOLOGICAL INVESTIGATION OF *ANOPHELES* MOSQUITOES IN FOREST-RELATED AREAS IN ACEH, INDONESIA

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Plasmodium knowlesi infections have been identified in Aceh Province, Indonesia, and are known to be associated with forest-based work, such as logging, rubber-tapping, mining, and farming. *Anopheles cracens* and *An. latens* in the *Leucosphyrus* group have been demonstrated as vectors of *P. knowlesi* in Malaysia. However, there is a lack of evidence to support a role for these species as *P. knowlesi* malaria vectors in Aceh. Our study aimed to characterize the distribution, abundance, and behavior of potential *P. knowlesi* vectors in the districts of Aceh Besar and Aceh Jaya, Aceh Province, Indonesia. Entomological sampling was conducted every other month between April 2017 to April 2018 at three collection sites representative of three ecologies: forest site, forest-fringe site, and settlement site. For each ecological type, adult mosquitoes were collected using outdoor/indoor human-baited night collection, morning collection, light traps, and mosquito magnets; larval collection was conducted during habitat surveys. We morphologically identified vector species and analyzed resting and feeding habits, seasonal densities, longevity and the types of water bodies used as breeding sites. A total of 22,266 mosquitoes were collected, of which anophelines comprised 0.6% (1,408) and 99.4% (20,858) were non-anopheline. A total 31 *Anopheles* species were morphologically identified. Out of these anopheline mosquitoes, 55% (775/1.408), 23% (321/1.408) and 22% (312/1.408) were collected from the forest, forest-fringes and village settlement, respectively. Molecular analysis to identify species and examine for sporozoites is ongoing. These results will provide essential baseline data for understanding forest transmission and finding suitable and effective vector control strategies for high-risk forested malaria areas in Aceh.

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FACTORS ASSOCIATED WITH OWNERSHIP AND USE OF LONG-LASTING INSECTICIDAL NETS IN UGANDA: A CROSS-SECTIONAL SURVEY OF 48 DISTRICTS

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A cross-sectional community survey was conducted to assess ownership and use of long-lasting insecticidal nets (LLINs) in Uganda, approximately 3 years after the last universal coverage campaign (UCC) to distribute LLINs. The survey will serve as a baseline for an ongoing trial to evaluate the impact of LLINs distributed in Uganda's 2017-18 UCC, and included 104 clusters (health sub-district) across 5 administrative regions. Households were randomly selected using two-staged cluster sampling; 50 households were enrolled per cluster. Outcomes included household ownership of LLINs (at least one LLIN) and adequate LLIN coverage (at least one LLIN per 2 residents), and use of LLINs by residents (slept under a LLIN the previous night). In March-June 2017, 5200 households were enrolled, including 29,649 household residents, and 6,953 bed-nets. Overall, 65.0% of households owned at least one LLIN (down from 94% in 2014), ranging from 55.6% in the mid-north east to 72.4% in the south-west. In an adjusted analysis, the factors most strongly associated with LLIN ownership were living in wealthier households (highest tercile vs lowest; adjusted odds ratio [aOR] 1.94, 95% CI 1.66-2.28, p<0.001) and less time since the last UCC (29-37 vs 42-53 months; aOR 1.91, 95% CI 1.60-2.28, p<0.001). Only 17.9% of households were adequately covered by LLINs (down from 65% in 2014), ranging from 10.6% in the mid-north east to 25.4% in the south-west. The factors most strongly associated with adequate LLIN coverage were fewer household residents (2-4 vs >7; aOR 6.52, 95% CI 5.13-8.29, p<0.001), living in wealthier households (highest tercile vs lowest; aOR: 2,32, 95% CI 1.88-2.85, p<0.001) and less time since the last UCC (29-37 vs 42-53 months; aOR 2.13, 95% CI 1.61-2.81, p<0.001). Only 39.5% of residents reported using LLINs. Age was strongly associated with LLIN use (5-15 vs <5 years; aOR 0.58, 95% CI 0.55-0.62, p<0.001), as were household wealth and time since the last UCC. LLIN ownership and adequate coverage have reduced markedly in Uganda since the last UCC, vary by region, and are lowest in poorer households. Larger households and school-aged children are also at risk.

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OPTIMIZING ENDECTOCIDE USE TO MITIGATE RESISTANCE DEVELOPMENT AND IMPROVE MALARIA CONTROL SUSTAINABILITY

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The most common methods of malaria vector control, long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), have significantly reduced the burden of malaria. However, several hurdles remain before elimination can be achieved: mosquitoes are increasingly reported to seek their blood meals outdoors; mosquitoes that are more generalist in their host choice can more easily avoid human contact while traditional control tools are at their most potent; and mosquitoes have developed multiple modes of resistance to the insecticides used in LLINs and IRS due to their widespread use. Endectocides, insecticides applied directly to blood-hosts to kill mosquitoes that take a blood meal, offer a promising solution that could improve current vector control methods. These chemicals can be deployed to target mosquitoes displaying a range of feeding behaviors. Although endectocides have been shown to effectively reduce malaria transmission for 1-3 weeks after being applied, they are currently only applied once or twice a year. Consequently, mosquito populations can recover between treatments and the transmission cycle of malaria is not broken. If long term vector control is to be accomplished, we need to increase the application frequency of endectocides while taking care not to promote resistance. Here, we describe a mathematical model that explores dosing regimens using endectocides for humans and livestock that effectively reduces malaria transmission long term while minimizing the selection for endectocide-resistant mosquitoes. We also describe scenarios whereby resistance to insecticides used in LLINs and IRS can be more effectively managed through co-deployment with endectocides.

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WIDESPREAD INSECTICIDE RESISTANCE REPORTED IN COUNTRIES WITH THE HIGHEST MALARIA BURDEN

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The decrease in malaria cases between the year 2000 and 2015 has largely been attributed to vector control using long lasting insecticidal

nets and indoor residual spraying. However, in 2016 the number of malaria cases worldwide increased compared to 2015. Insecticide resistance in malaria vectors is one of the challenges recognized by WHO for malaria elimination. The decreased ability of current vector control tools to effectively kill mosquitoes may be an early indicator to an increase in malaria cases and attributed deaths. Visualizing the confirmed reports of insecticide resistance in malaria endemic countries provides an indication where resistance may play a role in the persisting malaria burden. Launched in 2012, IR Mapper (www.irmapper.com) geospatially displays reports of insecticide resistance in malaria vectors. As of March 2018, the *Anopheles* IR Mapper platform consisted over 19,500 unique data points from 2,664 localities in 60 countries. 85% of the countries reported confirmed resistance to at least one of the four main insecticide classes for malaria vector control. According to the World Malaria Report, in 2016 only 15 countries accounted for 80% of all malaria cases globally. Confirmed resistance to pyrethroids was widely reported in all 15 countries. Confirmed resistance to carbamates was also reported in 14 of the 15 countries. Target site knockdown mutations (*kdr)* and overexpressed oxidase metabolic mechanisms were the main resistance mechanisms reported in these countries. IR Mapper is a useful tool for visualizing the spatiotemporal spread of insecticide resistance in *Anopheles* mosquitoes and can be used to assist in decision making for deployment of the most appropriate tools and development of insecticide resistance strategies.

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AN ENTOMOLOGICAL SURVEILLANCE PLANNING TOOL TO IMPROVE ENTOMOLOGICAL INTELLIGENCE FOR EVIDENCE-BASED VECTOR CONTROL DECISION-MAKING TOWARDS MALARIA ELIMINATION

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To accelerate progress towards malaria elimination, the World Health Organization (WHO) Global Technical Strategy 2016-2030 calls for maximizing the impact of vector control by strengthening entomological surveillance and capacity and managing insecticide resistance and residual transmission. In response to these efforts, we have developed a draft Entomological Surveillance Planning Tool (ESPT) to distil WHO guidance into an operational, decision-support tool for national malaria programs to support cost-effective, locally tailored, evidence-based vector control. The ESPT aims to support countries in generating entomological intelligence that guides vector control intervention selection, deployment in time and space, and provides a platform to evaluate complementary strategies and tools. To this end, the ESPT consists of a series of decision trees to help guide countries in the collection of the priority entomological indicators needed to make decisions through: 1) baseline surveys, 2) routine sentinel surveys, 3) focus investigations, and 4) entomological surveys based on priority programmatic questions. These decision trees link to the priority minimum indicators, a trapping methodology matrix to guide collections, data collection forms, and guidance on selecting sites for entomological investigations. In collaboration with national malaria programs and local partners, pilots of the ESPT have begun in three countries. Parallel evaluation activities are underway and include: 1) qualitative assessments to measure the ESPT's feasibility, acceptability, utility, and impact on vector control program decision-making; 2) costing of entomological surveillance activities and the cost per indicator; and 3) tracking decision-making on vector control strategy as related to implementation of the ESPT. Preliminary data from the pilot evaluations will be available by mid-2018 and will inform future iterations of the ESPT for malaria programs.

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COSTS AND COST-EFFECTIVENESS OF ITN DISTRIBUTION STRATEGIES IN SUB-SAHARAN AFRICA

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Insecticide-treated nets (ITNs) are one of the most cost-effective measures for preventing malaria. WHO recommends both large-scale mass distribution campaigns (MC) and continuous distributions (CD) as part of a multifaceted strategy to achieve and sustain universal access to and coverage of ITNs. A combination of these strategies has been effective for scaling up net coverage. For policy makers to make informed decisions on how to efficiently implement CD strategies, information on the costs and cost effectiveness of these delivery systems is necessary. To address this gap in CD cost data, costs of four types of delivery systems (mass campaigns, routine health services (ANC and EPI), schools, and community distribution), were collected in various country contexts. Data on costs were collected retrospectively from financial and operational records, stakeholder interviews, and resource use surveys. Additionally, a systematic search of published cost literature was conducted. The OpenMalaria platform was used to develop simulations of the health effects of various ITN distribution scenarios and these results were combined with the systematic review results to make cost-effectiveness (CE) estimates for each scenario. Results from the case series and systematic review demonstrate that the average economic costs of CD systems per net (3.21 (USD) (95% CI 2.55-3.86)) were not significantly higher than the MCs reviewed (2.34 (USD) (95% CI 1.63-3.04)), despite a higher mean cost per ITN distributed. Results also showed that CD systems involve more country contributions than MCs per ITN. Incremental Cost Effectiveness Ratios (ICER) and CE expansion paths were estimated under varying transmission contexts and intervention deployment histories. ANC and EPI distribution alone is likely to be the most efficient (i.e. lowest cost per case of malaria averted) use of resources for ITN distribution and favored under the most resource limited situations. ANC/EPI had the lowest ICER compared to no vector control in more than 80% of simulated scenarios. Other CD systems can perform at similar levels of efficiency to repeated mass campaigns.

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POPULATION SEASONALITY AND RELEASE TIMING SIGNIFICANTLY AFFECT THE PROBABILITY OF ESTABLISHMENT FOR SMALL RELEASES OF GENE DRIVE MOSQUITOES

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Highly efficient CRISPR/Cas9 gene-drive systems have recently been developed, targeting reproductive-capacity and malaria-competency loci of malaria transmitting vector species, such as An. gambiae. The resulting drive systems aim to either suppress the local wild-type population or alter its genome, conveying desirable phenotypes such as *P. falciparum* refractoriness. The potential for sustained spread of gene drive constructs as proposed for malaria and a variety of other applications (pest control, tick borne diseases, dengue) has raised concerns for unintentional or unauthorized organism release outside approved and strictly-regulated trial sites. Previous analyses posit that as few as one or two gene drive organisms carrying efficient gene drive cassettes may establish a permanent (sub)population of genetically-modified (GM) mosquitoes with probability >50%. While these results are broad and cautionary, we show that seasonality is a fundamental environmental characteristic to consider when modeling decision variables. For the first time, we investigate the impact of gene-drive release timing and numbers on the establishment probability of GM vectors in the context of realistic seasonal population variation. We model a male sex bias, driving-Y population suppression gene drive, targeting An. gambiae, since these are among the first field trials candidate constructs. We analyze gene-drive establishment in geographies of different seasonality and spatial vector population features. We show that releasing a small number of gene-drive mosquitoes over the few weeks in the beginning of the wet season facilitates population founder effects and high establishment probability: between 60% - 80% for releases of as few as one or two mosquitoes. However, releasing gene-drive mosquitoes outside this time results in much lower establishment probability, typically <20%. Our findings address crucial ethical and environmental concerns, which may guide whether, how, and where to set up gene-drive trials.

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RESULTS FROM A COMPARISON-CONTROL TRIAL EXAMINING DIFFERENT TARGETING STRATEGIES FOR IRS ZAMBIA 2017

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Indoor residual spraying (IRS) is a powerful intervention in the fight against malaria, but high cost prohibits most national malaria programs from achieving universal coverage. Most countries rely primarily on universal coverage of insecticide-treated mosquito nets (ITN) for malaria control and target IRS campaigns to prioritized areas. Yet, information is scarce on how IRS should be delivered to maximize impact and prevent the most malaria with limited resources. In collaboration with the National Malaria Elimination Centre in Zambia we conducted a comparison-control trial in 2017 and 2018 to evaluate different prioritization strategies for IRS. Six districts were divided into three groups (2 districts per group), with each receiving a different IRS targeting methodology for IRS operations (pirimiphos-methyl) in 2017. Group A received a geographic concentration strategy wherein all structures within a geographic area were targeted for IRS. This method (blanket spraying) aims for heavy saturation of IRS without spatial gaps. Group B used health facility incidence data from the health management information system (HMIS) to estimate malaria burden in each area and prioritize houses according to incidence at the nearest facility. Group C used a strategy of ecological targeting to allocate IRS based on predicted probability of *Anopheles funestus* as developed by the Malaria Atlas Project. IRS was implemented in late 2017, and all data will be analyzed by July 2018. The primary outcome is the incidence of uncomplicated malaria measured through the HMIS which will be analyzed using a difference-in-differences approach. The entomological inoculation rate (EIR) of local primary vector species is being measured through five routine entomological collections in 150 houses across the different arms and will serve as a secondary outcome; it will be assessed using post-only comparison between trial arms and between sprayed and unsprayed areas. Analyses will be finalized by September 2018. This presentation will discuss the results of this study and its implications for future targeting methodologies in Zambia and other countries using IRS.

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SOCIO-BEHAVIORAL SURVEILLANCE ON OUTDOOR SLEEPING AND OTHER OUTDOOR NIGHTTIME BEHAVIORS IN NORTHERN BENIN FOR INDOOR RESIDUAL SPRAYING DECISION-MAKING

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Indoor Residual Spraying (IRS) and insecticide treated nets (ITNs) in Benin have resulted in drastic reductions in entomological parameters that have not been accompanied by similarly large reductions in epidemiological indicators. To identify household (HH) practices that may impact vector control strategies, we performed nighttime socio-behavioral surveillance in northern Benin during the Harmattan (cold) season in January 2018 in two regions where IRS was performed and one where IRS was withdrawn. We selected a convenience sample of 96 HH with at least five HH members (HHM), of whom one had to have sufficient educational background to monitor and record nighttime HHM behaviors and locations. Surveillance occurred every 30 minutes between 7PM to 7AM every other night for one week for three nights in total. We interviewed heads of HH on perceptions and use of vector control strategies. Proportions were calculated using the number of HHMs ever observed in a location. Among 576 HHM observed during 7–10 PM, 5.7% slept indoors under an ITN and were potentially protected from mosquitoes; >90% were not protected because they were doing outdoor (50.3%) or indoor (25.3%) activities or were away from the HH (18.5%). Between 10 PM–7 AM, nearly all (98.1%) HHMs slept indoors; only 44.2% used an ITN. Approximately 30.1% of HHMs used other mosquito prevention measures, including coils (30.5%), insecticide bombs (28.5%), and local herbs (38.9%); use did not differ significantly between IRS and non-IRS areas. HHs were noted to have large amounts of belongings and clothing hanging on walls that served as potential mosquito resting places other than IRS-treated walls. The impact of IRS and ITNs on malaria transmission were likely impacted by HHM behaviors, such as outdoor nighttime activities and lack of ITN use, identified in our surveillance. However, these behaviors may have been influenced by the relatively cool, dry weather at the time of sur veillance. To provide more

information on how HHM behaviors may impact vector control strategies at other times of the year, we will repeat the surveillance during the hot season (March 2018) and rainy season (July 2018).

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THE IMPACT OF MASS BED NET DISTRIBUTION ON VECTOR SPECIES AND MALARIA PREVALENCE IN KILWA AND KASHOBWE, HAUT-KATANGA PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO

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A mass insecticide-treated net (ITN) distribution campaign took place in Haut-Katanga Province, Democratic Republic of the Congo, a high transmission setting with little prior history of vector control, during September and October 2016. To measure the impact of the ITN distribution, cross-sectional surveys were conducted in Kilwa and Kashobwe in Haut-Katanga Province before and after the ITN distribution, with two surveys conducted in the rainy season (February 2016 and 2017) and two surveys in the dry season (July 2016 and August 2017). The surveys consisted of administering a questionnaire, collecting blood for microscopy and molecular studies, and indoor mosquito collections. In total, 210 households were surveyed, and 1,742 participants were enrolled. Over 2,000 female anophelines were collected using CDC light traps and pyrethroid spray catches. Following the mass ITN distribution, there was a significant increase in reported ITN usage the night before the study visit (59% (95% CI: 53, 65) in July 2016 vs 92% (95% CI: 89, 94) in February 2017) and decline in parasite prevalence by microscopy (mean prevalence of dry season and rainy season: 32% (95% CI: 29, 35) in 2016 vs 18% (95% CI: 16, 21) in 2017). Both before and after the ITN distribution, children 5-15 years of age had the highest parasite prevalence and reported less ITN usage compared to younger children (< 5 years of age) and adults (> 25 years of age). The overall reported ITN usage declined in August 2017 to 88% and the decline was greatest for children 5-15 years of age. In Kilwa and Kashobwe, malaria vectors were abundant throughout the year, with *Anopheles gambiae* and *An. funestus* as the predominant vectors. Blood meal PCR revealed that both species were highly anthropophilic. Prior to the ITN distribution, *An. gambiae* was most prevalent during the rainy season whereas *An. funestus* was most prevalent during the dry season; however, *An. funestus* became the predominant vector following the rainy season in 2017. A mass ITN distribution in Haut-Katanga Province impacted both vector counts and parasite prevalence but additional interventions will be needed to further reduce transmission.

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INSECTICIDE-TREATED NET ACCESS AND USE IN LIBERIAN HOUSEHOLDS, 2016

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In Liberia, a malaria endemic country, one objective of the Malaria National Strategic Plan 2016-2020 is to achieve universal access to long-lasting insecticide treated nets. From 2010 to 2015, about 4.5 million insecticide-treated nets (ITNs) were distributed to a 2016 population of 4.6 million, through both mass campaigns and antenatal clinic visits. The National Malaria Control Program conducts ongoing countrywide social and behavior change (SBC) efforts to promote access to and use of ITNs. During the 2016 Malaria Indicator Survey (MIS), a nationally representative sample of 4,218 households (HH) was randomly selected and visited to assess ITN ownership, access (one ITN per two people), and use. Almost all women (99%) have heard of malaria, and 90% of them cited mosquitoes as a cause of malaria; of those 88% cited sleeping under a net as a way to avoid malaria. The night before the survey, 21141 persons slept in these HH, with an average number of five people per household. The proportion of HH with at least one ITN was 61% (95% CI 58-65), with a mean of 1.2 ITNs per HH. Over 80% of ITNs were obtained from mass campaigns. Thirty-four percent of HH reported having disposed of an ITN during the 12 months preceding the survey, for 1421 disposed ITNs, with tearing (89%) being the main reason. Forty-two percent of HH members had access to an ITN and 39% of HH members slept under an ITN the night before the survey, indicating high use: access ratio (93%). Net utilization was higher in rural areas (43%) than in urban areas (37%, p-value <0.001). In households with at least one ITN, 66% of 3315 children and 70% of the 304 pregnant women slept under an ITN the night before the survey. The high use:access ratio may reflect a strong culture of net use fostered through SBC efforts. To address its main challenge of ITN access, Liberia is exploring strategies to intensify its ITN program efforts especially in difficult-to-reach households. These strategies include using data from the 2016 MIS to target the 2018 mass campaign distribution geographically, considering new continuous distribution channels through schools and communities, and promoting proper net care.

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MINIMAL GENETIC DIVERSITY AND SPATIAL CLUSTERING OF CHOLERA CASES IN THE KATHMANDU VALLEY: IMPLICATIONS FOR A RING-VACCINATION STRATEGY

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A ring vaccination strategy targeting the population living around the case was found to be useful in eradicating smallpox and lately in controlling the Ebola outbreak in Guinea. However, such vaccination strategy has yet to be evaluated for its ability to stop the transmission of cholera. In mid-2016, a cholera outbreak occurred in Kathmandu Valley, Nepal. This study aims to determine if a reactive, ring vaccination strategy would have been useful in preventing cholera transmission during that outbreak. Data on cholera cases were collected as part of hospital-based surveillance for cholera in the Kathmandu Valley from June 30th to November 30th 2016. Cases were confirmed by bacterial culture and/or polymerase chain reaction testing and the genetic relatedness of positive samples was determined. Subsequent household visits were made to obtain GPS coordinates of the case households. Geographic clusters of cases were visually determined based on potential transmission patterns in space and time, and tested for clustering using Ripley's K and L functions. A total of 193 cholera cases were reported during the surveillance period. MLVA genotyping showed minimal genetic diversity, with only a single clonal complex. GPS coordinates were available for 69 case households. Six geographic clusters were identified, all of which showed significant clustering of cases. Approximately 85% of the cases within a cluster occurred more than seven days after the index case. The minimal diversity seen in the clinical samples combined with the shape of the epidemic curve seems to indicate a clonal outbreak consistent with a common source followed by secondary fecal-oral spread from person to person. Thus, it seems unlikely that there were multiple endemic environmental sources for *V. cholerae* in the Kathmandu Valley in 2016. Cholera cases during the outbreak were clustered in space, and the majority of cases

occurred over a week after the initial cases in the cluster, allowing for an opportunity to prevent transmission of the disease through use of the vaccine soon after the initial case was identified.

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BEYOND DRINKING WATER FOR CONTROL OF TYPHOID FEVER: NOVEL RISK FACTORS FROM A CASE-CONTROL ANALYSIS IN BLANTYRE, MALAWI

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Typhoid fever, caused by the bacteria *Salmonella* Typhi, remains a significant cause of mortality in low and middle income settings. Though well described as being spread via the fecal-oral route, the most important reservoirs are unknown in most endemic settings. Queen Elizabeth Hospital (QECH) in Blantyre, Malawi, experienced a sharp increase in cases of typhoid fever starting in 2011, the majority of which were multi-drug resistant. A case-control study was initiated to evaluate potential risk factors for typhoid fever in children. Pediatric cases were ascertained from diagnoses from QECH, while controls were selected at an approximate 4:1 ratio, and matched with cases by geographic city district. Stepwise variable selection was utilized to narrow the large survey of potential risk factors to a final variable set for analysis. A logistic regression with geographic city district as a fixed effect was conducted using R statistical software. Results from the variable selection indicated that variables evaluating cooking and cleaning water sources were more informative than drinking water sources for predicting disease. Other than healthcare-seeking, which controls for case-ascertainment, individuals using river water for cooking and cleaning have the highest estimated odds (odds ratio (OR)=3.7, 95% confidence interval (CI)=1.4-9.5)of being a case. Other identified risk factors present a complex but cohesive picture of water usage in Blantyre, including protective effects of having soap in the household (OR=0.52, 95% CI=0.31-0.90) and increased odds for those living further from their primary water source (OR=1.39 per standard deviation increase in distance, 95% CI=1.03-1.89). The results from this study implicate cooking and cleaning with river water as a probable exposure pathway for *Salmonella* Typhi in Blantyre, Malawi, and provide guidance for both city-level control strategies and environmental sampling. These results highlight the importance of capturing multiple water-related exposure pathways, not only drinking water, for diseases that are able to survive and propagate on surfaces and food after contamination.

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HOW DO INFANTS ACQUIRE A DYSBIOTIC MICROBIOTA EVENTUALLY LEADING TO PEDIATRIC ENVIRONMENTAL ENTEROPATHY? THE MITICA STUDY: INPUTS FROM THE FIELD

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Undernutrition contributes to more than 40% of the global child mortality rate. In 2016 stunting affected 155 million children. Stunting entails long-term repercussions for the child, such as impaired growth, defective immune response, and neuro-cognitive consequences. Epidemiological studies emphasize that undernutrition cannot be ascribed solely to food insecurity and that early life environmental exposures play a major role in the pathogenesis of the disease. Indeed, growing evidence indicates that a syndrome called pediatric environmental enteropathy (PEE) undermines proper nutrition. PEE is a chronic low-grade inflammation of the small intestine in response to repeated exposure to a highly microbiologically contaminated environment, including small intestinal bacterial overgrowth (SIBO). This entails changes in the resident gut microbiota and intestinal atrophy, eventually leading to nutrient malabsorption, and deficient responses to live-oral vaccines. Estimates indicate that up to 75-100% of children living in areas with high prevalence of enteric infections suffer from PEE. Identifying how the infant acquires a pediatric environmental enteropathy and a dysbiotic gut microbiota, and how it affects child nutrition and development, are the crucial questions to elucidate the pathological pathways associated with malnutrition and design future intervention strategies. The MITICA study intends to describe the composition of the infant gut microbiota at birth and its evolution during the first 6 months of life, as well as the possible presence of PEE. Therefore, we analyze the presence of a maternal dysbiotic microbiota and maternal malnutrition at delivery; the influence of external sources of microbial pollution; and the infant's nutritional patterns (breast-milk composition, duration of breastfeeding, time of weaning and diet). We conduct a longitudinal case-control study nested in a cohort of 112 mother-infant pairs in the Central-African Republic: 56 cases (malnourished pregnant women) and 56 controls (non-malnourished pregnant women) and their offspring are followed from delivery until 6 months of age.

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SAFETY AND EFFICACY OF NITAZOXANIDE ON DIARRHEA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Diarrheal diseases are marked as the fourth leading cause of mortality among under-five children and ninth among the population globally. Therapeutic interventions for diarrhea are still mostly limited to supportive management due to the variety of potential pathogens and different drug categories. Hence, we aimed to systematically review the evidences on the safety and efficacy of nitazoxanide for treating infectious diarrhea. We searched 12 databases, including PubMed, Scopus, and Embase. Data were extracted from included studies and pooled as risk ratio (RR). We conducted a meta-analysis for both direct and indirect comparisons of each pathogen using R studio and Comprehensive Meta-analysis software. The primary endpoint was the clinical response through the cessation of diarrhea. The secondary endpoints were the parasitological response and any reported adverse events. In *Cryptosporidiosis*, the overall estimate favored nitazoxanide in both clinical and parasitological responses in comparison with placebo (RR 1.892, 95% CI [1.297, 2.759]); (RR 2.297, 95% CI [1.520, 3.471]), respectively. Among patients with *Giardia intestinalis*, nitazoxanide in comparison with placebo, showed a 150% increase in the probability of diarrheal cessation (RR 2.50, 95% CI [1.12, 5.58]). In *Clostridium difficile* infection, the network meta-analysis

depicted the non-inferior clinical response effect of nitazoxanide to metronidazole at both 7 and 31 days after treatment (RR 1.09, 95% CI [0.91, 1.29]); (RR 1.21, 95% CI [0.87, 1.69]), respectively. Nitazoxanide enhanced rotavirus induced diarrheal symptoms resolution and shortened duration among hospitalized patients. We highlighted the effectiveness of nitazoxanide in cessation of diarrhea caused by *Cryptosporidiosis* and *Giardia intestinalis* infection. We also found the non-inferiority of nitazoxanide to metronidazole, so it might be beneficial in the treatment of *Clostridium difficile* infection. We recommend nitazoxanide as a good option for treating infectious diarrhea. Conducting further trials is highly suggested for other diarrheal conditions such as hepatic amoebiasis.

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GENERAL KNOWLEDGE ON CHOLERA IS HIGH, BUT SAFE WATER ACCESS STILL A RISK IN HAITI, EIGHT YEARS INTO PUBLIC HEALTH CAMPAIGN AGAINST CHOLERA

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Eight years into a cholera epidemic in Haiti, despite a decrease in the rate of reported cases, transmission and cholera deaths continue. In the setting of comprehensive interventions including water, sanitation and hygiene (WASH) and oral cholera vaccination (OCV) campaigns, recent data on continued drivers of transmission is not available. We conducted a population-based survey of 1,868 households in Mirebalais, Haiti - a major hotspot for reported cholera cases - between March and November 2017. We assessed demographic and socioeconomic data, knowledge and self-reported practices related to cholera, and water quality (free chlorine residual) in the household water supply using a DPD (diethyl paraphenylene diamine) indicator test. In descriptive analyses, 1849/1868 (99%) respondents had heard of cholera. However, despite years of public health messaging, 103/1849 (6%) did not know how one gets cholera and 93/1,849 (5%) did not know any ways to avoid cholera. While 1417/1849 (76%) responded "washing hands" as a known method of avoiding cholera, only 229/1849 (12%) listed "not defecating near water source," and 70/1849 (4%) listed "vaccine." Although 982/1868 (53%) reported using household bleach, and 1293/1868 (69%) reported using Aquatabs (active ingredient is a chlorine donor) to treat water, only 305/1459 (21%) samples tested were within the recommended potable range of 0.2-2.0ppm. Of those who reported "sometimes" or "almost never" treating their drinking water, 360/669 (54%) cited a lack of necessary treatment products as the reason. In addition, 956/1868 (51%) households reported that water from their usual source was unavailable for at least 1 day in the past month. These findings suggest that broad national messages for cholera prevention have been well-received, but more targeted interventions may be needed at this stage to reach those low-knowledge subgroups. Further analysis of these data will provide guidance on specific target groups for interventions. Inconsistent access to safe water sources and the tools of water treatment are likely drivers of sustained transmission despite generally high knowledge.

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DETECTION AND CORRECTION OF HYPOKALEMIA IN A MALNOURISHED PEDIATRIC POPULATION WITH DIARRHEA IN RURAL NIGER

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Serum electrolytes are commonly used to manage critically ill children, but this diagnostic is rarely available in pediatric humanitarian projects. Nonetheless, electrolyte disorders are significant in these contexts, in particular in malnourished children, where hypokalemia (serum [K+] <3.5 mmol/L) is frequent and associated with mortality risk. We document the prevalence of hypokalemia at admission and during hospitalization, and pilot the efficacy and tolerance of parenteral potassium (K+) correction in a malnourished population. Ill children (gastroenteritis or malaria) over 3 kg with MUAC ≥115 mm underwent point-of-care electrolyte testing. Those with capillary refill time ≥ 3 second or nutritional edema were excluded. Serum electrolytes were performed at admission and regularly until serum [K+] > 2.5 mmol/L. Intravenous K+ correction was carried out (0.6 or 1.0 mmol/kg over 3 hours) if serum [K^+] < 2.5 mmol/L. To date 51 children, 12 girls and 39 boys, among which 33 cases MUAC 115-124 mm, have been tested. 16 children have received parenteral K+ correction. 39 children (76 %) had hypokalemia, 22 of them severe (< 2.5 mmol/L) and 16 critical (< 2.0 mmol/L). Critical hypokalemia was more prevalent in children with MUAC 115-124 mm, non-significant trend, p = 0.17 No improvement was noted between the first and second K+ levels in 29 children receiving potassium-containing therapeutic milk (> 4 mmol K+/Kg/d). One third of all children tested (6/18 MUAC ≥ 125 mm, 10/33 MUAC 115-124 mm) received a K+ infusion without adverse event and with improvement in serum [K+] Hypokalemia is important in malnourished children in Niger: often severe and slow to correct with therapeutic milk suggesting a need to review the amount of K+ provided in the current formulation. Hypokalemia also appears to be common in pediatric patients, yet they do not benefit from K+ containing supplements, suggesting it is important to further describe electrolyte imbalances in hospitalized children in humanitarian settings and review K+ levels provided in intake. It remains to be determined whether correction of hypokalemia could improve the survival of these children.

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DISTINCT PROFILES OF ANTIMICROBIAL SUSCEPTIBILITY AND *SALMONELLA* SEROTYPES IN STOOLS OF CHILDREN ENROLLED IN THE GLOBAL ENTERIC MULTICENTER STUDY, 2007-2011

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The Global Enteric Multicenter Study (GEMS) determined the etiological agents of moderate-to-severe diarrhea (MSD) in children under the age of five years living in Africa and Asia. *Salmonella spp.* were associated with MSD in children from Kenya and Bangladesh. Here, we describe the serovars and antimicrobial susceptibilities of *Salmonella spp*. from GEMS stools. *Salmonella* isolates were identified by GEMS sites using clinical microbiological methods. We subsequently serotyped the isolates by agglutination with O and H antisera. Specimens came from both children with diarrhea and healthy controls. We evaluated antimicrobial susceptibility using the Kirby- Bauer disk diffusion method. We determined *S*. Typhimurium sequence types using multi-locus sequence typing and/or whole genome sequencing. We isolated *Salmonella* spp. from 370 children. Of these, 188 were from Asia and 182 were from Africa. *S*. Typhimurium (22.7%), *S*. Paratyphi B Java (11.4%), *S*. Enteritidis (5.1%), *S*. Hissar (2.4%), and *S*. Typhi (2.2%) were the most abundant serovars in

stools of MSD cases. *S*. Virchow (9.7%), *S*. Newport (8.6%), *S*. Heidelberg (3.0%) were the most abundant serovars in stools of MSD controls. 2-3% of MSD episodes among Kenyan 0-23 month olds were attributable to *S*. Typhimurium, while 3.7% of MSD episodes in Kenyan 24-59 month olds and 1.9% of MSD episodes in Bangladeshi 0-11 month olds were attributable to serogroup B isolates. 1.2% of MSD episodes in Pakistani 0-11 month olds were attributable to serogroup D isolates, and 1.2% of MSD episodes in Bangladeshi 23-59 month olds were attributable to serogroup C1 isolates. Multidrug resistant *S*. Typhimurium and *S*. Enteritidis were isolated in Kenya and multidrug resistant *S*. Typhi was isolated in India. *S*. Typhimurium sequence type 313 was the predominant genotype from Kenyan stools. *Salmonella* isolated in GEMS show regional differences in serotype distribution as well as antimicrobial susceptibility. *S*. Typhimurium ST313, a dominant strain from invasive NTS in Africa, was the predominant strain in GEMS stools from Kenya.

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CALIBRATING A RABBIT COLONIZATION MODEL TO TEST A CANDIDATE MULTI-EPITOPE FUSION ANTIGEN (MEFA) VACCINE FOR ENTEROTOXIGENIC *E. COLI*

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Enterotoxigenic *E. coli* (ETEC) is a leading cause of children's diarrhea and travelers' diarrhea across the globe. Adopting a multi-epitope fusion antigen (MEFA) approach, a candidate vaccine was generated using epitopes from characteristic colonization factor antigens associated with ETEC infection. Previous work has shown that this fusion antigen, combined with administration of a toxoid fusion, induced broadly neutralizing antibodies in mice and protected STa+ or LT+ ETEC diarrhea in a pig model. Protection against ETEC colonization was not quantified in these studies, but is known to be a crucial step for the establishment of disease in humans. Earlier studies utilized a rabbit model to investigate the colonization of *Shigellae, Vibrio cholerae* and *E. coli* in the rabbit small intestine. We have adapted these methods for use with ETEC strains. After a 24 h fast, we challenged New Zealand white rabbits with 10^9, 10^{10}, or 10^{11} colony forming units of ETEC using oral gavage; this was combined with administration of sodium bicarbonate buffer and famotidine. After 24 h animals were sacrificed, and 10 cm intestinal sections were taken from the proximal and distal small intestine. These were opened, vortexed in sterile PBS, and dilution-plated on MacConkey agar for colony-forming units. Colonization was found to be limited to the distal small intestine. Strain H10407 (a strain producing CFA/1 and LT/ST) showed an attenuated initial colonization when compared to strain B7A (a strain producing CS6 and LT/ST). Additionally, serum collected from immunized rabbits shows a multiple fold-change response in IgG antibody titers directed towards each of the colonization factor antigens in the fusion antigen. By combining direct colony counts with serology, we have developed a robust model to investigate the candidate MEFA vaccine and future candidate vaccines for ETEC.

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THE SYMPTOMS CHARACTERISTICS OF INFECTIOUS DIARRHEAL WHICH CAUSED BY DIVERSIFORM PATHOGENS

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38950 cases of acute diarrheal who were from 0-99 years old were collected in the hospitals among 18 provinces of China during January 2010 to December 2016. The feces sample of each cases was detected 5 virus and 17 bacteria. We compared the symptoms which caused by different pathogens by statistical methods. It was found that the symptoms of infectious diarrheal caused by rotavirus and norovirus were showed obvious watery feces (OR=1.87 and 1.47 respectively, P<0.05) with vomit (OR=3.46 and 1.88 respectively, P<0.05) while stool routine test was negative. The symptoms of *Vibrio* spp. infectious diarrheal were similar with rotavirus and norovirus infection but leukocyte (OR=2.10, P<0.05) and erythrocyte (OR=2.42, P<0.05) were present in feces. The symptoms of *Salmonella*, EIEC, *Shigella* and *Campylobacter jejuni* infectious diarrheal were similar that showed fever (OR=2.47, 1.91, 3.21 and 1.98 respectively), mucus feces with blood (OR=2.21, 1.75, 18.55 and 6.98 respectively, P<0.05), feces leukocyte (OR=2.57, 2.42, 8.07 and 9.78 respectively, P<0.05) and erythrocyte (OR=2.85, 1.89, 17.10 and 9.04 respectively, P<0.05) positive. The symptoms *Yersinia enterocolitic*a were mild but leukocyte (OR=3.32, P<0.05) were present in feces obviously. And it may led quite a bit of *Y. enterocolitica* infections didn't go to see a doctor. It was very difficult to distinguish which pathogen cause the infectious diarrheal just according to the symptoms. But the symptoms characteristics could provide clues to the laboratory detection.

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UNEXPECTED LOW PREVALENCE OF TYPHOID AMONG ACUTE FEBRILE ILLNESS CASES IN INDIA

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Globally, an estimated 11-20 million people have typhoid fever and 150,000 die from it annually. In 2014, as part of Global Health Security, we initiated a facility-based acute febrile illness (AFI) surveillance platform to identify the etiologies of AFI in India. We defined AFI patients as patients with documented or reported fever <15 days duration; we enrolled all admitted AFI patients from 32 district/sub-district hospitals in 10 states of India. We recorded demographic and clinical data; we collected blood and tested for bacterial, viral and parasitic diseases. A positive BacT/ALERT blood culture was diagnostic of typhoid. Antibiotic susceptibility of Salmonella isolates were tested using vitek 2. We enrolled 27,431 AFI patients from June 2014-July 2017; of these, 14,134 (51.5%) were positive for any pathogen. The major etiologies were influenza (16.6%), dengue (9.7%), scrub typhus (8.6%), leptospirosis (8.1%), malaria (4.7%), KFD (2.2%) and typhoid (0.7%). Among the 200 typhoid cases, 156 were positive for *S.typhi*; and 44 cases were positive for *S.paratyphi A*. The percentage positivity of typhoid among AFI cases varied from 0.2-3.1 by state and was highest in Maharashtra (3.1%) The median age of typhoid cases was 20 years (IQR 15,29); 67% were male. On admission, 38% of patients had vomiting, 38% had abdominal pain, and 10% of patients (21/200) had diarrhea. While 94% of isolates of *S.typhi* were sensitive to Ceftriaxone and Trimethoprim/sulfamethoxazole and resistant to Ciprofloxacin, 4.6% were sensitive to all the three. No Ceftriaxone resistance was recorded. Contrary to general belief, typhoid did not figure among the major etiologies of AFI in India. However, typhoid may be underestimated in our population as the majority of cases received antibiotics prior to hospital admission. Despite Ciprofloxacin resistance among both *S.typhi* and *S.paratyphi A*, all isolates were susceptible to third generation cephalosporins. Our results highlight the importance of etiological diagnosis at district and sub district healthcare facilities and these results will help in developing clinical guidelines for management of AFI cases.

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MONITORING *YERSINIA PESTIS* SUSCEPTIBILITY TO ANTIMICROBIALS IN MADAGASCAR

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Fight against antimicrobial resistance (AMR) is one of this 21th century challenges. Resistance in high pathogenic agent such as *Yersinia pestis*, a potential bioterrorism weapon, is even more a threat for global health. This pathogen continues to threaten the Malagasy population with around 300 reported cases/year. Current treatment regimen in Madagascar consists on high-dose Streptomycin. We present *Y. pestis* resistance to antimicrobial drugs. Also, to provide information on AMR strains will help to adapt treatment recommendations in the the Plague National Control Program (PNCP). This survey was conducted from 1995 to 2017 by the Plague Unit at the Institut Pasteur de Madagascar. Strains were isolated from human biological samples (bubonic and pulmonary plague) as well as rodents and fleas sampled from the field. Antibiotic testing using Kirby Bauer disk diffusion method was performed on each isolated strain. Susceptibility was assessed for Streptomycin, Gentamycin, Tetracycline, *sulfamethoxazole-trimethoprim,* Chloramphenicol, Ampicillin and Ciprofloxacin, and interpretation was done according to Clinical Laboratory Standards Institute guidelines. Among 4812 tested isolates (4364 from humans (H), 438 rodents and fleas (R&F)), *Y. pestis* harboring résistance profile have been identified, among which three strains shows resistance to the drug of choice recommended by the PNCP (Streptomycin), and one with multimicrobial resistance profile. The prevalence of drug resistance is relatively low (0.31%, 15/4812, 13H, 2 R&F), nonetheless it is alarming because of the high risk of dissemination, high mortality rate and the lack of rapid resistance detection tool. *Y. pestis* is classified as category A by the US CDC. Effectiveness of the national network surveillance for *Y. pestis* antimicrobial susceptibility is crucial to detect and monitor the occurrence of resistant strains. There is a need to reinforce capacity in rapid detection of AMR and to update the PNCP treatment recommendation to be aligned with the international guidelines.

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CHANGES IN HEALTH CARE SEEKING BEHAVIOR FOR DIARRHEAL ILLNESS IN MALI

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Diarrheal diseases are a leading cause of child mortality in Africa. Early recognition and seeking timely medical care are very important in preventing diarrheal mortality. To assess trends in health care-seeking behaviour of children under five with diarrheal illness residing in Bamako, Mali, we conducted health care utilization surveys (HUS) several times per year during the Global Enterics Multicenter Study (GEMS, 2009-2010) and, following rotavirus vaccine introduction as part of the Vaccine Impact on Diarrhoea in Africa (VIDA) study (2015-2017). We conducted 3 rounds of HUS during GEMS and 6 rounds during VIDA. For each survey, we randomly selected children in 3 age strata (0-11, 12-23, and 24-59 months) from a censused population and interviewed their primary caretakers. The questionnaire solicited information about a history of diarrhoea illness during the previous 7 days and the use of healthcare facilities. Responses were weighted to reflect the age structure of the underlying population. During the survey rounds conducted from 2009-2010, 3,730 eligible children of which 3,016 (81%) were enrolled. 12% children had a history of diarrheal illness in the past 7 days, of whom 65% sought care outside the home from a medical provider (14%), a pharmacy outside of a medical facility (13%), or a traditional medicine practitioner only (38%); 28% were administered Oral Rehydration Solution (ORS). From 2015-2017, 11895 eligible children of which 10,078 (85%) were surveyed, of whom 2% had a history of diarrheal illness in the past 7 days. 77% of children sought care outside the home from a medical provider (13%), a pharmacy outside a medical facility (29%), or a traditional medicine practitioner provider only (40%); 51% received ORS. The 7-day prevalence of diarrheal disease has decreased in our study area in Bamako. Concomitantly, there is an increase in care seeking related to greater use of pharmacies and uptake of ORS. Introduction of rotavirus vaccine and intensified televised public health messaging about ORS may have contributed to these trends.

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IDENTIFICATION AND CHARACTERIZATION OF *ORIENTIA CHUTO* IN TROMBICULID CHIGGER MITES COLLECTED FROM WILD RODENTS IN KENYA

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We present data that concurs with the reported geographical expansion of scrub typhus outside the "Tsutsugamushi Triangle" and addition of *Orientia chuto* as a second species in the *Orientia* genus. Wild rodents were caught in Marigat, Baringo County, Kenya and ectoparasites, including chiggers recovered. The rodents and the chiggers were speciated by taxonomic features. Genomic DNA was extracted from the chiggers and used to amplify and sequence the 47-kDa (*HtrA*) gene, the 56-kDa (*TSA*) gene and the 16S rRNA. The main rodent hosts identified were *Acomys wilsoni, Crocidura sp* and *Mastomys natalensis* which accounted for 59.2% of the total collection. Of these, *A. wilsoni* and *M. natalensis* harbored most of the chiggers that belonged to the *Neotrombicula* and *Microtrombicula* genera. A pool of chiggers from one of *M. natalensis* was positive for the *Orientia* bacteria by 47-kDa PCR, but did not amplify with the 56-kDa primers. On sequencing the 850 bp of the 47-kDa gene, the closest phylogenetic relative was *O. chuto* with 97.65% sequence homology compared to 84.63 – 84.76% for *O. tsutsugamushi*. 16S rRNA metagenomic sequences also revealed *O. chuto* as the closest phylogenetic relative with 99.75% sequence homology. These results and the existing immunological and molecular reports are strongly suggestive of the existence of scrub typhus in Kenya.

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O-SEROGROUPS OF MULTI-DRUG RESISTANT CERVICOVAGINAL *ESCHERICHIA COLI* HARBORING SEVERAL VIRULENCE GENES

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The occurrence of cervicovaginal *Escherichia coli* (CVEC) multi-resistant to antibiotics and harboring several virulence genes is considered a serious health problem. The present study is another effort to determine the antimicrobial susceptibility, the frequency of several virulence genes and the O-serogroups of CVEC strains. Two hundred *E. coli* strains were isolated from Mexican women suffering cervicovaginal infection attending at two medical units of the Instituto Mexicano del Seguro Social at State of Mexico. *E. coli*, the O-serogroups and virulence genes were identified by PCR. Antibiotic susceptibility was determined by Kirby-Bauer disk diffusion test. O25 (50%), O75 (9%), and O15 (7.5%) were the more frequent serogroups. Ninety-eight percent CVEC (n = 197) were resistant to 3-12 antibiotics; 97% were resistant to ampicillin, 93.5% to carbenicillin, 77%

to cephalotin, and 71% to nitrofurantoin. The most frequent virulence genes among the CVEC strains were *feoB* (91.5%), *fimH* (89.5%), *kpsMT11* (75%), *iutA* (66%), and *iron* (59%). This study revealed the main O-serogroups of these multidrug-resistant CVEC strains and their association with the virulence markers. These data can serve to orientate a better antibiotic prescription.

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A NOVEL SURFACE-EXPOSED PROTEIN OF *LEPTOSPIRA INTERROGANS* THAT INTERACTS WITH THE COMPONENTS OF HUMAN TERMINAL COMPLEMENT PATHWAY

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Leptospirosis is a zoonosis globally disseminated caused by pathogenic spirochetes of the genus *Leptospira*. Preventive measures to control leptospirosis are difficult to implement. Thus, understanding leptospiral pathogenic mechanisms is critical for the efficient development of vaccines and diagnostic tests. Our group has identified leptospiral surface proteins with the ability to bind extracellular matrix and plasma/serum components, which could mediate adhesion and facilitate invasion through the hosts. This work aims to assess the role of a probable lipoprotein encoded by the gene LIC13259 of *L. interrogans* in the host immune evasion. The gene LIC13259 was cloned into the expression vector pAE. The plasmid pAE-LIC13259 was employed to transform *E. coli* strains for protein expression studies. In order to assess the localization of LIC13259 at the bacterial surface, ELISA, proteinase K and immunofluorescence assay were performed. The ability of recombinant protein to interact with the complement components was evaluated by ELISA and immunoblotting assay. The effect of LIC13259 on C9 polymerization was assessed by SDS-PAGE. The coding sequence LIC13259 was cloned and expressed successfully in their soluble form. The recombinant protein was purified by nickel affinity chromatography and analyzed by SDS-PAGE (17 kDa). ELISA, proteinase K and immunofluorescence assays suggest the presence of LIC13259 at the cell surface. Furthermore, LIC13259 showed a dose-dependence interaction with the vitronectin, C7, C8, C9 components either purified or from normal human serum. Increasing concentration of heparin had effect on the binding of LIC13259 with vitronectin, suggesting that this interaction involve heparin binding sites. Most interestingly, the LIC13259 was able to inhibit C9 polymerization in a dose-dependent manner. In conclusion, our data suggest that LIC13259 is located at the cell surface and could be involved in host immune evasion processes by interacting with the components of the terminal complement pathway.

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ANTIBIOTIC SUSCEPTIBILITY OF THE FIRST *STREPTOCOCCUS CONSTELLATUS* ISOLATED FROM EPIDURAL ABSCESS IN DAKAR

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Streptococcus constellatus is member of the *Streptococcus anginosus* Group (SAG) that are considered commensal organisms; however, they can sometimes cause serious invasive infections of the liver, lung, brain abscesses, bacteraemia, endocarditis and intra-abdominal infections. *S. intermedius* and *S. constellatus* are the main causes of deep abscess. SAG is generally considered to be susceptible to beta-lactam antibiotics and macrolides; however, increasingly we are seeing the emergence of viridans and beta-haemolytic Streptococci resistant to antibiotics. In a 59-year-old patient hospitalized in intensive unit care for an epidural abscess, pus was collected and cultures were grown. Bacterial identification was performed by routine laboratory methods including Gram stain, catalase reaction and biochemical analysis using the API 20 Strep test (BioMérieux Vitek 2). Antimicrobial susceptibility testing was performed using standard disk diffusion method. Antibiotic disks of penicillin, vancomycin, teicoplanin, gentamycin, erythromycin, lincomycin, pristinamycin, norfloxacin, and rifampicin were placed on inoculated plates. Minimum inhibitory concentration (MIC) was determined by using the BioMérieux Vitek 2. *S.constellatus* was identified in abscess and was resistant to penicillin, aminoglycosides, macrolides, lincomycin, fluoroquinolones. This isolate was sensitive only to vancomycin, pristinamycin and rifampin. Difficulties in identification strains isolates of the SAG have caused confusion in determining their pathogenic potential, but using new powerful tool for rapid identification bacteria, makes accurate diagnosis and differentiatiation possible. Thus, it is necessary to equip the laboratories for good management of infectious diseases. This is the first known report of *S. Constellatus* from abscess in Dakar. SAG are generally susceptible to penicillin, other beta-lactam antibiotics and macrolides. However, in our study *S. Constellatus* were resistant to penicillin, aminoglycosides, macrolides, lincomycin and fluoroquinolones, demonstrating antibiotic resistance among SAG isolates.

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MOLECULAR EPIDEMIOLOGY OF LEPTOSPIROSIS IN PERU

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Leptospirosis represents an important public health problem in Peru (incidence 7 x 100 000 inhabitants). In 2017, 2239 new infections attribute to Leptospira were reported; however, only 50% of the cases were confirmed. Determinant factors of this lack of diagnosis are the location of reference diagnostic center, limitation of diagnostic methods, and limited knowledge of local etiological agent. Besides, a lack of molecular knowledge about the diversity of circulating strains makes difficult the development of new diagnostic tools, treatment or vaccine development. The present work proposes the use WGS-MLST as a tool to give an overview of the genetic diversity and initial epidemiological insights of Peruvian pathogenic Leptospira species and serovars. Ninety-one isolates from the Peruvian National Health Institute strains collection were randomly reactivated. Whole genome sequencing was performed using SBS platform. Genome assembly, annotation, and phylogenetic analysis were performed using free genomic analysis software. MLST data allowed determining 5 species and 12 serovars from 84 isolates. Comparative analysis of leptospiral genomes shows a size of 4.45 Mb with a core comprising 3483 proteins. L. interrogans, L. santarosai and L. noguchi were the most frequent species. Species distribution per time shows that L. interrogans was present in a wide period (2003 - 2013), followed by L. noguchi and L. santarosai. Among five regions of Peru who reported cases of leptospirosis, the majority of species were more frequent in Loreto (approximately 81% of isolates). The molecular identification of Leptospira species in field isolates contributes to increasing the knowledge of genetics and species/serovars distribution.

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CHARACTERIZATION OF MULTIDRUG RESISTANT GRAM-NEGATIVE BACILLI FROM HUMAN INFECTIONS IN NICARAGUA

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Antimicrobial resistance (AMR) represents a global public health crisis; yet much of the burden in limited resource settings remains unknown. In Nicaragua, high rates of AMR have been observed among gram-negative rods (GNRs), though scant data on clinical isolates has been published. The aim of this study was to characterize AMR among GNRs using 100 isolates collected from 6 departments in Nicaragua between 2014 and 2017. Isolates were sent to the Centro Nacional de Diagnóstico y Referencia in Managua, where susceptibility testing was initially performed by the Kirby-Bauer method. We selected 100 isolates with evidence of resistance to ≥ 2 classes of antibiotics, including carbapenems. These represented the most common GNRs identified: *Enterobacteriaceae*, 52 (*K. pneumoniae*, 50; *Escherichia coli*, 4); *A. baumannii*, 30; and *P. aeruginosa*, 16. Isolates were selected predominantly from sterile sites: blood (81 of 96 cultures with specimen recorded), pleural fluid (n=7), cerebrospinal fluid (n=4), peritoneal fluid (n=2), and bronchoalveolar lavage (n=2). Metallo-β-lactamase production was suspected as the primary mechanism of resistance based on imipenem-EDTA synergy disk testing. Carbapenem resistance was confirmed with repeat testing by disk diffusion in all but one isolate (*P. aeruginosa*). Genes for the New Delhi metallo-β-lactamase 1 (NDM-1) carbapenemase were detected in 62% of isolates by real-time PCR, including 50/54 (92%) *Enterobacteriaceae*, 10/30 (33%) *A. baumannii*, and 2/16 (12%) *P. aeruginosa*. The remaining 4 *Enterobacteriaceae* isolates had a *Klebsiella pneumoniae* carbapenemase gene detected. No evidence of colistin resistance was identified. This study reveals very high rates of NDM-1 detection in multidrug-resistant GNRs from Nicaragua, where the enzyme was first identified in a clinical sample in 2012. NDM producing bacteria have spread throughout the world, but little data exists regarding the prevalence of this resistance mechanism in most of Central America. Further research is needed to determine the burden of highly resistant bacteria in Nicaragua and to guide interventions to limit further spread.

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PREDICTORS OF GONORRHEA AND CHLAMYDIA AMONG PATIENTS ATTENDING FIVE SELECTED CLINICS IN GHANA

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Sexually transmitted infections (STIs) directly affect sexual and reproductive health worldwide. *Neisseria gonorrhoeae*, and *Chlamydia trachomatis* significantly contribute to DALYs. The goal of this study was to identify risk factors associated with the acquisition of gonorrhea and chlamydia in Ghana as well as determine their common clinical symptoms. Participants enrolled presented with symptoms consistent with urethritis or cervicitis were enrolled in some selected health institutions in Sekondi, Takoradi and Accra between June 2012 and March 2016. First void urine samples collected, were tested by a Nucleic Acid Amplification Test (NAAT), to detect both *N. gonorrhea* and *C. trachomatis*. Demographic data was collected and from a structured questionnaire that assessed various social and behavioral characteristics. Nine hundred and fifty individuals comprising 58% females and 42% males were enrolled. A total of 28% tested positive for gonorrhea and 11% for chlamydia, with more males testing positive than females. Commonly reported symptoms among gonorrhea positive patients that demonstrated statistical significance included painful urination and urethral discharge. Additionally, multiple sexual partners, alcohol use, and ages between 25-31 years were statistically associated with higher rates of gonorrhea in males while only the frequency of condom use was associated with gonorrhea in females. The frequency of condom use among females was inversely related to rates of gonorrhea. None of the symptoms or risk factors were associated with testing positive for chlamydia. Individuals presenting with painful urination and discharge in males, were at a higher risk of testing positive for gonorrhea, but not Chlamydia. Risk factors such as multiple sexual partnership, alcohol use and age represent a high-risk group of males who are more likely to test positive for gonorrhea. Identifying these symptoms and risk factors help inform health care delivery systems for STIs in Ghana.

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ESTIMATING THE BURDEN OF LEPTOSPIROSIS IN SRI LANKA; A SYSTEMATIC REVIEW

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Though Sri Lanka is considered as a hot spot of leptospirosis, true incidence of leptospirosis is unknown. The objective of this study was to provide valid estimates on burden of leptospirosis in Sri Lanka. A systematic review (SR) was carried out using Cochrane guidelines on SR. Pub Med, MEDLINE®, BIOSIS Previews, Zoological Record, Web of Science Core Collection, Current Contents Connect, KCI-Korean Journal Database, BIOSIS Citation Index, Data Citation Index, SciELO Citation Index and Google scholar databases were searched. In addition, quarterly epidemiological bulletin (QEB), Indoor mortality morbidity review(IMMR) and hand search of local literature was done in libraries of SLMA, MRI, PGIM and Universities of Peradeniya and Colombo. Forty relevant full texts, 32 QEBs, and 8 IMMR were included in the full text review. Incidence estimates were based on 87,075 patients reported in IMMR and 45,316 reported in QEB from 2005 to 2015. Adjustments for under diagnosis, underreporting and chance variability were done using the data from prospective studies included in full text review. After the adjustments, estimated annual caseload of leptospirosis was 10,423 and the cumulative annual incidence of leptospirosis that require hospitalization in Sri Lanka during 2008-15 periods was 52.1 (95% CI 51.7-52.6) per 100,000 population. The number of estimated annual deaths due to leptospirosis is around 730 (95% CI 542-980), with an estimated pooled case fatality ratio of 7.0% (95% CI 5.2-9.4). Our SR shows gross underestimation of true leptospirosis burden and this deadly disease needs more attention in the public health agenda.

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MULTI-DRUG RESISTANCE SUPPORTED BY PLASMID-ASSOCIATED GENES IN GEORGIA

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Infectious threats, and the consecutive emergence and spread of drug resistant bacteria have become a significant global health issue in the 21st century. Antimicrobial resistance (AMR) is steadily increasing in bacterial pathogens and makes treatment a challenging process that often leads to fatal outcomes. It has been estimated that unless hampered, rise of drug resistance will cause up to 10 million deaths a year and excess health-care associated costs in excess of 100 trillion USD by 2050. It has also been suggested, that most direct and indirect effects of AMR will fall on low and middle-income countries. Therefore, a boost in surveillance activities is needed to track the spread of AMR. Georgia, located in the Caucasus region, represents a middle-income country. Importantly, the prevalence and distribution of antimicrobial resistant bacteria in Georgia have not been adequately defined. Therefore there is a significant possibility that Georgia can be heavily impacted by the increasing numbers of multi-drug resistant organisms. The purpose of this study was to detect and examine antibiotic resistance patterns in Georgia. Bacterial isolates, collected at various sites of Georgia, were identified using the automated system Vitek 2. Antimicrobial susceptibilities were determined by both, automated and manual methods (disc diffusion and E-test). Antimicrobial resistance genes were identified by PCR and phenotypic analyses. 100 specimens were collected between July 2017 and February 2018. It was found that 100% of the *Acinetobacter spp.* and *Pseudomonas spp.* isolates were multi-drug resistant. In addition, *Klebsiella spp.* and *Serratia spp.* were found to harbor both, the CTX-M15 extended spectrum beta-lactamase and the OXA-48 carbapenem resistance genes. Multi-drug resistance has been observed in bacterial isolates recovered in the country of Georgia. In addition, detection of highly transmissible plasmid associated resistance genes is indicative of a potential for horizontal spread that in combination with already existing multi-drug resistance could lead to the emergence of a novel "superbug" in Georgia.

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DETERMINATION OF EXPRESSION OF EFFLUX PUMPS IN MDR STRAINS OF *ACINETOBACTER BAUMANNII* FROM A TERTIARY CARE HOSPITAL IN LIMA, PERU

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Acinetobacter baumannii is an important nosocomial opportunistic pathogen that has emerged in the clinical setting for its multidrug resistance. It affects mainly patients in intensive care unit. Mortality rates between 7,8-23% and 10-43% in intensive care unit (ICU). It has been reported 30% of multidrug-resistant (MDR) strains in ICU. It is associated with pneumonia, bacteremia, and less frequent with endocarditis, meningitis, and soft tissue injuries. Efflux pumps are divided into 6 superfamilies, between them exist the RND (Resistance-Nodulation-Division) complex that plays and important role in multidrug-resistance. To evaluate the mechanism of efflux pumps, inhibitors of efflux pumps have been used to avoid the exit of diverse molecules. Evaluate and identify the expression of efflux pumps in isolated strains of *A. baumannii* from a tertiary care hospital in Lima between the period of 2014-2016. Clinical isolates of Acinetobacter spp. have been identified by BD Automatized System PhoenixTM, and was confirmed as *A. baumannii* by the amplification of blaOXA-51 gene by PCR. The susceptibility to fluoroquinolones and aminoglycosides was performed by the disc diffusion method and the estimate of the MIC was determined by broth-microdilution, cutoff values were determined according to the CLSI 2018 guidelines. The expression of efflux pumps was analyzed by adding the inhibitor Phenylalanine-arginine β-naphtilamide (PAβN) and it was confirmed its presence if the MIC descend 4 fold times. There were obtained 51 strains positive to *A. baumannii* by PCR *bla*OXA-51. They showed high resistance levels to aminoglycosides: amikacyn was 69,49% (n=41), tobramicyn 67.79% (n=40). Meanwhile, fluoroquinolones: levofloxacin 81.35%(n=48). When PAβN was added to amikacyn it changed to 69,49% (n=41) into 49.15% (n=29). In conclusion, Results obtained suggest efflux pumps were present increasing the susceptibility of amikacin by the use of Phenylalanine-arginine β-naphtilamide (PAβN).

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GUT MICROBIOTA IN HOSPITALIZED CHILDREN UNDER FIVE YEARS WITH ACUTE INFECTIOUS GASTROENTERITIS FROM A TEACHING HOSPITAL IN CAJAMARCA, PERU 2011-2012

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Gut microbiota (GM) is a community of microorganisms from in the human gastrointestinal tract (HGIT) which plays a key role maintaining the host health. However, this microbial structure can be disrupted under an inflammatory process such as viral or bacterial infections. The main objective was to describe the gut microbiota profiles in hospitalized children under 5 years with the diagnosis of Acute Infectious Gastroenteritis (AIG). A retrospective, descriptive cross-sectional study was conducted using the Hospital Regional de Cajamarca physicians database between 2011 and 2012. A total of 117 samples from patients under 5 years old with AIG were analyzed for detection of common viral and bacterial etiologies and 13 gut microbiota agents via Polymerase Chain Reaction. Infants younger than 12 months-old were the most predominant age group in 36.7% cases. The most commonly detected microbiota bacteria were: Firmicutes (n=74 cases), Bacteroidetes (n=73 cases), Lactobacillus (n=70 cases), Prevotella (n=67 cases) and Proteobacterium (n=63 cases). Patients with exclusive breastfeeding or mixed feeding registered a higher number of gut microbiota bacteria, compared to those who received formula or were not breastfed. In conclusion, coinfections, type of lactations, nutritional status and the different etiologies altered the gut microbiota profiles in children under 5 years old.

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MAKING A MASS DRUG ADMINISTRATION (MDA) PARTICIPATOR: IDENTIFYING FACTORS ASSOCIATED WITH MDA PARTICIPATION FOR TRACHOMA CONTROL IN AMHARA, ETHIOPIA

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Mass drug administration (MDA) with azithromycin is a core part of the WHO recommended strategy to eliminate trachoma as a public health problem, but low participation rates in MDA campaigns may undermine the effectiveness of this intervention. Following an MDA in Amhara, Ethiopia in May 2017, we conducted multi-level cluster random coverage surveys in 4 districts to collect data on self-reported MDA participation. We then explored predictors for individual MDA participation at the individual, household, and guardian levels. Random-effects logistic regression modeling was used to identify predictors of MDA participation while adjusting for nesting of individuals within households. A total of 100 clusters were surveyed, which we obtained data on 6613 participants from 1629 households. The district-level self-reported MDA participation ranged from 78.5% to 86.9%. We developed a model for all participants and found several positively-associated factors for MDA participation: excellent and fair health status (Odds Ratio [OR] = 7.30; 95% Confidence Interval [CI]: 2.62, 20.33; OR = 9.35; 95% CI: 3.06, 28.55), length of household's residency (OR = 2.34; 95% CI: 1.32, 4.17), advanced knowledge of the MDA campaign (OR = 4.28; 95% CI: 2.62, 6.98), and knowledge of trachoma (OR = 1.67; 95% CI: 1.05, 2.67). A second model was run, which excluded heads of household and included head of household participation in the model. Factors associated with participation were similar to those found in the first model, in addition to the head of household participation (OR = 6.03; 95% CI: 3.90, 9.30). Conversely, older age (OR = 0.98; 95% CI; 0.98, 0.99) was negatively associated with participation. These results offer exploratory insight into the factors associated with MDA participation in Amhara, and suggest that heads of households hold a strong influence over their household's participation. To ultimately increase the coverage in Amhara, MDA mobilization strategies – inclusive of comprehensive trachoma and azithromycin messaging and MDA campaign awareness – should target heads of households as well as new residents and those in poorer health and older age.

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THE PREVALENCE, RISK FACTORS AND SEROTYPES DISTRIBUTION OF TRACHOMA IN LAIKIPIA, KENYA 2017

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Trachoma is one the leading causes of preventable blindness caused by a bacterial infection known as Chlamydia trachomatis. It is a Neglected Tropical Disease (NTDs) spread from person to person through direct contact with discharges from infected eye and nose, through mechanical vectors like flies or by contact with fomites. There are various serotypes of Chlamydia trachomatis, serotypes A, B, Ba, and C cause eye infections whereas serotype D and K cause genital infections. The objective of this study will be to determine prevalence and factors associated with Trachoma occurrence, transmission and to document the human Chlamydia trachomatis serotypes and their distribution in Laikipia County. This Will be a cross-sectional community-based survey, estimate the prevalence of TT in adults ages above 15 years old and trachomatous inflammation—follicular in children 1-9 years old. Multi-stage cluster sampling will be done using the existing trachoma clusters. The World Health Organization guidelines on mapping. an evaluation unit (EU) is a considered an administrative unit with a population of 100,000-250,000 persons for healthcare management. Twenty-five to thirty clusters will be randomly selected in each survey segment as recommended by World Health Organization. In each cluster, 30 households will be selected, and all residents living in those households ages one year and above will be invited to participate voluntarily. Participants will be examined by Tropical Data-certified graders, using 2.5× magnifying loupes under direct sunlight. In addition, the certified recorder will conduct direct observation and administer a standardized questionnaire on household-level data on access to water and sanitation.The finding of this study will be used to inform on the progress made in the elimination of trachoma in Laikipia county where the SAFE strategy has been under implementation. It will also bring new knowledge on the existing serotypes and their distribution and correlate the laboratory and clinical diagnosis. Results will inform policy and will be disseminated through workshops, conferences, and publications.

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TRACHOMA GRADER RELIABILITY RESULTS FROM NINE TRACHOMA IMPACT SURVEY TRAININGS CONDUCTED IN AMHARA, ETHIOPIA, 2011 - 2017

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Trachoma impact surveys (TIS) rely on the field grading of clinical signs of the disease using the World Health Organization's recommended simplified grading system as a tool for assessing clinical signs. Conjunctival clinical signs diagnosed by this system include: trachomatous inflammation - follicular (TF), trachomatous inflammation - intense (TI) and trachomatous scarring (TS). Before each TIS conducted in Amhara, Ethiopia since 2010, graders received training for 7-10 days consisting of classroom and field practice followed by a slide exam. Trainees who passed the slide exam moved on to take a field reliability exam. The field exam included grading the conjunctivae of both eyes from 25 children ages 1 to 9 years and were designed to test the ability of graders to diagnose TF, however, the accuracy of TI and TS diagnoses were also assessed. To participate as a grader, trainees were required to achieve a ≥ 84% agreement and a Cohen's kappa (κ) of ≥ 0.7 with TF compared to the consensus grade of three master trachoma graders. Graders were allowed up to 3 attempts to pass a field exam and all rounds of field exams use a unique set of children. Using the collective data from the passing field exam of all passing trainees for a given TIS, a group agreement and κ was calculated for each clinical sign to allow for comparisons between the groups of graders from different trainings. Graders that participated in multiple TIS from 2011-2017, had their data from different surveys treated as independent resulting in a total of 204 trainees taking a field reliability exam. Mean group size was 23 trainees (Standard Error (SE): 3) per survey. A mean of 63.4% (SE: 5.2%) of trainees per survey passed an exam, totaling 124 trainees. The mean group agreement and κ was: 89.1% TF agreement, 0.78 TF κ; 86.9% TI agreement, 0.58 TI κ; 94.5% TS agreement, 0.72 TS κ. The range of group TF reliability results across trainings was 86.9% (group κ: 0.74, n=20) to 96.0% (group κ: 0.92, n=8). Over 7 years, the Trachoma Control Program in Amhara has trained a large cadre of qualified trachoma graders and has demonstrated the ability to consistently achieve high reliability in grading TF.

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MASS AZITHROMYCIN DISTRIBUTION FOR TRACHOMA CONTROL AND SELECTION FOR ANTIBIOTIC RESISTANCE: A SYSTEMATIC REVIEW

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Mass distribution of azithromycin is effective for controlling trachoma and has been shown to reduce childhood mortality. As mass azithromycin is considered for child survival more broadly, understanding the potential for the selection of macrolide resistance is essential. In this systematic review, our objective was to synthesize existing evidence on macrolide resistance following mass azithromycin distribution. We searched electronic databases for articles and conference abstracts published through September 13, 2017 and we contacted authors of included papers to identify unpublished data. We included studies on the community-wide distribution of oral azithromycin for trachoma that included measures of prevalence of carriage and macrolide resistance for any organism. Heterogeneity in study design, setting, treatment frequency, and follow-up precluded formal meta-analysis. We identified 196 studies overall and 19 met inclusion criteria for this review. Twelve studies assessed resistance in *Streptococcus pneumoniae*, 3 in *Chlamydia trachomatis,* 3 in *Escherichia coli*, 2 in *Staphylococcus aureus,* and 1 in *Plasmodium falciparum.* Several studies on *S. pneumoniae* found increases in resistance immediately after mass azithromycin, with subsequent decreases to near baseline levels over time. Some evidence of resistance was found in *E. coli* and *S. aureus*. No resistance was identified in *C. trachomatis* or *P. falciparum.* The lack of resistance seen in *C. trachomatis* is encouraging as it provides evidence that mass azithromycin may remain effective for trachoma control. As mass azithromycin distributions continue for trachoma and are considered for broader interventions, ongoing monitoring of resistance in multiple organisms is required to mitigate unintended adverse effects.

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UNDERSTANDING TRACHOMA TRANSMISSION DYNAMICS AT LOW PREVALENCE LEVELS: THE TIMESCALES OF INFECTION AND DISEASE PROVIDE DIFFERENT SIGNALS BEFORE AND AFTER HAULTING INTERVENTIONS

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Trachoma remains the world's leading infectious cause of blindness, with an estimated 160 million people at risk of blindness. With falling levels of infection across multiple populations, the program is faced with a classic challenge of how to measure low levels of prevalence within the restricted technicaland budgetary options. For trachoma in particular, it can be challenging to verify elimination as, despite limited-to un-detectable PCR positivity, TF prevalence can persist around the target level of <5% for extended periods. In this study we use a validated transmission model to explicitly model the proportion of individuals in the community that were PCR only positive, those TF and PCR positive andthose only TF positive. We evaluatedhow the expected proportion of individuals in each diagnostic state changedover: a 3 year intervention period, 2 years after the implementation of an intervention program and during the endgame phase of elimination. To assess whether what we observed in our simulation model was correct we validated our model findings using paired individual level PCR and TF prevalence data from 4000 individuals across 5 different trachoma endemic or formally endemic regions: Kiribati, Solomon Islands, Ghana, Nepal and The Gambia. Our results suggested that at low-levels of prevalence the relationship between may be more linear when we account for the diagnostic state of individuals instead of aggregating the data at the community level. Disparities in prevalence estimates at low-levels of transmission could be an artifact of sampling size if the study is not sufficiently powered to determine very low-levels of PCR positivity. Our results suggested that a clearer understanding of the dynamics of infection can be understood from harnessing the power of individual level data that it routinely collected to understand the proportion of individuals in each diagnostic state, instead of only aggregate measures of PCR and TF prevalence. Despite the time lag to clear disease, TF prevalence is a more sensitive indicator of a community's elimination status when resources are constrained and is a more reactive indicator of re-emergence.

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RESULTS OF A TRACHOMATOUS TRICHIASIS SURGICAL QUALITY AUDIT IN TANZANIA

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A trachomatous trichiasis (TT) surgical quality audit was performed in Tanzania in August 2017. The goal of the audit was to identify surgeons requiring further training or removal from the program, and to evaluate quality outcomes program-wide. The audit was performed by Tanzania Ministry of Health TT Technical Supervisors. A random sample of patients treated by each of the 14 surgeons were selected using the Lot Quality Assurance Sample for Audits (LQAS) sampling procedure and examined for any post-operative trichiasis (PTT) and other poor surgical outcomes. The corresponding surgeons were classified into "high PTT rate" and "low PTT rate" groups rather than estimating actual PTT rates. The Tanzania Trachoma Technical Working Group (TTWG) established a rate of 20% as the highest acceptable rate of PTT for a surgeon; thus a sample size of 30 patients per surgeon was required with a maximum of 5 PTT cases allowed in the sample. A total of 388 patients were seen, and 404 eyes analyzed. Two surgeons were found to have a high rate of PTT. Furthermore, although it was not the primary outcome of interest, one surgeon was found to have unusually high rates of granuloma, severe over-correction, and eye contour abnormality (2, 4, and 10 patients respectively). After a review of the results by the TTWG, all three surgeons with poor outcomes were scheduled for an immediate supportive technical supervision visit from a TT Master Trainer before performing more TT surgeries, with further technical supervision visits as needed, and all three surgeons will be re-audited in six months. Overall, the surgical audit showed that most surgeons performing TT surgery had acceptably low PTT rates, and successfully identified the surgeons who were in need of further support and re-training. The audit protocol itself was found to be accessible and easy to follow, although some minor improvements to the method will be made before the next round of audits. Surgical audit was found to be a useful tool to provide additional support to surgeons needing technical improvements and to improve TT surgery outcomes in Tanzania.

1199

THE TRACHOMA ENDGAME IN NEPAL: TRACHOMATOUS TRICHIASIS-ONLY SURVEYS TO SUBSTANTIATE ELIMINATION AS A PUBLIC HEALTH PROBLEM

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Trachoma was endemic in Nepal. The surgery, antibiotics, facial cleanliness and environmental improvement (SAFE) strategy was implemented from 2002-2017. By August 2017, Nepal had drafted a dossier to request validation of elimination of trachoma as a public health problem. However, elimination of trachomatous trichiasis (TT) was uncertain in 12 districts where previous trachoma rapid assessments had demonstrated a measurable burden of TT (8 districts) or baseline surveys had shown TT prevalence to be >0.2% in ≥15-year-olds (4 districts). We describe the methodology and results of TT-only surveys undertaken to investigate if the TT elimination prevalence threshold (<0.2% in ≥15-year-olds) had been attained. Surveys were undertaken in 14 evaluation units (EUs) following the Tropical Data methodology. The sample size was calculated to estimate, with 95% confidence, an expected TT prevalence of 0.2% with absolute precision of 0.2%, a design effect of 1.47, yielding 2,818 as the minimum number of adults aged ≥15 years to be examined. Two-stage cluster sampling was used, whereby in each EU, 20 to 30 *wards* (clusters) were systematically selected with probability proportional to population size and 40 households selected in each cluster using the compact segment method. Training of trachoma graders was completed using Tropical Data methods for TT-only surveys. Only people aged ≥15 years were invited to be examined. Prevalence of TT was adjusted for age and gender. A total of 44,140 eligible participants were examined for TT, a slight majority (56%) of whom were female. TT prevalence by EU ranged from 0-0.10% (95% confidence interval 0.01-0.20). Accounting for TT known to the health system, TT prevalence ranged from 0% to 0.07%. Findings suggest all surveyed EUs had attained the elimination threshold for TT. These surveys provide important evidence for substantiating the claim of elimination of trachoma as a public health problem in Nepal.

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ELIMINATION OF TRACHOMA THE SAFE WAY IN NEPAL: KEY PROCESSES AND MILESTONES

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Trachoma was endemic in Nepal where implementation of the surgery, antibiotics, facial cleanliness and environmental improvement (SAFE) strategy was undertaken from 2002 to 2017. We describe: baseline surveys; implementation of SAFE; impact and surveillance surveys; and preparation of dossier for validation of elimination in Nepal. Data were collated from reports of SAFE implementation, scientific manuscripts, water sanitation and hygiene strategies (WASH), and national development plans. The trachoma elimination dossier narrative and data templates were completed according to the World Health Organization standard operating procedures. Trachoma rapid assessments (TRA) were done from 1996 to 2008 covering 57 districts and informed where further baseline surveys were required. A total of 53 districts were surveyed at baseline of which 20 were eligible for the AFE components of SAFE. A total of 29,468 trichiasis surgeries were done from 2002 to 2017. Annual mass drug administration (MDA) was undertaken from 2002 to 2014 in 20 districts. Implementation of facial cleanliness and environmental change (F&E) was targeted to trachoma endemic districts and nationally through WASH programmes. Impact surveys showed that all 20 eligible districts had achieved elimination of TF (<5%) and surveillance surveys showed that TF prevalence of <5% had been maintained 24 months after the impact surveys. A total of 12 districts were eligible for trichiasis only surveys because TRA results suggested that trichiasis was a public health problem (8 districts) and baseline surveys has showed trichiasis prevalence was >0.2% (4 districts). Trachoma dossier preparation started 6 months before the final surveys were conducted and took a year and half from start to submission. Nepal has made steady progress towards elimination of trachoma. The Nepal case study provides important lessons for other national programmes on implementation of SAFE and trachoma elimination.

1201

REACHING DISTRICT ELIMINATION THRESHOLDS, HOUSE BY HOUSE: A NEW APPROACH TO CASE FINDING FOR TRICHIASIS SURGERY IN OROMIA REGION, ETHIOPIA

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Oromia Region is the most trachoma-endemic region, in the most trachoma-endemic country globally and over 25 million people live in 216 trachoma endemic districts. In partnership with the Oromia Regional Health Bureau (ORHB), The Fred Hollows Foundation (FHF) has cleared nearly 65% (90,000 surgeries) of the TT backlog in the region. As districts approach elimination, case finding has become significantly more challenging. Those left untreated are typically those in remote areas, the immobile, and those reluctant to participate in surgery. The need for a more intensive and proactive approach to case finding has been identified. The current project sought to test the efficacy and cost of a systematic house to house approach to case finding. Focusing on rural areas of two districts (Haro Maya and Kombolcha) a team of health extension workers (HEWs) were trained and mobilised to visit each house in a designated area. The HEWs were closely supported and supervised to meet daily targets by Primary Health Care Unit Directors (PHCUD). In turn, daily targets were cascaded from PHCUDs to Woreda Health Office staff to Zonal staff. Collectively this highly organised, coordinated effort sought to ensure each HH was visited and each suspected TT case was actioned. Efforts are ongoing, but as of April 1st, HEWs have visited 62,212 households (HHs) in Haro Maya (80% of HHs), identifying 559 suspected TT cases (255 confirmed; 562 required to reach elimination). In Kombulcha, 38,764 HHs have been visited (90%), identifying 232 suspect cases (186 confirmed; 146 required to reach elimination). Surgeries are ongoing. These districts are expected to clear their TT backlog in May. The cost of the house to house approach per district is estimated to be $6000. Results suggest the house to house approach is an effective and efficient way to reach those 'hard to reach' and reluctant to participate in outreach services. Future plans include examining the feasibility of applying the strategy in low versus high backlog areas, the impact of providing low cost ophthalmoscopes to aid case finding, and exploring opportunities to integrate case finding with other health care activities.

1202

COMPLETING TRACHOMA BASELINE MAPPING IN UGANDA RESULTS OF SURVEYS IN 14 POPULATION BASED SURVEYS CONDUCTED IN 2014 AND 2018

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Trachoma is endemic in 46 districts of Uganda where implementation of the SAFE strategy (surgery, antibiotics, facial cleanliness, environmental improvement) started in 2007. Some districts neighbouring known endemic areas had not previously been mapped. We aimed to estimate the prevalences of trachomatous inflammation-follicular (TF) and trichiasis in those districts. Population-based prevalence surveys were undertaken in 14 evaluation units (EUs) covering 12 districts. The sample size was calculated assuming an expected prevalence of 10% with absolute precision of ± 3%, at 5% level of significance, 95% level of confidence using, a design effect of 2.71 and inflation by 1.15 to account for 15% non-response. Therefore a minimum sample of 1,200 children aged 1-9 years was required in each EU. In each EU, two-stage cluster sampling was used to select the sample. Consenting participants aged 1 year and above were examined for trachoma using the World Health Organization simplified grading system. Prevalence estimates were adjusted for age and gender. A total of 11,796 households were surveyed, and 47,117 people were examined for TF and trichiasis signs. EU-level TF prevalence in children aged 1-9 years ranged from 0.3 (95% confidence interval [CI] 0.1-0.7) to 3.9% (95% CI 2.1-5.8). Among adults aged ≥15 years, trichiasis prevalence ranged from 0.03 (95% CI 0-0.09) to 0.65% (95% CI 0.25-1.31). Trachoma was not a public health problem in 8 of 14 EUs surveyed. In four EUs, trichiasis prevalence in adults aged ≥15 years was ≥0.2%, thus further trichiasis surgery interventions at public health-level are warranted to achieve elimination. These findings will facilitate planning for elimination of trachoma in Uganda.

1203

THE "LAST MILE" OF TRICHIASIS MANAGEMENT IN CAMEROON: ALIGNING IMPLEMENTATION AND EPIDEMIOLOGICAL DATA AT THE THRESHOLD OF TRACHOMA ELIMINATION

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In trachoma elimination programs, epidemiological surveys guide service provision and demonstrate evidence of progress towards elimination. In partnership with the Cameroon Ministry of Health, the USAID-funded Morbidity Management and Disability Prevention (MMDP) Project uses survey data to plan its provision of intensive trachomatous trichiasis (TT) management services in two regions of the country. As Cameroon is in the 'last mile' of TT elimination, TT management services are carefully coordinated with epidemiological surveys as districts approach the elimination threshold. Survey estimates of TT cases, however, sometimes do not match with the number of cases found at the community-level, as demonstrated in Touboro district in the North region of Cameroon. In 2016, trachoma impact survey data demonstrated a TT prevalence of 0.48% in adults >15 years, resulting in an age-and sex-standardized backlog of ~438 people. The MMDP Project then supported a TT campaign with robust, district-wide social mobilization, trained traditional healers to identify and refer TT cases, and organized community meetings in all 12 of the district's health areas. This effort identified 117 TT cases, of which 104 received surgery. In November 2016, the national program conducted a TT-only survey in Touboro, using WHO's Tropical Data platform. Given the prior investment in TT case identification, the program anticipated finding few unidentified TT cases. Preliminary results showed a TT prevalence of 0.77% in adults >15 years, suggesting a backlog of >1,000 people unknown to the health system. In response, additional social mobilization and outreach were conducted in 2017 in four of the district's 12 health areas, identifying only 55 TT cases (54 received surgery). Two campaigns are planned for 2018 to provide services to the district's remaining health areas, though they may yield a low number of cases. The Touboro experience raises the challenge of determining appropriate next steps for program implementers, as findings from implementation do not always align with the latest surveys that may produce wide confidence intervals and imprecise backlog figures.

1204

WATER SANITATION AND HYGIENE (WASH) IMPACT ON THE ELIMINATION OF TRACHOMA: POSITIVE CHANGES BETWEEN IMPACT AND SURVEILLANCE SURVEYS IN TANZANIA

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The World Health Organization (WHO) recommends a surveillance survey after two years after the last trachoma impact survey which showed a result of trachomatous inflamation - folicular (TF) prevalence < 5%. Both surveys evaluate the prevalence of TF, trachomatous trichiasis (TT) and water sanitation and hygiene (WASH) indicators. The Tanzania Neglected Tropical Disease Control Program (TZNTDCP) investigated the prevalence of TF and TT in people aged ≥1 in six districts. Impact surveys were completed in 2015 and surveillance surveys in 2018. Two-stage cluster survey sampling was used, and households were selected using systematic random sampling. In sampled households, all people aged ≥1 were examined for TF and TT using the WHO simplified trachoma grading system. Heads of households were interviewed on sanitation and hygiene facilities. Results from the 2015 impact survey showed Masasi TC had the highest proportion of people using an improved water source (51.4%) followed by Newala DC (37.8%), Mtwara DC (30.4%), Masasi DC (26.6%) and Tandahimba DC (25.5%). Only 8.8% of Nanyumbu DC population reported to be using protected/improved water sources. Approximately two years later (2018) the surveillance survey showed an increased proportion of the population using improved water sources compared to the impact surveys. Results included the following: Newala (44.24%), Mtwara DC (57.66%), Masasi DC (38.44%), Tandahimba (34.16%), and Nanyumbu (18.8%). There was no significant change in water source status in Masasi TC despite having a high proportion of population using improved water source two years earlier. This improvement in water source has significantly contributed to keeping the TF prevalence to less than 5% as evidenced by surveillance surveys. The results suggest that WASH activities are being undertaken in these formerly endemic districts which have attained the criteria for stopping mass drug administration to prevent trachoma.

1205

TRICHOMONAS VAGINALIS INFECTION IN SOUTHERN GHANA: AS SOCIATED RISK FACTORS, CLINICAL CHARACTERIZATION AND OUTCOMES

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Trichomonas vaginalis is the causative agent for the most prevalent non-viral sexually transmitted infection (STI) among women and the infection is associated with varied clinical presentations and outcomes. We conducted a cross-sectional study on 479 women visiting gynaecological and STI clinics in Southern Ghana between January and April 2016 to assess the risk factors associated with *T. vaginalis* and clinically characterize the infected groups. Vaginal samples were analysed using wet preparation microscopy and polymerase chain reaction (PCR). Of the 479 women, 63 (13.2%; 95% CI 9.5-17.6) and 78 (16.8%; 95% CI 12.2-21.1) had *T. vaginalis* infection based on wet preparation and PCR respectively, and diagnosis by PCR was significantly more sensitive ($P < 0.0001$). Univariate analyses found that *Trichomonas vaginalis* infection was significantly associated with vaginal itch (OR = 1.38, $P = 0.04$), oral sex (OR = 1.10, $P = 0.04$) and a prior history of stillbirth (OR = 3.62, $P = 0.04$). We used a model averaging approach to examine the relationship between *T. vaginalis* infection and predictor variables for clinical signs and risk factors. Vaginal itch was ranked highest in variable importance for clinical signs, occurring in 62% of the top eight models; discharge colour and discharge consistency were other variables also occurring in the top set of models. Scores were assigned based on the presenting clinical signs to categorise the pathogenicity levels of *T. vaginalis* into four groups; 6 (6.7%) corresponded to non-pathogenic group, 21 (23.6%) to the very low pathogenic, 39 (43.8%) to the low pathogenic and 12 (13.5%) to the high pathogenic group. A history of engaging in oral sex was ranked highest in variable importance for risk factors, occurring in 72% of the top-ranked models; cleaning material used after toileting and educational level also appeared in more than 50% of the top-ranked models. The varied clinically characterized groups suggest the existence of genetic polymorphism in the *T. vaginalis* infecting these individuals. Keywords: *Trichomonas vaginalis*, Women, Southern Ghana

1206

SUCCESSFUL INTRODUCTION OF AN ANTIMICROBIAL STEWARDSHIP PROGRAM TO IMPROVE ANTIBIOTIC USAGE IN A LARGE, URBAN HOSPITAL IN BLANTYRE, MALAWI

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Ceftriaxone is the first and last-line broad-spectrum antibiotic available for treatment of sepsis in Blantyre, Malawi, where there has been a recent, rapid spread of extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae isolated from blood cultures. Antibiotic misuse is a major driver of antimicrobial resistance (AMR), yet there are little data on stewardship from low-income countries and none from Malawi, where there are challenges both in achieving timely access to antimicrobials and in preventing emergence of AMR. This study describes the first introduction of an antimicrobial stewardship programme to the largest government hospital in Malawi, and its effect on ceftriaxone prescriptions. An antibiotic prescribing survey was carried out on the medical wards prior to the introduction of an antibiotic guideline. The guideline was written by a committee of locally experienced physicians and microbiologists, and took the form of a smart-phone application (MicroGuide). Weekly point prevalence surveys of ceftriaxone usage were carried out and presented to prescribers with reminders to review all antibiotic prescriptions at 48-hours. Antibiotic prescribing surveys were repeated 3 months and 2 years after guideline introduction. Prior to the guideline, 80.0% of all antibiotic prescriptions were for an intravenous 3rd generation cephalosporin, falling to 62.7% (percentage difference 17.3 [95% CI, 6.7-27.9, p<0.01]) at 3 months and to 55.5% (percentage difference 24.5 [95% CI, 2.7-15.6, p=0.01]) 2 years after guideline introduction. The proportion of 48-hour reviews increased from 62.2% to 93.3% (percentage difference 31.1 [95% CI 23.0-39.3, p<0.01]). Mortality did not change over the study period. We report the first successful introduction of a low-cost antimicrobial stewardship approach in Malawi. An antibiotic guideline, supported by weekly 'reminders' led to a reduction in ceftriaxone usage and an increase in antibiotic reviews. This study provides urgently needed feasibility data to support larger scale stewardship interventions in this and other resource-limited settings.

1207

INDUCING IRON DEFICIENCY IN TRANSGENIC SICKLE MICE: WILL LESS BE BETTER?

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Sickle cell disease (SCD) is caused by polymerization of deoxygenated hemoglobin-S (HbS) within the red blood cell (RBC). The kinetics of hemoglobin polymerization are dependent on the intra-erythrocytic concentration of HbS; specifically, the time from deoxygenation to polymerization is inversely related to HbS concentration[30]. Iron-deficient erythropoiesis (IDE) produces RBCs with decreased intracellular Hb concentration, which could reduce sickling and reduce the severity of SCD. In a pilot experiment, we determined the effect of dietary iron restriction on erythropoiesis in mice. Six-week-old female C57Bl/6J mice (Jackson Labs) were fed iron-restricted or control diet for 12 weeks. Complete blood count (CBC) results were obtained by Advia® 120 Hematology System, and erythropoietin was measured by ELISA. In iron-restricted mice, RBC parameters including MCV, MCH, MCHC, and reticulocyte hemoglobin content were all decreased over time. Total hemoglobin, reticulocyte percentage, RBC count, red blood cell distribution width (RDW), and platelet count remained constant. Erythropoietin levels were unchanged by iron restriction. Next, we will investigate the impact of dietary iron restriction on hematologic and pathologic endpoints in a transgenic mouse model of SCD. Townes SCD mice will be fed custom diets (AIN-93G, Research Diets, Inc.) containing either 3 ppm iron or 48 ppm iron and followed over a period of 6 months. We will monitor hemoglobin, hematocrit, MCHC, and sickling time longitudinally at 2, 4, and 6 months. At month 6 we will examine pathologic endpoints including markers of hemolysis and organ damage to brain, liver, and kidney. This study will provide a picture of the hematologic changes that occur in blood during the onset of iron deficiency anemia and evaluate the potential of iron deficiency to delay sickling and reduce the severity of organ damage in the context of SCD. This may inform a strategy of optimizing iron intake as a potential treatment for SCD.

1208

BENEFITS AND COSTS OF HYDROCELE SURGERY IN MALAWI

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Hydrocele, the accumulation of fluid around the testis or testes that expands the volume of the scrotal sac, is the most common manifestation

of lymphatic filariasis, endemic in 72 countries. Hydrocele afflicts about 25 million men in Africa, Asia, Oceania, and the Americas. It is frequently a painful condition that leads to reduced mobility, social withdrawal and exclusion, depression, and reduced work productivity. Some men with hydrocele work fewer days per week or hours per day, may be less productive at work, or simply cannot work. In poor, highly endemic regions, the total earnings loss due to hydrocele perpetuates low income and consumption for the whole community. Surgery provides an effective cure that is safe, inexpensive, and can be performed in field situations. This study measures the economic benefits of hydrocelectomy by estimating the gain in productivity over the patient's lifetime as a result of hydrocele surgery. This benefit-cost analysis is based on pre-operative interviews with 201 hydrocelectomy patients in Malawi who reported their work-time loss due to hydrocele. We calculate the present discounted real (inflation-adjusted) value of the lifetime earnings loss due to hydrocele. In post-operative surveys, respondents reported that surgery almost completely eliminated the disability and earnings loss imposed by hydrocele. We estimate average discounted present value of future earnings gain from hydrocelectomy for all respondents from the present to the end of their working lives to be $614, which is approximately 12 times the cost of the surgery and more than twice the Malawian income per capita in 2016.

1209

DEATHS OF EMERGING AND RE-EMERGING INFECTIOUS DISEASES OUTBREAKS, EPIDEMICS, AND PANDEMICS IN THE LAST 10 YEARS: A SYSTEMATIC REVIEW

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The emergence and re-emerge of infectious diseases in the last two decades has heightened concerns about the possibility of global outbreaks of disease and the ability of national and international health systems to respond and to overcome such infections successfully with the least possible number of victims. This study aimed to determine the death of the major emerging and re-emerging infectious disease outbreaks, epidemics, and pandemics during the last 10 years. We searched on 10 following electronic databases: PubMed, Scopus, GHL, VHL, POPLINE, ISI, Google Scholar, SIGLE, NYAM to assess the death of major emerging and re-emerging infectious disease outbreaks from 2006 to 2015. We used NIH tool to assess the risk of bias. The study protocol was registered on PROSPERO, number CRD42016038138. After searching in 10 databases, 8045 studies initially found, 167 were eligible for analysis. Overall case fatality rate is 0.052 (0.044 – 0.062) with 95% CI. The highest overall CFR was at South East Asia Region 13.2% and the lowest was in western Pacific Region 3.5%. The CFR peaked at 2006, 2010 and 2015 (14.9%, 8.6%, and 16%, respectively) with a declination of CFR in-between these years. *Burkholderia pseudomallei* and Nipah virus had the highest CFR (80%, 78.6%) while Enterovirus and Vibrio had the lowest CFR (0.0%, 0.4%). Significant risk of bias found with p-values of (0.0001) using Egger's regression intercept. South East Asia Region had the most death cases of infectious disease outbreaks. *Burkholderia pseudomallei* and Nipah virus were the causative pathogens that caused the highest number of cases of death.

1210

DECIPHERING ETIOLOGIES OF ACUTE FEBRILE ILLNESS IN WEST AFRICA: A JOINT MILITARY - CIVILIAN COLLABORATION

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The Joint West Africa Research Group (JWARG) is a global health program facilitating standardization of laboratory methods for disease surveillance in Nigeria, Ghana, and Liberia. Through the protocol "Severe Infectious Disease: Surveillance, Detection, Risks and Consequences in West Africa", it and the U.S. Military HIV Research Program, along with in-country partners, is enrolling military and civilian participants at the 68 Nigerian Army Reference Hospital. In addition to clinical diagnostics, JWARG labs use a MAGPIX multiplex platform and real-time RT-PCR to test for viral hemorrhagic fever and arthropod-borne viruses. To date 43 participants have enrolled. Participants with respiratory symptoms had sputum testing by GeneXpert MTB/RIF to detect of Mycobacterium tuberculosis and Rifampin resistance. HIV testing followed the national testing algorithm and malaria testing at T0 and T28 was performed using BinaxNOW and microscopy. For immunodetection, patient serum was screened with the Luminex MAGPIX platform. Magnetic beads were conjugated with antibodies against Ebola, Marburg, Lassa, and Crimean Congo hemorrhagic fever viruses, Rift Valley fever virus, alphavirus family, and flavivirus family. For molecular detection, real-time PCR utilized virus-specific primers and probes and synthetic RNA controls. Testing so far resulted in 15 HIV positive out of 32 tested. In addition, 11 participants were known to be HIV positive at enrollment. 5 tested malaria positive at T0. Only *Plasmodium faliciprum* was identified on microscopy. 17 participants that tested malaria negative by microscopy were taking antimalarial medications. 11 participants reported contact with animals along with tick, mite, and mosquito exposure. None reporting contact with mosquitoes were malaria positive. Of 11 enrollees tested for MTB, only 3 were positive and no Rifampicin resistance noted. No pathogens were detected by the MAGPIX assay or real-time RT-PCR. The JWARG is developing crucial front line diagnostic and surveillance laboratories in West Africa to discern the prevalence of infectious diseases as well as to improve the ability to respond to outbreaks.

1211

PROGRESS MADE TOWARD THE ELIMINATION OF TRACHOMA AS A PUBLIC HEALTH PROBLEM IN NIGER

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Trachoma is endemic in 35 out of 42 districts in Niger. The National Eye Health Program (PNSO) began baseline mapping in 1999, with prevalence of trachomatous follicular (TF) as high as 62.3% and 45.7% in health districts (HDs) in Zinder and Maradi region, respectively, and 28.6% in Dosso region. The PNSO plans to eliminate trachoma as a public health problem by 2020 through the WHO-recommended SAFE strategy, operational research, capacity building for PNSO staff, and monitoring and evaluation activities. This strategy involves impact assessments after one, three, five or seven years of mass drug administration (MDA) based on prevalence. Niger started MDA progressively with two HDs in 2002, to which 32 HDs were added between 2004 and 2009 and two additional HDs in 2015, reaching 100% geographic coverage of endemic HDs. Currently, no HD in Niger requires more than three years of treatment. The government reorganized the HDs in Niger from 42 to 72 in 2014. Currently, 10 HDs have a TF prevalence between 5% and 9%; 12 HDs have a prevalence between 10 and 29%; 6 urban and peri-urban HDs are waiting baseline assessment to determine whether they are endemic for trachoma; and 44 HDs are under surveillance (TF <5%). Of the 44 under surveillance, seven HDs have undergone their first surveillance survey, 21 are in progress and 16 are waiting to conduct surveillance surveys. Surveillance surveys conducted in 2017 in Ouallam and Banibangou HDs (Tillaberi region) found a TF prevalence between 5 and 9% and these HDs must conduct an additional year of MDA. The region borders Mali and has been insecure since 2012, experiencing repeated attacks since 2014. It is likely that cross-border migration and internal displacement of populations to refugee camps has contributed to the resurgence of trachoma, which proliferates in areas where water, sanitation and hygiene is poor. To maintain low levels of trachoma in the 16 HDs that have successfully completed their surveillance survey, the PNSO will continue to increase awareness through radio stations.

1212

INFANTILE ANEMIA AND ASSOCIATED MEDICAL CONDITIONS IN GUINEA-BISSAU

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Infantile anaemia is a severe public health problem in sub-Saharan Africa and is known to be associated with reduced cognitive development, immune function, and survival. The strength of association between anaemia and common tropical infections is however still poorly understood. Communities of the Bijagos Archipelago in Guinea-Bissau, have an average life expectancy of 55 years and an under 5 mortality of 223 per 1000 live births. Previous research in the archipelago has shown a high prevalence of trachoma, which shares risk factors with soil-transmitted helminths (STH) and *Plasmodium falciparum* infections. The first ever community-based cluster-randomized surveys were conducted in the archipelago to measure the prevalence of anaemia as well as infection with STH and *P. falciparum* during the rainy season between August to October 2017. The findings were analysed to identify the associations between these conditions and help leverage future protective interventions. Estimated hookworm prevalence was found to be 23.23% (95% CI 19-28%; N=419), and estimated malaria parasitaemia was 5.81% (95% CI 3.55-9.36; N=404). 65.35% of participants were estimated to be anaemic by WHO standards (95% CI 61-70%; N= 823). Analysis using a generalised linear model with forward univariate and multivariate logistic regressions showed significant associations between hookworm and *Pf* infection, acute and chronic malnutrition, age groups, sanitation practices and severity of anaemia. The results of these surveys map the links between infantile anaemia and common chronic tropical infections, nutritional status, and sanitation practices. The unique geography of the archipelago is ideally suited for a cluster randomised control trial of biomedical (including mass drug administration and nutrition supplementation), sociological and educational interventions to sustainably disrupt these links.

1213

INITIAL DEATH NOTIFICATION RESULTS FROM THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) SIERRA LEONE PILOT PHASE, OCTOBER 2017 TO FEBRUARY 2018

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The Child Health and Mortality Prevention Surveillance (CHAMPS) Network aims to integrate and share standardized data with key national and global stakeholders on the causes of death in children under-5 and stillbirths. As part of a phased implementation approach, the CHAMPS site in Sierra Leone piloted death notification in three Makeni City Hospitals, two health centers, and two peri-urban communities. From October 2017 to February 2018, the CHAMPS surveillance team received 250 unique death notifications from facility and community reporters. Two-hundred and forty-one of the notified deaths (96%) died at a health facility. Seventy-four of the deaths (30%) were reported as stillbirths. Of the under-5 deaths, 41 (23%) were classified as early neonatal [0 – 6 days], 16 (9%) as late neonatal [7 to 27 days], 62 (35%) as post neonatal deaths [28 days to 1 year], and 54 (31%) children 1-4 years. Eighty-one deaths (34%) were reported with residence in the rural areas of the chiefdom, while 139 (59%) were from the urban areas and 16 (7%) had an unknown residence. To inform planning for advanced diagnostics using minimal invasive tissue sampling (MITS), which must be performed within 24 hours after death, we analyzed time between death and notification. Notification time decreased from a median of 24 hours (interquartile range [IQR] 7 to 58 hours) in October 2017 to 12 hours (IQR 4 to 19 hours) in February 2018. In February 2018, 80% cases were reported within 24 hours of death compared to 48% in October 2017. Although not representative for the CHAMPS site, and mostly from health facilities, pilot data provide initial descriptive information on child and stillbirth mortality, and inform planning for advanced diagnostics starting later in 2018.

1214

PREDICTORS OF LINEAR GROWTH FALTERING IN CHILDREN WITH ENVIRONMENTAL ENTERIC DYSFUNCTION

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Stunting in children under five is enigmatic in the third world countries. A significant proportion (44%) of children have linear growth faltering in Pakistan. A fraction of these children suffer from Environmental Enteric Dysfunction, an acquired syndrome in children with multi-factorial disease processes of environmental insults, inadequate food and repeated infections. These children thought to manifest an underlying

inflammatory condition of the small intestine that translates into linear growth faltering. The objective of our study is to assess the association of co-morbids and biomarkers on growth outcome from birth to 18 month. In a rural setting of Pakistan, we prospectively enrolled a birth cohort and followed up to 18m for monthly anthropometric measurements. Blood and fecal samples were collected at 6 and 9 ms. In a linear Mixed Effects Model, the outcome of change in LAZ (length for age Z score) at 18m was independently analyzed for various exposures, stunted at birth, co-morbid and levels of systemic and gut inflammatory bio-markers. Principal Component Analysis (PCA) was applied to reduce the bio-markers (n=23) in to 5 main components. Mixed model used child as a random effect and PCAs of bio-markers, stunted at enrollment ,low birth weight, gestational age <37 wks, fever, diarrhea, cough and chest in drawing as fixed effect. Adjusted analysis showed stunted at enrollment (β: -1.06 ; 95%CI: -1.26,-0.86) , low birth weight (β: -0.36 ; 95% CI : -0.56,-0.16), gestational age <37wks (β: -0.45 ; 95% CI: -0.63,-0.26), PCA2 at 9 month (Ferritin, C-reactive protein, Insulin like Growth Factor and α1-Acid Glycoprotein) (β: -0.12; 95% CI : -0.21,-0.03) and PCA5 at 9 month (fecal Myeloperoxidase and Regenerating Protein-1β) (β: -0.09; 95% CI -0.18,0.00) were associated with significant change in LAZ from birth to 18m. Our data suggests that low birth weight or stunted at birth are major determinants of linear growth faltering independent of diarrhea and acute respiratory illnesses. Of 23 bio-markers, systemic inflammatory bio-markers were predominantly involved in linear change in LAZ, most likely through the pathway of gut inflammation.

1215

DESIGNING A NOVEL MULTIPLEX MULTI-ANALYTE DIAGNOSTIC PLATFORM (MAPDX) TO ADDRESS SEVERE FEBRILE ILLNESS

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Despite advancements in multiplex diagnostics in high-income countries, multiplex testing is rarely available in low-resource settings (LRS). A limited test menu hinders clinical decision-making and antimicrobial stewardship, leading to empiric treatment and suboptimal patient outcomes. This is particularly true for management of severe febrile illness without a known source (SFWS), since many pathogens present with similar symptomology. To revolutionize multiplex capabilites in LRS, Médecins Sans Frontières (MSF) partnered with FIND to examine the feasibility of developing a new diagnostic for near-patient testing of several pathogens and different analyte types called MAPDx. Together with the World Health Organization (WHO), a target product profile (TPP) was drafted to inform developers of minimal and optimal system requirements. Utilizing a Delphi process, 60 stakeholders (industry, experts, and end-users) reviewed the TPP through two survey rounds, an online public consultation, and an in-person consensus meeting. The final TPP contains 41 characteristics describing the envisioned MAPDx intended for management of patients with SFWS in a district hospital setting, but ideally for diagnosis of other diseases. The instrument must be capable of simultaneous detection of different analytes types (e.g., nucleic acids and serological markers) in either an integrated assay cartridge (optimal) or dedicated cartridges for each analyte type (minimal) to identify at least 6 targets, and ideally > 15 targets from a single specimen. The assay cartridge must contain all reagents, require little to no sample pre-processing, and meet 'semi-open' design specifications so that multiple assay developers can make a variety of tests for use on the platform. MAPDx must be robust to environmental conditions such as dust, temperature extremes and high humidity, while maintaining an affordable price (optimally < USD 5,000 / instrument and < USD 5 / cartridge). In summary, this rigorous TPP process resulted in an ambitious but attainable vision of MAPDx. If realized, this innovation would be a transformative advancement for diagnostics in LRS.

1216

BRAIN MRI IN IMPORTED MALARIA

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Previous studies have documented a spectrum of brain MRI abnormalities in patients with cerebral malaria, but less is known about findings in patients with non-cerebral malaria. Inpatients at the University Medical Centre Hamburg-Eppendorf (Germany) with microscopically confirmed *Plasmodium falciparum* malaria could participate in this study. Brain MRI was acquired preferentially in the first 24 hours after admission using a 3T Magnetom Skyra (Siemens, Erlangen, Germany). Brain MRI was independently assessed for structural lesions by two radiologists blinded to clinical information. A total of 17 patients underwent MRI and were included in the analysis. Half (n=8) were immigrants from malaria-endemic countries who were visiting friends and relatives (VFR), while the other half were non-immune travellers. Patients' age ranged between 20 and 64 years. Severity of malaria ranged from mild (with few clinical symptoms and less than 0.1% of infected erythrocytes) to severe (with neurological involvement and very high parasitaemia of 40% of infected erythrocytes). Five patients were classified as complicated malaria according to WHO criteria. Four patients had reduced vigilance and/or confusion (Glasgow Coma Scale scores between 12 and 14). No focal neurological deficits were noted in any of the included patients. Structural brain abnormalities detected included a hyperintense lesion of the splenium on T2-weighted imaging (n=3), accompanied by visible diffusion restriction in two cases. An isolated single brain microhemorrhage was detected in 3 patients. Small focal T2-hyperintense signal abnormalities of the deep and periventricular white matter were frequent findings, ranging from absent to diffuse. We did not observe significant correlations between parasitemia and the apparent diffusion coefficient in any of the locations (p>0,05). In conclusion, we describe a considerable frequency of T2-hyperintense splenial lesions in returning travellers with malaria, which appears to be independent of the degree of parasitemia. Future studies may reveal whether an immunologic or therapy-related etiologiy could explain this phenomenon.

1217

A CASE OF INITIALLY UNEXPLAINED CARDIOMYOPATHY AND THE ROLE AND OPERATING CHARACTERISTICS OF REMOTE TELECONSULTATION

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Dilated cardiomyopathies are prevalent worldwide; however, etiology often remains unexplained, making it difficult to diagnose without specialized care. Here, we describe a clinical presentation of endomyocardial fibrosis, a relatively common but underrecognized cause of cardiomyopathy in tropical regions, and describe the role of asynchronous remote teleconsultation in increasing capacity to interpret echocardiograms. We present a 50 year old Guyanese man who was admitted to an urban referral hospital with several weeks of progressive shortness of breath, cough, and several days of altered mental status. The clinical assessment was concerning for heart failure, so bedside echocardiography was performed, which revealed a dilated cardiomyopathy of unclear etiology. The case summary along with echocardiography clips were sent for review to a cardiology team via an asynchronous teleconsultation organization, The Addis Clinic, and the diagnosis of endomyocardial fibrosis was made. In this case, teleconsultation was key in determining diagnosis, prognosis,

and management plan. We review operational aspects of teleconsultation, including patient selection, development of referral templates, steps in allocation, and response to the referrer. We see that asynchronous teleconsultation is most effective for cases that are less acute, less common, and require more specialized knowledge to diagnose, such as this case. Inclusion of photos, or in this case, echocardiographic clips, can be an important component of the consultation. Turn-around-times of 24-48 hours can be achieved in an asynchronous telemedicine network, but requires very short turn-around-times of individual steps in the referral. In summary, we describe a case of endomyocardial fibrosis, a relatively common but less well recognized cause of cardiomyopathy. Through this case and through review of operational aspects of teleconsultations, we illustrate how telemedicine can play a role in diagnosis, prognosis, and management of tropical medicine conditions, particularly those that are chronic and require specialized skills to diagnose or manage.

1218

METABOLOMIC ASSOCIATIONS WITH STUNTING IN EARLY CHILDHOOD IN A BANGLADESHI COHORT

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Malnutrition is a challenging global health problem affecting almost one in four children under age 5. It significantly impacts survival in early childhood, leads to growth stunting, and has long-term negative consequences on neurocognitive development and performance. Data compiled from a comprehensive longitudinal study of a birth cohort of children in an urban slum in Bangladesh (PROVIDE) indicated that stunting was associated with environmental enteropathy, systemic inflammation and maternal malnutrition. However, there remains a gap in knowledge of biological factors driving stunting, and better understanding of metabolic associations with disease would aid in devising effective interventions to limit its effects. In a pilot study to investigate potential metabolic alterations associated with stunting, we conducted targeted metabolomics on plasma from 25 stunted children (HAZ<2) and 25 non-stunted controls in the PROVIDE cohort. Plasma was analyzed at 10 months and 1 year of age, and stunting assessed at age 2. The 34 metabolites, chosen on the basis of previous metabolic modeling studies, included amino acids, short chain fatty acids (SCFA) and tricarboxylic acid (TCA) cycle intermediates. Differences in metabolites between stunted and non-stunted groups were analyzed using a Mann-Whitney test and a critical value of 0.0121 calculated using the Benjamini-Yekutieli False Discovery Rate procedure. Stunted children were found to have significantly lower levels of tryptophan both at 10 months (p=0.0095) and at 1 year (p=0.0013). Tryptophan is a precursor for synthesis of a wide range of metabolites impacting growth, immune modulation and neurodevelopment, and our results suggest that limitation may contribute to the development of environmental enteropathy or stunting. Stunted children also had reduced levels of arginine at 10 months (p=0.0004) and threonine at 1 year (p= 0.0010). Efforts are ongoing to validate these findings in a larger cohort, and to explore their biological relevance. Further investigation of metabolite profiles could potentially identify early biomarkers for stunting or targets for intervention.

1219

CHAGAS DISEASE SEROLOGY AND CO-INFECTIONS WITH ARBOVIRUSES IN SOUTHERN COASTAL ECUADOR

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Chagas disease and arboviral infections are neglected vector borne diseases co-endemic throughout the Americas, but co-infection rates and immunological implications are largely unknown. This study examined the proportion of individuals positive for Chagas disease and co-infections with dengue (DENV), chikungunya (CHIKV), and Zika viruses (ZIKV) in an ongoing arbovirus surveillance study in Machala, Ecuador. We also evaluated five serological tests for Chagas disease. Samples were collected from individuals between January 2014 - December 2015. Serological positivity for Chagas disease was evaluated by three commercially available enzyme-linked immunosorbent assays (ELISAs), and a western blot assay (TESA blot). Chagas disease seropositivity was defined by agreement in at least two conventional tests. Additionally, the rapid test Chagas Detect Plus (CDP), was also evaluated. DENV infections were identified by NS1 rapid test, NS1 ELISA, IgM ELISA and RT-PCR, and acute CHIKV and ZIKV infections by RT-PCR. We found that 6/658 individuals (0.9%) were positive for *T. cruzi*. The average age for *T. cruzi* positive individuals was 63 years and 67% were female. We found one individual with *T. cruzi*/DENV co-infection and another with *T. cruzi*/CHIKV/DENV co-infection (0.1%), corresponding to an acute secondary and a primary DENV infection, respectively. Chagas serology test performance was as expected in all tests, however, we observed discrepancies by the Hemagen Chagas Kit and the rapid test CDP, with false positive percentages of 3.9% (1/26) and 15.4% (4/26), respectively. This preliminary finding suggests further test validation of the InBios rapid test CDP is needed in other endemic areas. This study revealed a low proportion of Chagas and DENV/CHIKV co-infections in Machala, and provides the first evidence of *T. cruzi*/arbovirus co-infections in the area to our knowledge. Future studies should rigorously investigate *T. cruzi*/arbovirus co-infection prevalence, and potential disease severity co-modulation effects to guide intervention strategies in high-risk populations.

1220

CULTURE ISOLATION AND GEOGRAPHICAL DISTRIBUTION OF *LEPTOSPIRA* SPP FROM PATIENTS WITH LEPTOSPIROSIS IN SRI LANKA

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The most recent culture isolation and serotyping of *Leptospira* spp. in Sri Lanka was reported in 1973, although the country is globally considered as a hot spot for leptospirosis. Changes in circulating *Leptospira* spp since 1960s is lacking. This knowledge gap hinders preventive and control measures. We carried out a multi centre hospital based study since June 2016 to fill this knowledge gap. Patients presenting with acute undifferentiated fever to six hospitals located in different geographical regions including wet and dry zones were included as the study population. Two to four drops of venous blood was collected on admission from each patient and bed side inoculation was done into EMJH semi solid media. Samples were subsequently incubated at 30^0C. Initial inspection of the samples was done after three weeks and on monthly basis thereafter. Positive samples were sub cultured in to liquid media and an aliquot was saved in -80^0C. Total of 728 cultures were collected over a period of 18 months and *Leptospira* spp were isolated from eighteen (2.5%) samples from five districts. Out of the 18 isolates 3 had a short incubation period of three weeks while others took longer to show positivity.To determine the species, a validated multi-gene targeted qPCR was done using four primer pairs. These were specifically designed to differentiate *L.interrogans,*

L.kirschneri, L.noguchi and L.borgpetersenii. All eighteen samples were subjected to this assay. Fourteen isolates were turned out to belong to *L.interrogans* and three were *L.borgpetersenii.* One isolate didn't belong to any of the species tested. In this study, we culture isolated *Leptospira* spp for the first time from the dry zone in Sri Lanka. *L.interrogans* was isolated from both dry and wet zones whereas *L.borgpetersenii* isolates show space and time clustering which were only found in the dry zone in three consecutive patients admitted to the ward within a month period. Our ongoing studies on whole genome sequencing of these isolates will enable to enhance the current knowledge on leptospirosis pathogenesis and will help in establishing possible reservoir hosts to facilitate preventive and control measures.

1221

DIARRHEA HOME MANAGEMENT AND DEHYDRATION IDENTIFICATION IN BAMAKO, MALI: PARENTS OF CHILDREN UNDER-5 LACK KNOWLEDGE AND CONFIDENCE

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Diarrhea is among the top five causes of child mortality globally. The Integrated Management of Childhood Illness (IMCI) guidelines for management of Acute Watery Diarrhea (AWD) contains evidence-based recommendations for improving outcome of diarrheal illnesses, but research demonstrates incomplete adherence in Mali. Greater understanding of parental knowledge and attitudes about management may lead to more effective messaging for adherence. Using stratified random sampling of children under-5, we interviewed 30 mothers in a censused population of Bamako, Mali. Stratification was by age, gender, and neighborhood. Interviews used diarrhea-related scenarios to assess syndrome recognition, perceived cause, and factors that influence care-seeking. Wilcoxon two-sample and Fischer's exact tests assessed associations between categorical variables from interviews and parental preference for seeking care from the informal or formal sector (formal sector includes professionals held to IMCI guidelines: physicians and pharmacists). Our data show Malian parents believe that 1) diarrhea is part of normal child development, 2) the need to increase fluids and continue feeding for children with AWD is under-appreciated, and 3) most parents cannot identify 2 signs of severe dehydration that should prompt care-seeking from the formal sector. Perceived quality of provider treatments, age >1 year, and low confidence in home management were associated with preference for the formal sector. Demographic factors and perceived syndrome cause were not significantly associated with sector preference. Strategic efforts to promote cultural shift in care-seeking behaviors for AWD could improve rates of morbidity and mortality attributed to childhood diarrhea in Mali. Parent education should focus on 1) the dangers of untreated diarrhea for children, 2) increased fluids and continued feeding at home, and 3) symptoms of dehydration that should prompt formal sector consultation. Collaboration with communities and providers from both sectors are desirable to develop consistent messaging about evidence-based home management and care-seeking.

1222

FROM INFECTION TO DISEASE IN LOIASIS: A SYSTEMATIC REVIEW OF CLINICO-EPIDEMIOLOGICAL CASE REPORTS

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Loiasis, a filarial infection caused by *Loa loa*, is a neglected tropical disease (NTD) estimated to affect over 10 million people in heavily forested countries of central Africa. Calabar swellings (transient migratory subcutaneous swellings) and eye worm passages are the hallmarks of the disease. It was previously considered benign and solely viewed as a public health impediment to mass ivermectin administration for onchocerciasis and lymphatic filariasis. Challenging this conception, a recently published cohort study conducted in Cameroon demonstrated increased mortality in those with high *Loa loa* microfilaraemia. Furthermore, case reports on atypical cardiac, respiratory, gastrointestinal, renal, and neurological clinical manifestations are becoming increasingly prevalent in the scientific literature. We undertook a systematic review of all published case reports containing individual-patient data on loiasis. The aim was to determine the full spectrum of clinical manifestations and assess whether these can be correlated to infection severity and/or markers of infection. We also wanted to quantify worm longevity from data on the interval between last exposure and presenting with signs of active infection. Information on patient demographics, co-infection with other parasites, typical and atypical symptoms, serum biochemistry, microfilaraemia, clinical imaging, pharmacological management and patient outcomes were collected. This study is the largest systematic review of individual-patient data on loiasis, tripling in number of patients, its closest comparable predecessor published in 2005. Results suggest that the atypical presentations cause significant patient morbidity and confirm previous findings that patient demographics play a role in determining clinical presentation and markers of infection. This project is part of a wider undertaking to develop a model for the global disease burden due to loiasis. It will serve as a foundation towards estimating the true morbidity associated with loiasis and will help to support the argument that loiasis should be included on the World Health Organization NTD list.

1223

NEUTRALIZING ANTIBODY AFTER 2 INTRADERMAL DOSES PRE-EXPOSURE PROPHYLAXIS OF PURIFIED VERO CELL RABIES VACCINE (PVRV) TO 12 TO 24 MONTH-OLD CHILDREN, CONCOMITANTLY WITH JAPANESE ENCEPHALITIS CHIMERIC VIRUS VACCINE (JE-CV)

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Rabies Pre-exposure prophylaxis is recommended for children living in endemic area. Our institute had studied the 2-site i.d. PrEP on day 0 and 21 with 0.1 ml of PVRV in adults and found this regimen effective, safe and cost saving compared to the usual 3-doses i.m. regimen. In Thailand it is mandatory to children aged 9-12 months to receive JE vaccine according to national immunization program and boost 1 year later. We studied the immunogenicity and safety of two-visit i.d. regimen compared with conventional i.m. regimen when co-administered with a live attenuated JE chimeric virus vaccine (JE-CV) in 9-18 months old children to improve further the acceptability of childhood rabies immunization. The trial was controlled, randomized 2:1, open-labelled. Forty-nine seronegative healthy Thai children were allocated to Group A (n=32) given 0.1 ml of PVRV i.d. at two sites on day 0, 28 and Group B (n=17) given 0.5 ml of PVRV i.m. on day 0, 7, 28. Both groups received JE-CV subcutaneously on day 0 (concomitantly with rabies vaccine day 0) and 365. Rabies virus

neutralizing antibody (RVNA) titers were measured on days 0, 42 and 365. JE neutralizing antibody titers were determined on day 0, 365 and 379 (14 days after JE-CV booster vaccination). All subjects had RVNA titers ≥ 0.5 IU/ml (protective level) on day 42 after PrEP vaccination, the geometric mean titers (GMTs) of RVNA titers in group A and B were 14.35 IU/mL (range 2.10 - 43.62 IU/mL) and 14.83 IU/mL (range 5.68 - 41.77 IU/mL), respectively. The GMTs of RVNA for group A and B on day 365 were 1.49 IU/mL (range 0.15-6.16 IU/mL) and 2.00 IU/mL (range 0.47-4.96 IU/mL), respectively. There was no statistically significant difference between GMTs of RVNA titers for group A and B on day 42 and 365. All subjects were seroprotected after receiving the JE-CV primary and booster dose one year later. Mild local adverse events e.g. injection site pain, pruritus, erythema and fever were observed. In conclusion, there was no evidence for any interference between rabies vaccine and JE vaccine, supportive that 2-site i.d. PrEP regimen on day 0 and 28 with 0.1 ml of PVRV and JE-CV are safe and immunogenic when co-administered.

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SPLENOMEGALY WITH A SIDE OF SINISTER PORTAL HYPERTENSION

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Introduction: Splenomegaly is increasingly identified among refugees resettling in the United States. In one study 15% of screened Congolese refugees had splenomegaly. In the absence of prior diagnosis the etiology can be difficult to determine. Case Presentation: A 33 year old male refugee from DRC with PMH significant for appendectomy presented to primary care clinic three weeks after arrival to the U.S. with episodic chest tightness, and fatigue for at least 6 months. On exam patient had normal vitals, splenomegaly, and caput medusa. Labs were pertinent for leukopenia (WBC 1.7 k/ul), thrombocytopenia (Pct 52 k/ul), normal Alt (14 u/l), Ast (25 u/l), elevated T. bilirubin (1.7 mg/dl) with (0.6 mg/dl) D. bilirubin. Patient had normal albumin (4.1 g/dl) and mildly elevated INR (1.3). Hepatitis serologies were negative for acute/chronic HBV or HCV and showed immunity to HAV. Abdominal US with Doppler showed enlarged spleen measuring (16.4 cm) and no evidence of portal or hepatic vein thrombosis. O&P testing x3 and schistosoma mansoni serology were negative. Patient was referred to Gastroenterology for further evaluation. EGD showed large >5 mm esophageal varices that were banded. Liver biopsy showed normal liver parenchyma with no evidence of cirrhosis and no infectious organisms. Hepatic pressure measurements showed Hepatic venous pressure gradient pressure of 1 mmhg which is not consistent with portal hypertension (PHTN). CT abdomen/pelvis was obtained, and is pending at this time. Other pending studies include serum sample that will be sent to CDC for repeat Schistosoma testing and malaria PCR testing. Discussion: Our patient had normal liver biopsy and intrahepatic pressures despite having clinical PHTN. Left sided PHTN is a rare clinical syndrome and accounts for less than 5% of all cases of PHTN. It occurs as a result of isolated splenic vein pathology. Patients with this syndrome have a normal liver biopsy and hepatic pressures. Differential diagnoses include spleen infiltrative disease (hyperreactive malaria hypersplenism syndrome, lymphoma, TB), pancreatic disease or schistosomiasis with falsely negative serology.

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PRIORITIZING PATHOGENS TO SUPPORT DIAGNOSTIC PRODUCT DEVELOPMENT FOR FEBRILE ILLNESS MANAGEMENT: A NOVEL YET PRAGMATIC APPROACH

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Febrile illness is a frequent reason for admission to hospital in low-resource settings (LRS). Management of patients with severe febrile illness without a known source (SFWS) is a challenge for clinicians and diagnostic developers due to numerous reasons. In LRS, diagnostic capacity, including at the hospital, is limited and empiric treatment is commonplace; resulting in suboptimal patient outcomes and antimicrobial resistance. MSF partnered with FIND to examine the feasibility of developing a novel diagnostic capable of simultaneous testing for multiple pathogens and analyte types, to improve management of SFWS. As a part of this endeavor, a list of priority pathogens was developed to allow more targeted treatment and advance surveillance capacity. Herein, we describe our pragmatic approach, combining peer-reviewed data overlaid with expert knowledge and experience to identify a list of priority pathogens. This two-pronged quantitative/ qualitative approach aimed to address the lack of comprehensive etiology data and allow for continuous improvement when new data become available. As per earlier prioritization by health institutions, e.g. WHO, an analytical hierarchy process (AHP) was applied to a pre-defined list of pathogens selected from Prasad *et al.*'s (2015). The AHP was made up of 5 categories (annual cases, severity, morbidity, patient impact, and public health impact). Weights were assigned to each category based on a pairwise comparison performed by nine experts (MSF, DNDi). This data derived list was subsequently subjected to the input of the wider scientific community via an online survey (n~100) to re-define and re-rank pathogens based on expert opinion with evidence-based justification. The resulting list of priority pathogens will inform a target product profile for a SFWS multi-analyte and multi-pathogen assay universal across geographical regions/ patient groups. This represents a unique and pragmatic approach to define global health priorities in diagnostics and an opportunity to catalyze research and development efforts in this important area of public health need.

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FREQUENCY AND ASSOCIATED FACTORS OF TYPHOID CARRIER ON DUODENAL FLUID CULTURE IN A TERTIARY CARE HOSPITAL, KARACHI

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Typhoid fever is a serious public health problem and remains a major cause of morbidity and mortality worldwide. Typhoid carriers serve as a reservoir in the ongoing transmission of typhoid fever. This study aims to determine the frequency of typhoid carriers in a typhoid endemic country by performing duodenal fluid culture in patients undergoing upper gastrointestinal (GI) endoscopy. We conducted a crossectional study at Aga

Khan University Hospital, Karachi from February-August 2017. Individuals of age ≥1 year who underwent upper GI endoscopy for any reason were included. Duodenal fluid culture was performed for identification of *Salmonella. typhi* and *paratyphi*. Statistical analysis carried out using Stata. For socioeconomic status factor analysis was performed. Overcrowding was assessed by estimating crowding index. GIS mapping was done for visualizing distribution of study participants. Out of 801 participants, 477 were enrolled. The mean±SD age (years) of the individuals was 42.4±15.5. Majority 287/477 (60.2%) were males. Two thirds (74.5%) of the Participants were from the province of Sindh (54% from Karachi), 13.6%, 3.4% and 3.4% from Baluchistan, Punjab and KPK respectively. 205/477(42.9%) reported use of unsafe water for drinking in their homes. 389/477 (81.5%) underwent upper gastrointestinal endoscopy for the diagnosis of gastrointestinal illness. 73(15.3%) reported past history of typhoid fever. Only 9/477 (1.9%) stated that they had received antibiotics in last two weeks of the procedure. Out of 477 cultures, 250 (52.4%) were positive for a bacterial isolate. None of the cultures showed growth of *salmonella. typhi* or *paratyphi* . However, common pathogens isolated were *Escherichia coli* 68 (27.2%) followed by *Pseudomonas species* 58(23.6%), *Klebsiella pneumonia* 35 (14%). We were not able to identify any typhoid carrier on duodenal fluid culture among subjects undergoing upper GI endoscopy. Carrier detection remains one of the best ways to stop transmission of salmonella. We propose molecular detection methods to determine the prevalence of carrier state after an episode of typhoid.

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UNUSUAL DEATHS IN TWO HEALTH DISTRICTS IN THE EAST REGION-CAMEROON: A CASE CONTROL STUDY, 2016

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In November 2016, the Districts of Abong-Mbang and Doumé Cameroon, reported 22 unexpected deaths clustered in 3 areas. All cases had a common history of sight loss, headaches and/or coma leading to death following the consumption of locally manufactured alcohol ("odontol"). A multidisciplinary team investigated the event and conducted a case control study. An active case finding took place in community and health facilities. A case was any person who suddenly died or complained of one or more of the following signs and symptoms: headaches, tiredness, nausea, drowsiness, coma, partial or total sight lost from the 8th -15th November 2016 in Abong-Mbang and Doumé Health Districts. Controls were persons in the neighborhood of cases who were not ill. The ratio of 1 case for 2 controls was adopted. Data was collected using a standardized questionnaire including sociodemographic characteristics, date of onset, sign and symptoms. In addition, blood, urine and post mortem gastric and hepatic samples were collected for analysis. Logistic regression was used for analyses. A total of 83 cases were identified, 53 women (64%); the median age was 40 years (range 20-60 years) with 27 deaths (CRF=33%). Symptoms included asthenia 74% (n=60), visual impairment 68% (n=56) and blindness 16% (n=13). Local alcohol was identified as a risk factor (OR=11, IC 95% 5.8-21). Thirty two blood and urines sample were collected and 2 post mortem gastric and hepatic samples taken. Methanol and formate were found in 33% (n=11) blood samples with concentrations up to 510mg/l (norm 5mg/l); and in 19% (n=6) urine samples up to 380 mg/l (norm <2mg/l). Toxicological analysis revealed 1.25 mg/l of methanol and 837 mg/l of formate in post mortem samples. The unusual deaths were due to methanol intoxication. Administrative measures must be taken for a better quality control of alcoholic beverages.

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CLINICAL RECOGNITION AND MANAGEMENT OF DRUG-DRUG INTERACTIONS IN A RURAL TANZANIAN HIV COHORT

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Increasing numbers of people living with HIV have access to antiretrovirals (ARV) resulting in significant prolongation of life with more age-related co-morbidities and consequently greater use of co-medications. Polypharmacy increases the risk of drug-drug interactions (DDI) which can lead to clinical toxicity or treatment failure. To date, potentially clinically relevant DDIs (PCR-DDI) have mainly been analyzed retrospectively using cohort data, therefore not providing information on clinical recognition of PCR-DDIs nor whether management was appropriate, e.g. switch to a non-interacting drug, dosage adjustment, or close clinical monitoring. This study aims to prospectively assess the recognition and management of PCR-DDIs by clinicians of the Chronic Diseases Clinic of Ifakara and patients included in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO), Ifakara, Tanzania. Information on demographics, clinical parameters, co-morbidities, prescription of ARVs and co-medications, treatment adherence, and laboratory monitoring are systematically captured during visits in an electronic patient chart. For the purpose of this study, an additional paper form was used to collect information on the recognition and management of PCR-DDIs. From 17 March to 13 July 2017, 378 paper forms were filled in for all consenting patients on ARVs and at least one co-medication. DDIs will be screened using the Liverpool Interaction tables (www.hiv-druginteraction.org) and categorized as deleterious (red), of potential clinical relevance (amber), or of weak clinical significance (yellow). The recognition and management of PCR-DDIs will be evaluated in a standard way using a defined algorithm including following criteria: DDI recognized by clinician (yes/no), planned appropriate management (yes/no), application of management plan (yes/no due to operational issues). This study will generate evidence for the first time on the recognition and management of PCR-DDIs in HIV-infected persons in rural Africa, leading to recommendations on how to minimize PCR-DDIs such as by consolidating education or improving operational issues.

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REDUCED INPATIENT MORTALITY IN UGANDAN CHILDREN WITH SEVERE ANEMIA DIAGNOSED AND MANAGED ACCORDING TO CLINICAL GUIDELINES COMPARED TO THOSE NOT MANAGED ACCORDING TO GUIDELINES

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In resource limited settings, adherence to clinical guidelines in the management of children presenting to hospitals with common clinical conditions like severe anemia (SA) varies markedly. However, there is limited data on how adherence to clinical guidelines affects inpatient outcomes. Inpatient records of 1,131 children aged 0 to 5 years assigned a clinical diagnosis of 'severe anemia (SA)' in two regional referral hospitals in Uganda, were retrospectively reviewed to determine whether or not they were diagnosed and managed according to clinical guidelines. The rate of in-patient deaths were then compared for SA patients diagnosed and managed according to clinical guidelines to those not diagnosed and managed according to these guidelines.Logistic regression analysis was

conducted to evaluate the relationship between clinical care factors and inpatient deaths amongst patients managed for SA. Children diagnosed and managed according to clinical guidelines were less likely to die during hospitalization compared to those managed inappropriately (10/376 (2.7%) vs. 67/755 (8.9%), $p< 0.001$). Factors associated with decreased risk of death while in hospital included having pre-transfusion hemoglobin done to confirm diagnosis (OR 0.5; [95% CI; 0.29-0.87]), a co-morbid diagnosis of severe malaria (OR 0.4; [95% CI;0.25-0.76]), and being reviewed after admission by a clinician (OR 0.3; [95% CI; 0.18-0.59]), while a co-morbid diagnosis of severe acute malnutrition was associated with increased risk of inpatient death (OR 4.2; [95% CI; 2.15-8.22]). Children with suspected SA who are managed according to clinical guidelines have lower in-hospital mortality than those not managed according to clinical guidelines. Efforts to reduce inpatient mortality in children with SA in resource-limited settings should focus on training, supervision, and support of health workers to adhere to clinical guidelines.

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RAPID ASSESSMENT OF LYMPHEDEMA BURDEN USING MOBILE PHONE BASED TEXT MESSAGES BY COMMUNITY HEALTH VOLUNTEERS

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Lymphatic filariasis (LF) and other neglected tropical diseases (NTDs) have adopted a community-directed treatment strategy such that volunteers, referred to as community health volunteers (CHVs) are responsible for conducting mass drug administration (MDA) campaigns within their own communities. CHVs have gained valuable knowledge on the health status and conditions of their community, including LF symptoms amongst NTDs. The proposed study will be conducted in Kilwa District Lindi Region, aiming at expanding the use of CHVs to play a key role in identifying and recording of household-level information on LF morbidity cases. Knowledge of the geographical distribution of lymphedema (LE) patients is essential to ensure that morbidity management programs are targeted at the populations that need it most. Data reporters who are CHVs will be trained on how to recognize lymphedema and morbidity management. Information collected will include the case's community of residence, individual ID, age, sex, condition (lymphoedema), data is sent to a server at the National NTD office. The results of the study will be out in by June 2018 and will be reported to the in-country project team. All relevant parties within the project country will have access to the results obtained. It is anticipated that results will increase awareness of the morbidity symptoms to the local health workers and communities which will facilitate developing of a comprehensive morbidity management plan.

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INVESTIGATING THE CONTRIBUTION OF SEVERE MALNUTRITION TO CHILD MORTALITY AMONG CHILDREN AGED 6-59 MONTHS THROUGH THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK

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Nutritional factors are estimated to contribute to 45% of all deaths in children under five years old. However, many children die without any record of their nutritional status. Our objectives were to identify severe malnutrition (SM) among deceased children aged 6-59 months in the Child Health and Mortality Prevention Surveillance (CHAMPS) Network. Cases were classified as severely malnourished based on (a) mid-upper arm circumference <115mm, (b) WHO Child Growth Standards weight-for-length or weight-for-age z-score < -3 , (c) wasting, marasmus, or kwashiorkor noted in clinical records or listed as a cause of or contributor to death. Anthropometric measurements were collected postmortem. An expert panel systematically assigned cause of death based on laboratory, clinical, and verbal autopsy findings. In total, 101 deceased children aged 6-59 months from four countries - Mozambique, South Africa, Kenya, and Mali—were assessed after 376 cases aged <6 months of age, and 11 cases with missing data or implausible measurements were excluded. Of the included cases, 52 (52%) were severely malnourished. Clinical records were available for 85 cases; of these, four (5%) had been diagnosed with marasmic kwashiorkor, two (2%) with kwashiorkor, and 15 (18%) with wasting. Seven (8%) cases had no clinical record of malnutrition but met our anthropometric criteria for SM. The proportion of cases with SM did not differ based on age group or sex; however, all kwashiorkor cases were ≥11 months old. At the time of analysis, expert cause of death panels had reviewed and assigned cause of death in 24 cases. Of these, 10 (42%) met our definition of SM, including six cases that had malnutrition listed as a cause of or a contributor to death. SM was common among deceased CHAMPS cases aged 6-59 months with available data. As data accrue, CHAMPS will examine relationships between nutritional deficits and cause-specific mortality, improve understanding of nutrition-disease dynamics, and help identify strategies to prevent nutrition-related deaths.

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A STUDY OF MODIFIABLE RISK FACTORS CONTRIBUTING TO NEONATAL DEATHS (NNDS) AT BWAILA MATERNITY HOSPITAL IN LILONGWE, MALAWI

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Malawi has had success in reducing child mortality, however neonatal deaths (NNDs) remain high at 29 per 1000 live births. The objective of our study was to determine the contribution of modifiable risk factors in NNDs. We randomly sampled 110 records from a total of 375 NNDs that occurred from July 2016 to June 2017 at Bwaila maternity Hospital in Lilongwe, Malawi, a facility with 12,000 deliveries annually. We identified socio-demographic characteristics, causes of death, potentially preventable issues, and explored associations between modifiable factors and the leading cause of NND in our sample, birth asphyxia (mean 52.30%, 95% CI 42.8%-61.8%), followed by prematurity (mean 29.4%, 95% CI 20.7%-38.0%). Mothers ranged in age from 14 to 45 years old (mean 24.8, SD 6.8). Close to half had attended at least 2 antenatal visits (44.6%). Estimated gestational age of the neonates ranged from 28 to 42 weeks (mean 36.1, SD 2.9; median 37). Median weight was 2600 grams (mean 2486.9, SD 769). There was at least one of 28 modifiable factors in 92 % of the NNDs. Suboptimal intrapartum care was the most common modifiable factor. When problems were identified, interventions commenced on average 1 hour 15 minutes after detection. Labor was monitored with partograms (n 94 , 85.4%), but only 16% were completed according to standard parameters, the remaining 79 had scant documentation of Fetal Heart Rate or maternal vital signs. We found a significant inverse association between death from birth asphyxia and a complete error-free Partogram (Fisher's exact 0.010, Corr -0.2790, P>0.01); birth asphyxia was significantly and positively associated with delayed referral (Fisher's exact 0.043; Corr 0.2021, P>0.05), delayed care, (Fisher's exact 0.006; Corr 0.2786, P>0.01), and prolonged labor (Fisher's

exact 0.006; Corr 0.2760, P>0.01). Despite extensive funding and training initiatives in Malawi, neonatal deaths have not dropped. We found that the majority of neonatal losses at a large maternity centre during 2016-2017 were to infants born at a healthy weight and at term, due to largely preventable health system factors during the intrapartum period.

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DETERMINANTS OF MATERNAL MORTALITY IN SEVERE POSTPARTUM HEMORRHAGE IN MZIMBA DISTRICT, MALAWI

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Severe Postpartum Haemorrhage is one of the main causes of maternal mortality in Malawi. This study aimed to determine the factors associated with mortality versus survival in severe PPH at both a district hospital and a tertiary care centre in Mzimba District, Northern Region, Malawi. Using a case control design we drew, via stratified random sampling, 45 cases and 45 controls of women identified as having had severe PPH (per WHO : blood loss of >1000 ml or bleeding with hypotension or blood transfusion). We used STATA 15 to calculate odds ratios (or), chisquare, and fisher's exact tests to measure the association between sociodemographic, biological, and health system factors to maternal mortality. We found very poor documentation of partograms, particularly for women transferred into the district/tertiary care facility due to complications during delivery. Our results suggests that living in a rural areas (OR 3.826, 95% CI 1.501-9.751, p=0.008), poor neonatal outcomes (OR 10.857, 95% CI 3.927-30.015, p=0.000), having to receive a c-section (OR 3.025, 95% CI 1.519-8.652, p=0.006), parity greater than 5 (OR 3.296, 95% CI 1.205-9.018, p=0.032), requiring referral prior to delivery (OR 2.985, 95% CI 1.266-7.039, p= 0.020), and pre-eclampsia (OR 6.143 , 95% CI 1.262-29.895, p=0.027) are associated with higher odds of death as compared to those that lived in urban areas, had good neonatal outcomes, parity of less than 5, not requiring referral, and those with no evidence of pre-eclampsia.Spontaneous vaginal delivery was significantly associated with survival from PPH (OR 0.297, 95% CI 0.124-0.712,p=0.011). Of note, the type of health provider in attendance was not significantly associated with either increased odds of death or survival. We found that a combination of factors were linked to increased odds of death from PPH, as such, solutions to reducing mortality due to PPH should be multimodal, among them, outreach to women with high parity to provide access to contraceptive options, access to timely transport for women with obstetric emergencies, improved provider documentation , and collaboration between the health sector and local communities.

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SERIOUS SIDE EFFECTS REGISTERED POST MECTIZAN® MASS DISTRIBUTION FROM 2006 TO 2017 IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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The Democratic Republic of the Congo is a country endemic to Neglected Tropical Diseases including onchocerciasis It has 23 operational coordinations, of which 15 with Onchocerciasis-Loase co-endemicity; In these areas of co-endemicity there is a high risk of serious side effects post Ivermectin (Mectizan®) mass distribution (MD). First serious side effects (SSE) cases with death were noted in 2003 in 2 co-endemic coordinations. The objective of the study is to evaluate the evolution of the occurrence of SSE after mass distribution by analyzing ivermectin distribution and SSE cases records since 2006. Results Since 2006 in the coordinations, it has been organized more than 10 cycles of annual MD concerning about 17 million in coordinations with co-endemic onchocerciasis and loasis. Between 2006 and 2010, the cases of SSE went from 289 to 33. And from 2011 to 2017 the cases went from 33 to 6. For deaths between 2006 and 2010 the number has increased from 9 to 0. And since 2010 no case of death has been recorded. The most frequent SSEs physical asthenia is found in 90% followed respectively 50%, 40%, 30%, 30% and 10% for intense pruritus, language disorders, sphincter relaxation, conjunctival hemorrhages and unconsciousness. The average time of occurrence of the SSEs is 2 days. Ringer's lactate and 10% glucose serum are administered to all patients in addition to the symptomatic treatment that is specific. Conclusion Since 2010 the number of SSE has greatly reduced and the cases of death are no longer observed. All cases observed in 2017 occurred among people who were taking Mectizan for their first time.

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ACCEPTANCE OF MINIMALLY INVASIVE AUTOPSIES - FROM THEORETICAL INSIGHTS TO PRACTICAL EXPERIENCE IN MOZAMBIQUE

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The Minimally invasive Autopsy (MIA) consists of post-mortem tissue sampling for histopathological and microbiological assessments for cause of death investigation. It may potentially increase consent to post-mortem investigation compared to the full autopsy. This study aimed to predict and monitor the acceptance of MIAs among relatives of deceased individuals and factors determining it. The study was conducted in two health facilities in Southern Mozambique. Theoretical acceptance data was abstracted from 46 interviews with relatives of recently deceased individuals (17 were children) before the implementation of MIAs. Interviews generated data on whether and why respondents would agree to a MIA on the deceased relative. Actual acceptance was derived from 120 MIA eligible cases during the first year of MIA implementation. Direct observations of 47 informed consent sessions complemented the information on barriers to acceptance. Data analysis comprised calculation of proportions of theoretical and actual acceptance of MIAs and a description of acceptance determinants. Theoretically, 80% of respondents would consent to a MIA on a deceased relative of any age, and 94% would consent to MIA if the deceased was a child. Barriers to theoretical acceptance were personal principles (emotional attachment, religious convictions, confidentiality and scepticism about the value of the MIA), as well as practical aspects of family decision-making, incompatible timings, and economic constraints. Actual consent rate during the MIA implementation reached 82%. Barriers to actual acceptance were related to health facility preparedness, logistics and decision-making power. High rates of acceptance were similar between the theoretical insights and practical experience of the MIA implementation in Mozambique. It is crucial to assess acceptance and its determinants in advance of the implementation of a complex intervention. Further dimensions of acceptability besides consent must be studied and caution is needed not to overlook health-system and logistical barriers beyond personal, relational, social and cultural barriers.

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QUALITY OF LIFE CHANGES AND POST-OPERATIVE FOLLOW-UP OF HYDROCELE SURGERY PATIENTS

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Over the past two years, the USAID-funded Morbidity Management and Disability Prevention (MMDP) Project supported Burkina Faso's National Lymphatic Filariasis Control Program in its efforts to provide quality surgical services to patients suffering from hydrocele. In partnership with the ministry of health, the MMDP Project provided capacity building and hydrocele surgery training activities to local surgeons in select project districts. The training was conducted using the FASTT (Filaricele Anatomical Surgical Task Trainer) training package, which consists of didactic presentations, videos on patient management emphasizing the resection technique, and supervised practice on a surgical simulator and then on patients. Trained surgeons operated on hydrocele patients during and following the training, and as a patient care and quality assurance measure, the project conducted patient follow-up in collaboration with the ministry of health. Sixty-one individuals who had hydrocele surgery as a part of the project and who were in the 6-12 month post-surgery period during the study were identified, and all were enrolled. All patients expressed that they were very satisfied and/or would recommend the surgery to others suffering from hydrocele. All patients, mostly farmers, also reported that they noticed improvements in their capacity to conduct daily tasks, and 59 of 61 (97%) patients reported improvements in their social interactions. One patient, who initially had a bilateral hydrocele, had a unilateral recurrence and was referred to the hospital for surgery. Patients were enthusiastic about their outcomes and participation in this study to be able to tell their stories. Patient outcome results reveal positive quality of life changes and successful hydrocele surgeries, which would support the training approach used and confirm the importance of patient follow-up to ensure the provision of quality surgical services.

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COMMUNITY-BASED APPROACH TO IDENTIFY HYDROCELE CASES: RESULTS OF A PILOT IN FIVE HEALTH DISTRICTS IN CAMEROON

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In 2017, the USAID-funded Morbidity Management and Disability Prevention (MMDP) project partnered with the national lymphatic filariasis (LF) control program in Cameroon to implement a pilot project focused on the management of LF-related morbidity in five health districts in the North and Far North regions. We selected these areas as they were believed to have the highest number of cases, estimated using data gathered through other neglected tropical disease interventions for LF. The pilot included the development and implementation of a community-based approach for the identification and referral of hydrocele cases, with the hopes of providing a more refined estimate and identification mechanism to ensure those with hydrocele could be identified in their community and referred for services. Using a community-based approach enabled potential cases to be screened closer to home, without needing to travel sometimes long distances to a health center. The project supported the development and distribution of 2,000 visual guides to assist with case identification. These were used by 1,932 community mobilizers for initial case identification. In addition, 88 health area nurses were trained in preliminary hydrocele diagnosis techniques and referral. The community mobilizers held community meetings to provide information on LF, screen and register suspected cases, and refer them to a health facility for clinical confirmation. Using this community-based approach, the community mobilizers identified 834 suspected hydrocele cases, out of which 300 (36%) were subsequently confirmed by the trained nurses at the health facilities. Of these, for logistical and planning purposes, a total of 95 cases were referred to a district hospital where surgeons trained under the MMDP Project confirmed the diagnosis in all 95 cases and operated on the patients. The remaining 205 registered cases are awaiting surgery. In view of these results, the community-based approach to identify hydrocele cases using community mobilizers and nurses appears to be feasible as a method to detect and refer hydrocele cases for management.

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ALTERED LEVELS OF ANGIOGENIC AND INFLAMMATORY MEDIATORS DURING PREGNANCY ARE ASSOCIATED WITH PRETERM BIRTH IN MALAWIAN WOMEN

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Preterm birth (delivery <37 weeks gestation; PTB) is the leading global cause of death in children under five. Despite the increasing global burden of PTB, we know little about its underlying pathobiology. Tight regulation of angiogenic and inflammatory mediators are essential for healthy pregnancies. We hypothesize that disruption of pathways controlling placental vascular development and maternal inflammation precedes and contributes to PTB. Here, we validate and extend our previous findings in Tanzanian women to an external cohort of Malawian women. Samples were collected as part of a larger randomized control, multi-site trial of malaria prevention strategies in pregnancy. Longitudinal plasma samples were analyzed at three windows across gestation (16-24, 28-33, and 34-37 weeks; ultrasound dated) by multiplex luminex assays for a panel of angiogenic and inflammatory mediators previously implicated in PTB. Of the total cohort (n=1506), 304 (20.2%) delivered preterm. Multivariate log-binomial regression (adjusted for confounding factors) showed women with plasma levels of sTNFR2 ($p<0.005$), CHI3L1 ($p<0.05$), and IL18BP ($p<0.05$) in the highest quartile at enrolment (16-24 weeks) had an increased relative risk of PTB, compared to women with plasma levels in the lowest quartile. Furthermore, longitudinal analysis of biomarkers across pregnancy using linear mixed effects modeling showed that preterm pregnancies had altered kinetics over pregnancy for Ang2, sEng, sFlt1, CRP, CHI3L1 (all $p<0.05$), IL18BP ($p<0.01$), PlGF, and sTNFRII (both $p<0.001$) compared to term pregnancies. Malawi has the highest burden of PTB worldwide, with close to 1 in 5 babies born preterm each year. Understanding the pathobiology of PTB is critical to developing effective interventions. Our data indicate a role for altered longitudinal dynamics of angiogenic and inflammatory pathways in the etiology of PTB, and confirm previous findings that early biomarker profiles may identify pregnancies at risk of PTB.

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MASS SCREENING CAMPAIGNS TO INCREASE AWARENESS OF CERVICAL CANCER TREATMENT AND PREVENTION IN EQUATORIAL GUINEA

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Cervical cancer is the fourth most frequent cancer in women and represents 7.9% of all female cancers. In 2015 it was estimated that approximately 90% of all deaths due to cervical cancer occurred in low and middle-income countries. The risk of cervical cancer can be reduced with current vaccines that protect against cancer-causing types of the Human Papilloma Virus (HPV), which in conjunction with early diagnosis, effective screening, and treatment may further reduce mortality and increase prevention. Screening aims to detect and treat cancers at early stages. Cervical cancer awareness, access to screening and early diagnosis are very limited in Equatorial Guinea, therefore posing a barrier to effective prevention. In October 2016 Medical Care Development International (MCDI), funded by Noble Energy Inc, launched a Cervical Cancer Screening and Treatment (CCST) project. As part of this project Cervical Cancer Screening Corners (CCSCs) were established in two regional hospitals in the cities of Malabo and Bata as well as mass screening campaigns. The screening campaigns, which target women aged 20-26 years of age, aim to detect, treat and refer cases, as defined by lesions in the cervix, for specialized follow-up. Screening methods include visual inspection, Acetic Acid (VIA), and cold coagulation therapy. Other campaign activities include the sensitization of women to attend CCSCs, through Information, Education and Communication (IEC), and capacity development of government officials to implement prevention programs. Since the inception of the CCST project, about 2,500 women have been screened on the continental region of the country against almost 1,500 on Bioko Island. Moreover, for each campaign that is organized, community members receive training on IEC to support sensitization amongst the population of the areas they live in; and over 60 health workers across the country have attended short courses to implement screenings and cold coagulation treatments as part of their training for cervical cancer management.

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INCIDENCE AND RISK FACTORS FOR CERVICAL CANCER AND PRE-CANCEROUS LESIONS IN EQUATORIAL GUINEA

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According to the World Health Organization, 34 out of every 100,000 women are diagnosed with cervical cancer and 23 out of 100,000 cases are fatal. Routine screen and treat programs can prevent 80% of cervical cancers as it allows for early detection of cervical cancer and pre-cancerous lesions and at earlier stages there are more effective treatment opportunities. The Human Papilloma Virus (HPV) has been associated with the risk of cervical cancer and the HPV vaccine, along with screen and treat programs, has proven to reduce incidence of cases. In Equatorial Guinea the Cervical Cancer Screen and Treat (CCST) project, funded by Nobel Energy Inc., aims to provide early routine screening, early diagnosis, and effective treatment of cervical cancer and pre-cancerous lesions to women in areas where diagnosis and treatment is limited. The project was launched in 2016 in the Regional Hospitals in Malabo (Insular Region), and Bata (Continental Region) and has screened about 4,000 women. Routine data from the project provides insight into the background and potential risk factors, as well as symptoms at the time of screening. Descriptive data analysis and linear regression models will provide an overview of the associations between potential risk factors and symptoms and the outcome of the screening test. The data analysis will also allow for insight into the profile of women who participate in the cervical screening program. This study will be valuable to support future Information, Education and Communication (IEC) campaigns, targeted mass screening campaigns and treatment programs which include training of health care workers.

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ENVIRONMENTAL SUITABILITY FOR LYMPHATIC FILARIASIS IN NIGERIA

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Lymphatic filariasis (LF) is a mosquito-borne parasitic disease and a major cause of disability worldwide. It is one of the neglected tropical diseases identified by the World Health Organization for elimination as a public health problem by 2020. Maps displaying disease distribution are helpful tools to identify high risk areas and target scarce control resources. Using site level occurrence data consisting of 1378 data points collected during extensive mapping surveys by the Federal Ministry of Health, Nigeria, we implemented an ensemble of machine learning modelling algorithms (generalised boosted models and random forest). We mapped the ecological niche of LF at a spatial resolution of 1km^2. And by overlaying gridded estimates of population density, we estimated the human population living in LF risk areas on a 100m x 100m scale. Our maps demonstrate that there is a heterogeneous distribution of LF risk areas across Nigeria, with large portions of northern Nigeria having more environmentally suitable conditions for the occurrence of LF. Here we estimated that approximately 103 million individuals live in areas at risk of LF transmission. Machine learning and ensemble modelling are powerful tools to map disease risk, and are known to yield more accurate predictive models while decreasing the uncertainty of single models. The resulting map provides a geographical framework to target control efforts and assess its potential impacts.

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MODELLING LYMPHATIC FILARIASIS TRANSMISSION IN AMERICAN SAMOA LONG-TERM POPULATION DYNAMICS AND SPATIALLY HETEROGENEOUS RISKS

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Although considerable success has been achieved to eliminate lymphatic filariasis since 1999, there are significant knowledge gaps about LF transmission dynamics, particularly at the end stages of elimination programs when prevalence has reached very low levels. Current mathematical and computational models of lymphatic filariasis do not capture small-scale spatial heterogeneity of risk factors, restricting our understanding of LF dynamics at low prevalence. A new agent-based model was developed to fill the knowledge gaps about lymphatic filariasis transmission and elimination in American Samoa, but also to be readily adaptable to model other infectious diseases. The modelling framework was characterized by statistically realistic population, high-resolution geographic locations and long-term projections of the population dynamics. The population in the model is characterized by major dynamic processes including population renewal (birth/death), couple formation and separation, and migration, with daily commuting patterns predicted with the radiation model. The risk of infection was estimated based on the mosquito abundance (mosquito bites) and the prevalence of infectious individuals in the neighbourhood areas. The agent-based model in the study provides not only valuable insights into further LF dynamics based on the transmission assessment survey, but also a highly flexible modelling framework for a variety of infectious diseases, especially for diseases with long incubation and long infectious periods, or diseases with highly focal transmission hotspots.

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EVALUATING THE TRANSMISSION ASSESSMENT SURVEY CRITERIA FOR ELIMINATING LYMPHATIC FILARIASIS USING MATHEMATICAL MODELLING

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Regions endemic for lymphatic filariasis (LF), a debilitating vector borne parasitic disease, have made substantial progress toward the global goal of interrupting transmission by 2020. Despite this achievement, there remains significant uncertainty surrounding the transmission thresholds which serve as program targets for stopping mass drug administration (MDA). The proposed target to be eligible for implementing Transmission Assessment Surveys (TAS) and potentially stopping MDA is 1% microfilariae (mf) or 2% antigenaemia prevalence in a population. Here, we employ mathematical modelling to assess whether meeting the TAS criteria implies that transmission has been interrupted, and, if not, what the risk of recrudescence after stopping MDA might be. Surveillance data from six LF endemic sites chosen for their geographic distribution and range of endemic prevalences were included in this study. Using a Bayesian Melding data assimilation framework, the models were fitted to site-specific baseline mf data in order to capture local transmission dynamics, and the calibrated models were used to simulate the impact of observed MDA on mf prevalence. The estimated timelines to achieve 1% mf prevalence in the sites ranged from 5-13 years, and the probability of interrupting transmission after stopping MDA (i.e. the probability of sustaining mf prevalence < 1%) ranged from 30-84%. Sites with lower baseline prevalence exhibited more favorable elimination outcomes compared to sites with higher endemicity. We also modeled the effect that different intervention strategies have on recrudescence risk after meeting the 1% mf threshold and found that the lowest probabilities of recrudescence were achieved when MDA was supplemented with vector control (VC). The optimal scenario was to include VC both during and after stopping MDA (1-35% probability of recrudescence) compared to MDA alone (16-61% probability of recrudescence). These results question the current criteria used for making stopping decisions and highlight the need to validate transmission thresholds with field studies to avoid the consequences of prematurely stopping MDA.

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SOIL-TRANSMITTED HELMINTH AND *SCHISTOSOMA MANSONI* INFECTIONS DO NOT EVOKE CROSS-REACTIVE ANTIBODIES TO THE *ONCHOCERCA VOLVULUS* PEPTIDE EPITOPES OVMP-1 AND OVMP-23

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The Ov16 IgG4 ELISA or lateral flow test is considered the reference method for *Onchocerca volvulus* epidemiological mapping. Recently, two linear epitopes encoded in OvMP-1 and OvMP23 peptides were introduced as serological markers, but the observed antibody cross-reactivity in samples originating from *O. volvulus* non-endemic areas required further investigation. In this study, we evaluated these markers in an *Onchocerca* meso-endemic setting in Jimma, Ethiopia. For all individuals (n = 303), the infection status with soil-transmitted helminths and *Schistosoma mansoni* was known. In total, 11 (3.6%) individuals were positive for anti-Ov16 IgG4 antibodies, while 34 (11.2%) and 15 (5.0%) individuals had antibody responses to OvMP-1 and OvMP-23, respectively. Out of the 34 OvMP-1 positive samples, 33 were negative on the Ov16 IgG4 ELISA. Similarly, out of the 15 OvMP-23 positive samples, 14 scored negative on this reference method. Upon further analysis of the "false positive samples" for infection with non-*Onchocerca* helminth infections, they were not significantly correlated to soil-transmitted helminth ($p > 0.05$) or *S. mansoni* infections ($p > 0.05$). This suggests that these individuals are either infected with *O. volvulus* and were not picked up by the Ov16 IgG4 ELISA, or that they have an immune response against other agents that cause cross-reactivity. For OvMP-1, there appeared to be a significant trend towards increased seroprevalence in older individuals. The results of this work demonstrate that both OvMP-1 and OvMP23 do not cross-react with soil-transmitted helminth or *S. mansoni* infections. The discordancy with the Ov16 test requires further investigation in *Onchocerca* endemic populations.

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ONCHOCERCA VOLVULUS TRANSMISSION IN THE MBAM VALLEY (CENTRE REGION, CAMEROON) FOLLOWING 18 YEARS OF ANNUAL CDTI

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The onchocerciasis focus surrounding the lower Mbam and Sanaga rivers was historically the largest in southern Cameroon. Control through annual community directed treatment with ivermectin (CDTI) commenced in 1998 and takes place around July each year. However, recent surveys indicate that *Onchocerca volvulus* transmission is ongoing and the disease remains endemic. This study aimed to evaluate blackfly biting and parasite transmission rates after 18 years of annual CDTI. Anthropophilic blackflies were collected using human bait along the lower Mbam River near Bafia between July 2016 and June 2017. Collections were made for three consecutive days each month at four sites (two riverside; two ≥8km from the riverside). Specimens were either dissected to determine parity rates and intensity of *O. volvulus* infection or were preserved for pool screening. In total, 93,563 adult female *Simulium damnosum* s.l. were collected. Biting peaked between September and March and annual biting rates of ≥606,370 bites/person/year were recorded at riverside sites. Provisional results show that parasite transmission peaked between January and May and coincided with high blackfly parity rates. Transmission occurred almost exclusively at riverside sites, where annual transmission potentials of ≥4,488 were recorded. Cytotaxonomy of *S. damnosum* complex larvae indicated that a new variant of *Simulium squamosum* E was the predominant cytoform breeding in the area, making this the first time *S. squamosum* E has been found east of Lake Volta in Ghana. Current and pre-CDTI data suggest that *O. volvulus* transmission is sustained by high blackfly biting rates along the lower Mbam River, where it occurs mainly during the late dry and early rainy seasons. Similar seasonal patterns of transmission have been reported elsewhere in Africa where *S. squamosum* is the vector. The current timing of CDTI which takes place around July each year may not be ideal, and communities could benefit from earlier treatment, before blackfly parity rates and *O. volvulus* transmission increase.

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PREDICTED DISTRIBUTION AND BURDEN OF PODOCONIOSIS IN CAMEROON

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Understanding the number of cases of podoconiosis, its geographical distribution and the population at risk are crucial to estimating the burden of this disease in endemic countries. We assessed each of these using nationwide data on podoconiosis prevalence in Cameroon. We analysed data arising from two cross-sectional surveys in Cameroon. The dataset was combined with a suite of environmental and climate data and analysed within a robust statistical framework, which included machine learning-based approaches and geostatistical modelling. The environmental limits, spatial variation of predicted prevalence, population at risk and number of cases of podoconiosis were each estimated. A total of 214,729 records of individuals screened for podoconiosis were gathered from 748 communities in all 10 regions of Cameroon. Of these screened individuals, 882 (0.41%; 95%CI 0.38-0.44) were living with podoconiosis. High environmental suitability for podoconiosis was predicted in three regions of Cameroon (Adamawa, North West and North). The national population living in areas environmentally suitable for podoconiosis was estimated at 5.2 (95% Confidence Interval [CI]: 4.7-5.8) million, which corresponds to 22.3% of Cameroon's population in 2015. Countrywide, in 2015, the number of adults estimated to be suffering from podoconiosis was 41,556 (95% CI, 1,170- 240,993). Four regions (Central, Littoral, North and North West) contributed 61.2% of the cases. In Cameroon, podoconiosis is more widely distributed than was initially expected. The number of cases and the population at risk may pose a challenge to the national health system. Strengthening of the health system for early diagnosis of podoconiosis, morbidity management and follow up of cases is of utmost necessity. Promotion of footwear use and regular foot hygiene should be at the forefront of any intervention plan. Elimination of podoconiosis requires firm political will, policy formulation, operational and financial commitment by the Cameroon Ministry of Health and donors.

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DELINEATION OF ONCHOCERCIASIS TRANSMISSION ZONES IN ETHIOPIA; A CRITICAL ELEMENT FOR THE ELIMINATION OF ONCHOCERCIASIS

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Onchocerciasis, a parasitic disease caused by a nematode worm *Onchocerca volvulus,* is transmitted through the bites of *Simulium* vector flies that breed in fast flowing rivers and streams. In its advanced form, onchocerciasis can cause visual impairment, blindness and severe skin diseases. Recently onchocerciasis intervention efforts have reoriented from control to transmission elimination. With this new goal in mind, new mapping approaches are needed to delineate transmission zones. These transmission zones are targeted for mass drug administration (MDA) with ivermectin and/or vector control programs. A transmission zone (or 'focus') is defined as an ecological or geographical area where *Onchocerca volvulus* transmission occurs by locally breeding *Simulium* vectors. Under the control paradigm of the African Onchocerciasis Control Program endemic countries were not required to delineate transmission zones. Instead Rapid Epidemiological Mapping of Onchocerciasis (REMO) used palpation examinations to determine subdermal onchocercomata (nodule) rates to define intervention areas. In the elimination era, more sensitive serological tools (Ov-16 ELISA or RDT) are being applied to define transmission zones. We describe our experience in Ethiopia using OV-16 ELISA to delineate the borders of two transmission zones (Metema and Metekel) separated by a "buffer" zone of sufficient size to allow halting of MDA in Metema. The threshold for delineating this buffer zone was the finding of <2% Ov-16 in the adult (>20 years of age) residents of buffer area of >20km separating Metema and Metekel transmission zones. It is sufficient to prevent infected *Simulium* vectors from crossing one transmission zone (Metekel) to the another (Metema). In 2017 the Metema transmission zone met the WHO criteria for stopping MDA based on OV16 ELISA rates in 7,572 young children of <0.01% and PCR results from 28,465 *Simulium* flies of 0.14 flies with L3 per 2000 (95% CI, 0.01-0.39). MDA continued in Metekel due to ongoing transmission and very high OV16 rates (25.6%) in adults.

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DRAMATIC AGE DISTRIBUTION SHIFT OF PEOPLE WITH EPILEPSY IN A HIGHLY ENDEMIC ONCHOCERCIASIS FOCUS OF CAMEROON WHICH HAD BENEFITED FROM MASS IVERMECTIN TREATMENT OVER 19 YEARS

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Surveys conducted in 1991-93 in the Mbam valley (Cameroon) showed that onchocerciasis was highly endemic, with community microfilarial loads (CMFL) >100 microfilariae/snip in some villages. In 1991, a census of suspected cases of epilepsy (SCE) using a questionnaire applied to individuals identified by key informants identified 746 SCE revealing prevalences up to 13.6%. From 1998, annual mass ivermectin treatment (MIT) was implemented to control onchocerciasis. In 2017, a door-to-door household survey was conducted in 3 villages visited in 1991, using a 5-item epilepsy screening questionnaire. A total of 2286 individuals living in 324 households were screened (582 in Bayomen, 553 in Ngongol and 1151 in Nyamongo) and 112 SCE were identified (4.9%). Neurologists

confirmed the diagnosis of epilepsy in 81 (3.5%), rejected the diagnosis in 11; 20 SCE could not be examined. Between 1991 and 2017, the prevalence of SCE decreased from 13.6% to 2.5% in Bayomen (p=0.001), from 8.7% to 6.6% in Ngongol (p=0.2), and from 6.4% to 5.4% in Nyamongo (p=0.28). The median age of SCE shifted from 20 (IQR 12-23) to 29 years (IQR 18-33; p=0.018) in Bayomen, from 16 (IQR 12-21) to 26 years (IQR 21-39; p<0.001) in Ngongol and from 16 (IQR 13-19) to 24 years (IQR 19-32; p<0.001) in Nyamongo. The proportions of SCE aged ≤15, 15-30 and >30 years were 41.7, 51.2 and 7.1% in 1991 and 11.6, 58.9, and 29.5% in 2017, respectively. This age shift is probably due to decrease in the incidence of onchocerciasis associated epilepsy resulting from the dramatic reduction in the CMFL following 19 years of MIT. The fact that the prevalences recorded in 1991 and 2017 did not differ significantly in Ngongol and Nyamongo might be due (i) to a lower MIT coverage (the coverage was probably higher in Bayomen, where the village chief was also the drug distributor), (ii) to a better access to anti-epileptic drugs and thus a longer lifespan of SCE cases after 1991, or (iii) to an under-estimation of the SCE prevalences in 1991.

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IMPACT OF FORTY YEARS INTERVENTION TO CONTROL ONCHOCERCIASIS IN COTE D'IVOIRE

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To better coordinate and plan the elimination of onchocerciasis in Cote d'Ivoire we reviewed the impact of past interventions performed between 1974 and 2013. Onchocerciasis control started with aerial insecticide spraying and continued with community directed treatment with ivermectin (CDTi) from 1992 to present. We analysed microfilaria (MF, skin snip) prevalence and community MF load (CMFL) data reported from 39 health districts during two major epidemiological assessment periods. Data from 1975 through 1991 provided information on vector control impact, and data from 1992 through 2013 provided information on CDTi impact. Weekly aerial insecticide spraying in 8 endemic districts between 1974 and 1991 reduced the overall MF prevalence by 54% from 43.1% to 17.2%. The CMFL also decreased in 7 out of 8 surveyed communities by 69% from 9.85 MF/snip to 1.1 MF/snip. The CDTi coverage target for control was reached in most endemic districts, and some areas achieved 80% coverage. Two sets of surveys were conducted to assess the impact of CDTi. The first set of repeat surveys (1992-2002) showed a significant drop in overall MF prevalence by 73.9% (from 29.8% to 7.78 %). The second follow up evaluation (2007-2013) showed a significant reduction in overall MF rates by 71.0% from baseline (from 29.8% to 8.65%). The first and second follow up surveys also documented strong reductions in CMFL compared to baseline (from 7.67 MF/ snip to 0.61 MF/ snip and from 7.67 MF/snip to 0.70 MF/ snip, respectively). Between the first and the second set of follow up surveys the MF rates and CMFL were fairly stable at low levels without much further reduction. Currently, onchocerciasis and lymphatic filariasis (LF) are co-endemic in 46 of the 81 health districts, and 12 districts are endemic for onchocerciasis only. In conclusion, data collected over 40 years showed significant success of interventions conducted by the national onchocerciasis control programme during challenging times. The Health Ministry has now integrated efforts to control and eliminate neglected tropical diseases, and based on the most recent data it is working hard toward onchocerciasis elimination.

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LINEAR EPITOPES IN *ONCHOCERCA VOLVULUS* VACCINE CANDIDATE PROTEINS, EXCRETORY-SECRETORY PROTEINS, AND SERODIAGNOSTIC CANDIDATES

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There is a growing need for diagnostic tools to monitor infection with the helminth parasite *Onchocerca volvulus*. In previous studies, a proteome-wide screen was conducted to identify linear epitopes in this parasite's proteome, resulting in the discovery of 1110 antigenic peptide fragments. Here we investigated whether such peptides were found in proteins that were already known as vaccine candidates, excretome/secretome proteins, and/or serodiagnostic protein candidates for *O. volvulus*. This approach led to the identification of 35 immunoreactive peptides in 23 proteins. We developed peptide ELISA's and evaluated their diagnostic sensitivity and specificity. For 8 of those peptides, epitope mapping was performed and peptides were constructed that contained only the minimal epitope, flanked by a linker. The following plasma samples were available for ELISA peptide testing: 101 samples from onchocerciasis patients, 159 from non-helminth infected individuals, and 50 from individuals infected with other helminths (10 with *Wuchereria bancrofti*, 20 with *Brugia malayi* and 20 with soil-transmitted helminths). Investigation of the performance of these minimal epitope peptides demonstrated that three of them (OvNMP-14, OvNMP-16 and OvNMP-18) have a specificity of 100%, low cross-reactivity (8.0%, 6.0%, and 4%, respectively), but intermediate sensitivity (36.9%, 46.5%, and 41.2%, respectively). A theoretical assessment of the performance of a combination of these three peptides showed that sensitivity could be further increased to 77.2% without compromising specificity. In this work, the linear epitopes discovered were found in secretory/excretory proteins or serodiagnostic candidates, but none of them was found in vaccine candidate proteins. This would indicate that of all the vaccine candidate proteins that were included in this analysis, none would bear immunodominant linear epitopes that are recognized by infected non-vaccinated individuals.

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IMPLEMENTING THE SUPERVISOR'S COVERAGE TOOL IN AN URBAN SETTING: A CASE STUDY IN PORT-AU-PRINCE, HAITI

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The ability to achieve and maintain high drug coverage during mass drug administration (MDA) for lymphatic filariasis (LF) is critical for achieving the Global Programme to Eliminate Lymphatic Filaraisis goal of interrupting transmission by 2020. In Haiti, the urban communes of metropolitan Port-au-Prince (PaP) have struggled to achieve adequate drug coverage (≥ 65%) during each round of MDA for LF. In order to rapidly assess drug coverage during the 2017 MDA, the Ministry of Public Health and Population (MSPP) and partners implemented the World Health Organization (WHO) recommended Supervisor's Coverage Tool (SCT) in Butte Boyer, a sub-section of Tabarre commune, in PaP one week following the MDA. The community leader and 12 promoters (first-line supervisors) used purposive and random sampling to select six supervision areas (SA) in which to

conduct SCT. Twenty households were randomly selected from each of the SAs; one respondent per household was asked to report if they were offered and consumed the drugs during MDA, as well as reasons for non-participation. Among 120 respondents, mean age was 31 years, 45 (38%) were male. The SCT results indicated inadequate coverage in all six SAs, defined as 10 or fewer respondents consuming the medication. The primary reasons given by respondents for not being offered or not consuming the drugs were absence during MDA and fear of side effects, respectively. Results were consistent with the low reported administrative coverage for Tabarre commune (56%). Following the SCT implementation, promoters and leaders discussed reasons for low coverage and proposed new strategies to address low coverage in their SAs. These strategies were the basis for a new urban MDA protocol aiming to improve coverage in this complex setting. Further, the SCT should be considered as a tool to rapidly assess drug coverage if MSPP adapts the new triple drug therapy in Haiti.

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THE RELATIVE IMPACT OF ANNUAL AND SEMIANNUAL MASS DRUG ADMINISTRATION (MDA) ON LYMPHATIC FILARIASIS AND ONCHOCERCIASIS IN COTE D'IVOIRE

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Lymphatic filariasis (LF) and onchocerciasis are co-endemic in 64 of 82 health districts in Côte d'Ivoire. Both filarial infections are targeted for elimination and mass drug administration (MDA) using ivermectin alone (onchocerciasis) or in combination with albendazole (LF) is the main intervention strategy. We compared the impact of annual and semi-annual MDA with ivermectin and albendazole in Abengourou and Akoupé districts in eastern Côte d'Ivoire. Baseline surveys were performed in 4 sentinel villages per district in 2014, and follow-up surveys were carried out in 2015, 2016 and 2017. We assessed microfilaria (Mf) prevalence for both infections and circulating filarial antigen rates by FTS for LF. Three annual rounds of MDA in Abengourou district decreased the LF Mf prevalence from 9.5% to 3.5% (63% reduction) and the onchocerciasis Mf prevalence from 23% to 9.6% (59% reduction). Five semiannual rounds of MDA in Akoupe decreased the LF Mf prevalence 7.6% to 2.8% (63% reduction), but the prevalence was less than 1% in 2 of the 4 sentinel villages. FTS prevalence decreased from 24.2% to 12.6% (48% reduction) in the annual MDA area and from 25.6% to 11.6% (55% reduction) in the semiannual MDA area. Thus, 5 rounds of MDA was slightly more effective than 3 rounds for reducing LF and onchocerciasis prevalence over a 3 year period. However, the differences observed were less impressive than those predicted by modeling studies, and they may not justify the increased cost and effort required for providing semiannual MDA. Given resource restraints, we believe that disease elimination programs should focus on achieving high rates of therapeutic coverage and not struggle to increase the frequency of MDA.

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SOIL TRANSMITTED HELMINTHS INFECTION AND ASSOCIATED RISKS STILL A PREVALENT ISSUE IN NIGERIA; PRIMARY SCHOOL CHILDREN ASSESSMENT IN OWO LOCAL GOVERNMENT AREA OF ONDO STATE, NIGERIA

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Soil transmitted helminths' infections are one of the major public health problems in many tropical countries including Nigeria. The objective of the present study was to assess the prevalence of soil transmitted helminths' infections and associated risk factors among primary school children of Owo LGA in Ondo State. The design of the study was a cross-sectional survey involving 224 children aged 5-15 years old who were chosen using random sampling technique from four primary schools. Data were gathered by means of questionnaire survey and laboratory parasitological examination procedures. .A structured and pre tested questionnaires were administered to study participant to collect information on socio-demographic characteristics and risk factor. The pupil BMI was measured, blood samples was assessed for hemoglobin (Hb) and PCV level while stool samples were examined for STH infection using direct wet-mount and Formol-Ether concentration methods. Data were analyzed using SPSS version 20.0. Of the total 224 study participants examined, 50.4% were males and 49.6% female children. It was found that 54 (47.8%) male and 59 (52.2%) female children were infected with STH parasites. Thus, the overall prevalence of infections of STH parasites was 50.4%. The STH parasites species identified in school children were *Ascaris lumbricoides*, hookworm and *Trichuris trichuira* with prevalence of 25.9%, 16.1% and 2.2% respectively. The prevalence of STH parasite infections among school children in age group 6-10 and 11-15 were 59.3% and 40.7%, respectively. There was no significant association between STH parasite infections and parent occupation, knowledge of hygiene/sanitation ($p>0.05$). Factors like source of attitude/practice of hygiene, drinking water source, type of toilet facility, hand washing habit before meal and after toilet were significantly associated with STH parasites infection ($P<0.05$). Local health sector should collaborate with school health program for delivering health education to increase the knowledge, attitude and practice of school children.

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INVESTIGATING THE IMPACT OF REPEATED, COMMUNITY-WIDE, MASS DRUG ADMINISTRATION ON THE AGGREGATION OF SOIL-TRANSMITTED HELMINTH INFECTION

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It has been widely shown that human helminth infections are highly aggregated, with a minority of individuals harbouring most worms. At equilibrium, the negative binomial distribution is used to describe this overdispersion, with aggregation denoted by the clustering parameter, *k*. Recent observations have suggested that repeated rounds of mass drug administration cause aggregation to increase. However, the causative mechanism by which this may occur has not yet been researched. An individual-based stochastic model was used to simulate the effect of annual mass drug administration on hosts' worm burdens. The Bliss & Fisher method was used to estimate the change in effective aggregation of worms in the host population under the effect of treatment and across a range of ages. We also analysed the contribution of age-intensity infection profiles and treatment events to the variance of worm burden in a sampled population. From this overall variance, the effective aggregation can be estimated. Further, by analysing the contribution of various sources of variance we can identify which factors are driving changes in variance

and hence aggregation. Immediately after each round of treatment, mean worm burden drops and worm aggregation spikes. In both cases, equilibrium is rapidly restored. Upon cessation of treatment, aggregation remains at equilibrium levels in villages which do not reach elimination whereas it increases drastically in those which successfully eliminate. Analysis of the variance in worm population pre- and post-treatment produces *k* estimates closely matching those observed from the simulation. Estimates for the change in *k* incorporating age-structure also showed high levels of accuracy. A relationship was identified between the change in aggregation, the efficacy and coverage of treatment, and the initial aggregation value. We have shown that repeated, community-wide mass drug administration causes worm aggregation to spike in the short-term, and either maintain equilibrium or drastically increase in the longer-term, depending on a village's elimination success.

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VERMOX® (MEBENDAZOLE) CHEWABLE TABLET - DEVELOPING A CHILD-FRIENDLY SOLUTION TO ELIMINATE SOIL-TRANSMITTED HELMINTHS THROUGH PREVENTIVE CHEMOTHERAPY

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An estimated 1.5-2 billion people are infected with Soil-Transmitted Helminths (STH), with the highest prevalence in children living in communities without adequate sanitary facilities, safe potable water and/or basic hygiene practices. The World Health Organization (WHO) recommends regular deworming of target populations through preventive chemotherapy (PC), to reduce the negative impact of STH on nutritional status, and cognitive and physical development. PC consists mainly of the annual or bi-annual administration of the benzimidazole anthelmintics albendazole and mebendazole (MBZ). Johnson & Johnson annually donates 200 million MBZ tablets to the WHO for distribution to endemic countries. In line with WHO recommendations on deworming young children, J&J recently developed a new chewable 500-mg MBZ tablet, which rapidly disintegrates after addition of a small amount of water. This makes the tablet much more suitable for young children and will likely reduce the risk of choking. Readily available, non-proprietary technology was used to develop the chewable tablet. Non-clinical studies were limited to two dog PK studies, as the preclinical characterization with previous MBZ formulations was considered to remain valid. Four clinical trials were performed: two Phase 1 trials (one relative bioavailability and one food effect study), one Phase 3 safety trial in children, and one Phase 3 efficacy and safety trial in children with analysis of PK parameters in a subset of subjects. The MBZ 500-mg chewable tablet was approved by the FDA in October 2016 for the treatment of STH infections by *Ascaris* and *Trichuris* in patients as of 1 year of age. A dossier to seek Prequalification for the treatment of infections by *Ascaris*, *Trichuris* and hookworm species is currently under review by WHO. The authors will describe the development path followed for this new chewable tablet, as well as the plans to include it in J&J donation programs, once WHO prequalified. Its physicochemical properties will make the tablet easier and safer to administer, esp. in young (12-23 months) and preschool children (24-59 months).

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COMPARISON OF PCR DIAGNOSTIC WITH KATO KATZ TECHNIQUE FOR DIAGNOSIS OF SOIL TRANSMITTED HELMINTHS AND SCHISTOSOMIASIS INFECTIONS IN THE AYALOLOO CLUSTER OF SCHOOLS IN THE ACCRA METROPOLIS OF GHANA

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The greatest impact of Soil Transmitted Helminth (STH) infections andschistosomiasis (SCH) is on the impairment of physical and intellectual development in children.According to WHO, 2012, In Ghana, STH infections are reported to be more prevalent amongchildren of school – going age, hence the renewed commitment by major stakeholders toincrease current school-based MDA coverage in the country to 75% by 2020. However, underprevalence report is a major setback to this control targets. Accra Metropolis is listed as one of the low prevalence region in Ghana. A total of 120school children between the ages of 5-17 years were recruited from two schools within theAyalolo cluster of schools in the Accra Metropolis. Stool samples collected and processed at theparasitological and molecular biology laboratories of the Biomedical and Public Health ResearchUnit (BPHRU), CSIR-Water Research Institute respectively. All helminth infections recorded in this exercise were from the Central Mosque Primaryschool. The most prevalent infection for STH was *Ascaris lumbricides* at a prevalence of 9.62%,however, *Ancylostoma doudenale* (hookworm) was observed at a prevalence of 3.3%. There wasno infection recorded at the Ayalolo Primary school. In conclusion, the Kato-Katz technique which is widely employed in STH/SCH parasiticdiagnosis alone cannot accurately detect infections. More accurate, simple and affordablediagnostics are needed to detect the infections in humans. This is having a major consequence indiseases control programmes. Though PCR techniques is expensive and required highly skilledpersonnel, its robustness and sensitiveness indicates it effectiveness in diseases surveillance,hence the need to develop affordable and field friendly PCR machines such as the MagneticInduction qPCR Cycler in endemic countries.

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THE HIGH POTENTIAL OF *BACILLUS THURINGIENSIS* DERIVED CRY5B AGAINST *STRONGYLOIDES STERCORALIS IN VITRO*

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Strongyloidiasis is one of the Neglected Tropical Diseases (NTDs). The disease has the global prevalence more than 100 million people and also causes a public health issues in livestock. Strongyloidiasis can cause a chronic infection, resulting in high mortality rates (69-87%), due to the autoinfection and dissemination of the parasites. Current anthelmintics for strongyloidiasis, including ivermectin (ivm) and albendazole (abz), still have disadvantages. Importantly, ivm-resistance has been found in *Strongyloides* spp. infecting livestock. This data suggests that ivm-resistance would be found in the major pathogenic species in humans, *Strongyloides stercoralis*. Consequently, the finding of new anthelmintics is still required. An interesting candidate is *Bacillus thuringiensis*-derived Crystal (Cry) protein named "Cry5B". The Cry5B has been demonstrated

the anthelmintic activity against a broad range of nematodes, especially on two main species of soil-transmitted helminthes (STHs), including *Ancylostoma ceylanicum* and *Ascaris suum*. Additionally, the Cry5B has been shown to be effective against abz-resistant and nicotinic acetylcholine receptor agonist-resistant *Caenorhabditis elegans*. We investigated the anthelmintic activities of the Cry5B against *S. stercoralis*, ivermectin-resistant (ivm^r), and albendazole-resistant (abz^r) *C. elegans*. Our findings demonstrated that the Cry5B was able to intoxicate the first larval stage (L1), infective stage (iL3), and adult free-living stage of *S. stercoralis*. Moreover, the Cry5B was active against ivm^r-*C. elegans*. The Cry5B impaired larval development, fertility, and viability of the worms. Interestingly, the Cry5B was more effective against ivm^r-*C. elegans* than wild-type *C. elegans*. The data showed that the Cry5B was highly effective against *S. stercoralis in vitro*. For the further investigations, we aim to demonstrate the effects of the Cry5B on the parasitic stage of *S. stercoralis* and the therapeutic efficacy in *S. stercoralis*-infected animals. We expected that the Cry5B can be used in treatment and transmission control of strongyloidiasis.

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ELIMINATION OF THE SOIL TRANSMITTED HELMINTHS FROM GRENADA

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The soil-transmitted helminths (STH) are present in all Latin American and Caribbean (LAC) countries, but the prevalence and intensity is unknown as only a few current national and subnational studies are available. The objectives of this study were to conduct a behavioral and intervention program and included: 1) Investigation of the prevalence of STH in Grenada and implementation of an elimination program; and 2) examination of the knowledge, attitudes, and practices among 9 and 10 year old students before and after the intervention. The intervention consisted of an educational presentation and a school STH poster campaign. Fresh stool samples from students were obtained and examined before and after the intervention. Automatic response systems (ARS) were used to assess short and long-term knowledge retainment of students over an eight-month period. Annual country level data (2006-17) on STH and feco-orally transmitted protozoan species were used to determine effects on national parasite prevalence. There was a positive significant short-term effect from the educational intervention on STH knowledge among the original 903 students. A long-term recall decrease in knowledge occurred in one out of eight questions (N= 767, p = 0.011). National STH baseline prevalence for school aged children in 2012 was 1.3% (7 of 527) and decreased significantly post intervention (0.0%) in 2014. STH prevalence among all ages significantly decreased from 0.77 to 0.25 % post-intervention, as did the prevalence of other protozoan species 9.83 to 5.92%. These data indicate that successful educational interventions can play a powerful role in the reduction of STH. Long-term knowledge retainment occurred and was attributed to the use of novel educational tools. This study demonstrated that the elimination of STH from a small island nation is feasible.

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A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL TO ASSESS EFECTIVENESS OF ALBENDAZOLE IN CHILDREN WITH ASYMPTOMATIC TOXOCARIASIS

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Human toxocariasis is highly prevalent and its global importance may be greatly underestimated. Toxocariasis occurs mainly in childhood by ingestion of parasite eggs from contaminated environment. During migration in human tissues the *Toxocara* larvae can cause several symptoms including eye involvement, but the majority of patients are asymptomatic. Eosinophilia is a marker of activity of this disease. There are no studies supporting the treatment of asymptomatic patients. To evaluate the effectiveness of albendazole (10-15 mg/kg/day BID for 15 days) a randomized, placebo-controlled trial was carried out in asymptomatic children (ClinicalTrials.gov #NCT00755560). Treatment response was defined as mean absolute reduction in eosinophil counts, 12 months after treatment. Demographic, complete blood count, liver and renal function and stool parasite exam were assessed at diagnosis, and every 4 months during follow up. Inclusion criteria: age 2 to 15 years, reactive toxocara excretory-secretory substances (TES) by ELISA, eosinophils >1100/mm3, normal fundoscopy and non geohelmintic infection.The 45 enrolled subjects, median age 5.3 years (range 2-13), were randomized in a 1: 1 relationship.At 12-month follow-up, eosinophil median was: 1008/mm^3 (IQ $_{25\text{-}75}$ 680 to 2023) in placebo group and median of 1360/mm^3 (IQ $_{25\text{-}75}$ 761 to 2226) in albendazole group (p = 0.37).Kinetics of specific antibody titers by the ELISA showed an erratic pattern. No differences were observed in the values between the 2 treatment groups. Both groups had minor adverse events and no patient needed to discontinue medication. In asymptomatic patients with toxocariasis, albendazole was not effective to reduce eosinophil counts. Given that toxocariasis is a neglected disease, in order to evaluate the effectiveness of albendazole or other drugs, further studies need to be conducted.

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MOLECULAR DETECTION OF *CAPILLARIA PHILIPPINENSIS* IN THE PHILIPPINES

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Intestinal capillariasis caused by *Capillaria philippinensis* (Cp) has been continually infecting Filipinos since its first detection in 1963 in the Philippines. The parasite is acquired from handling or eating raw or undercooked infected fish. Informing the public, especially those in remote provinces, about the dangers of capillariasis infection from consuming raw fish remains to be a challenge of the Philippines' Department of Health (DOH) in mitigating the disease. Another challenge is that the only current diagnostic method for this parasite in the country is through microscopic screening which has a low sensitivity and specificity. If left untreated, capillariasis can be fatal due to electrolyte depletion, cardiac arrhythmia and/or superimposed bacterial infections in patients. Thus, there is a need to develop or adapt a more reliable diagnostic method to confirm Cp infection. This study is the first to utilize nested PCR (nPCR) and Sanger sequencing to confirm Cp infection from an outbreak of diarrheal disease in a rural municipality in the Philippines. Samples (n=50) from patients suspected to have acquired Cp infection were analyzed using microscopy

and PCR. Results demonstrated a higher PCR detection rate of 14% as compared to the 2% positivity in microscopy. The nPCR assay limit of detection was determined to be at 10 DNA copies/µL. Therefore, the PCR assay utilized in the study is more sensitive than microscopy. 18S sequences (n=3) obtained herein clustered with *Paracapillaria philippinensis* (bootstrap value = 87) and *Baruscapillaria obsignata* (bootstrap value = 70), further confirming Cp detection and providing the first partial 18S sequences of Cp in the country since 1963. Thus, sequences from the multiple *Capillaria* genera used in the analysis clustered according to their genera with moderately high bootstrap values (*Bosmina longirostris* as an outgroup). The study suggests that molecular detection of parasitic infections such as that of Cp can be used as a confirmatory test or used in conjunction with microscopy screening to prevent misdiagnosis, provide guidance for treatment, and aid diarrheal outbreak investigations.

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CHARACTERIZATION OF CELL PHENOTYPES INVOLVED IN ASYMPTOMATIC HOOKWORM AND MALARIA PARASITE CO-INFECTIONS IN ENDEMIC GHANA

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IntroductionHookworm infections are associated with sources of poor drinking water, poor sanitation and poor hygiene. Malaria parasite infections also occur at places plagued by hookworms. We characterized cell phenotypes that are involved in the immune response to hookworm and malaria parasite co-infections in Ghana, West Africa.MethodCross-sectional community survey of 1826 participants randomly recruited at household level. Biological samples were collected over 12-month period. Flow cytometry analysis with labelled antibodies to cellular markers were used on selected peripheral blood mononuclear cell (PBMCs) to stimulate in culture to characterize the cell phenotypes present among those with hookworm and malaria parasite infections.ResultsCompared to those treated with no stimulation, PBMCs exposed to *Pf* parasitized RBCs in culture, revealed significant difference in the cell population for CD4+/IFN-γ+ (p=0.025), CD4+/Foxp3+/IFN-γ+ (p=0.016), CD4+/HLA-DR+ (p=0.019), CD4+/HLA-DR+/IL-4+ (p=0.032) and CD4+/HLA-DR+/IFN-γ+ (p=0.020). Significantly, CD4+/IFN-γ+ increased about twice among malaria infected individuals compared to other infection groups. CD4+/Foxp3+/IFN-γ+ cells were significantly higher in malaria infected individuals (p=0.016). CD4+/HLA-DR+/IFN-γ+ (p=0.020) and CD4+/HLA-DR+/IL-4+ (p=0.032) cells were higher in malaria infected individuals. CD8+/IFN-γ+ (p=0.029), CD8+/Foxp3+/IFN-γ+ (p=0.026), CD11c+/HLA-DR+/IFN-γ+ (p=0.031) and CD3+/TCR-γδ+ (p=0.026) were significantly different across infections. Discussion and ConclusionRegulatory T-cells population induced in the asymptomatic hookworm infection, malaria parasite infection and in their co-infected states were not significantly different. Hookworm infected individuals with malaria parasites co-infection significantly produce CD4+IL-4+ and CD8+Foxp3+IFN-γ+. In hookworm infections, Th2 effector cells are significantly different and cytotoxic T-cells are different among malaria infected people. There was also a significant increase in CD11c+/HLA-DR+/IL-4+ cells associated with hookworm infection.

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HELMINTHIC INFECTION IS ASSOCIATED WITH MALNUTRITION IN URBAN BANGLADESHI CHILDREN UNDER TWO YEARS OLD

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The first two years of life is a critical time period in infant development. When children in low-income settings start exploring their environment, they are exposed to soil-transmitted helminth (STH). A total of 490 children were recruited between January 2008 and June 2009. Children were followed for two years with household visits twice a week. Stool samples were collected once a month and all stool samples were examined using the Kato Katz technique. Length and weight were measured at baseline, at 12 months, and 24 months. 8.6% of the children had a STH infection by one year and 2.7% of these had STH ova identified on more than one occasion. 43.7 % of the children had a STH infection by 2 years, and 49.5% of these had a STH ova identified on more than one occasion. The mean age at first acquisition was 16.2 months (standard deviation (SD) 4.8 months). Microscopic examination revealed ova of *Ascaris lumbricoides* (0.6%), *Trichuris trichiura* (0.07%), and mixed infestation (0.02%) by one year but it was found higher *Ascaris lumbricoides* (3.6%), *T, trichiura* (0.7%), and mixed infestation (0.2%) by 2 years. About 26.5% children were underweight and 16.1% children were stunted at base line. At 12 months, 32.2% of children were underweight and 37.8% were stunted. At 24 months, 42.5% of children were underweight and 56.2% were stunted. STH infection in the first year of life was not associated with anthropometry at one year of age. *T. trichiura* infection during the first two years of life was associated with both stunting (P<0.02) and being underweight (P<0.03). *A. lumbricoides* infection was significantly associated with stunting (P<0.04), but not being underweight (P<0.2) by two years. In conclusion, in the first year of life STH infection is not associated with growth. However in the second year of life, preceeding STH infection is associated with both stunting (*T. trichiura* and *A. lumbricoides*) and being underweight (*T. trichiura*).

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CAPILLARIA OVA CONFOUND DIAGNOSIS OF *TRICHURIS TRICHIURA* IN HUMANS BY KATO KATZ SMEAR IN LIBERIA

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Kato Katz smears are commonly used to detect and quantify ova of soil-transmitted helminths (STH). The barrel-shaped eggs of *Trichuris trichuria* are considered to be characteristic and easy to identify by microscopy. To assess the impact of mass drug administration (MDA) for the elimination of lymphatic filariasis (LF) on STH, stool samples were tested from Lofa and Maryland counties in Liberia. Duplicate Kato Katz smears from a single stool sample were examined for each participant. Aliquots of 578 randomly selected specimens were preserved on FTA cards or in RNAlater for analysis by qPCR. While qPCR confirmed the Kato Katz results from Maryland, its sensitivity was unexpectedly low for *T. trichiura* for samples from Lofa county: Twenty-seven samples (7.6%) were positive for *T. trichiura* by Kato Katz, but only 2 (0.6%) were positive by qPCR. qPCR-negative samples included 7 stools with more than 1,000 barrell-shaped eggs per gram. Therefore stool samples preserved in RNAlater were re-examined by microscopy with 100X and 400X magnification. Surprisingly qPCR-negative samples contained eggs with less pronounced plugs and a thick, striated egg shell. These features were consistent with eggs of *Capillaria* sp. (*Trichuridae*). Since human capillariasis has not been previously reported from Liberia we confirmed the presence of *Capillaria* sp. by PCR and DNA sequencing. Using conserved primers we were able to

amplify a 288 bp fragment of the 18S rDNA (GenBank MG859285) from samples that contained exclusively *Capillaria* eggs. Their DNA sequence was 100% identical to those of *Calodium hepaticum* (*C. hepatica*) (Genbank MF287972.1), *Aonchotheca putorii* (*C. putorii*) (Genbank LC052356.2) and *Pearsonema plica* (*C. plica*) (Genbank MF621034.1). While the life cycle and the medical importance of the *Capillaria* found in humans in Lofa remain to be elucidated, our results show that *Capillaria* eggs can easily be misidentified by Kato Katz as *T. trichiura*. *Capillaria* infection/pseudo-infection in known to be not affected by ivermectin and albendazole MDA which is used for LF elimination in Africa and qPCR is useful for validating Kato Katz results in STH surveys.

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NO EVIDENCE OF SOIL TRANSMITTED HELMINTH INFECTION IN A SURVEY OF STOOL SAMPLES FROM THE STATE OF MISSISSIPPI

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Recent studies have highlighted a potentially unrecognized disease burden of soil-transmitted helminths (STH) in the American South. However, there have been no large-scale surveys conducted recently in Mississippi. We collected de-identified stool samples with corresponding subject age and zip code data from the clinical microbiology lab at the University of Mississippi Medical Center. Stool microscopy (saturated salt flotation) was used to identify evidence of STH infection. In parallel, real time PCR (as described previously) for *Necator americanus, Ancylostoma duodenale., Ascaris lumbricoides, Trichuris trichiura,* and *Strongyloides stercoralis* is being performed on DNA extracted from the stool samples. We evaluated 650 samples from 650 subjects [average age 52 (range, 3-91, median 56)]. Based on consistency/volume available, 522 were microscopically analyzed, and all were negative. 600 DNA extracts tested thus far for *Necator americanus* by real time PCR are negative. A further 254 samples that have been analyzed by PCR for *Ancylostoma duodenale*, *Ascaris lumbricoides, Trichuris trichiura,* and *Strongyloides stercoralis* have also yielded negative results. Analysis of the remaining DNA extracts ongoing. Data thus far suggests that prevalence of STH infection is low in this population. Further, larger studies of at-risk populations are indicated.

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STRONG TREAT 1 TO 4: A RANDOMIZED, OPEN-LABEL CLINICAL TRIAL ON MULTIPLE VERSUS SINGLE DOSE OF IVERMECTIN FOR THE TREATMENT OF STRONGYLOIDIASIS

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Strongyloides stercoralis infection is a soil-transmitted helminthiasis with a wide distribution, primarily in tropical and subtropical regions. Most infected individuals are asymptomatic or have minor non specific symptoms. However, in the presence of immune suppression, strongyloidiasis can turn into a disseminated, life-threatening disease. Ivermectin is the drug of choice for treatment, but there is no consensus on the dosage regimen. Strong Treat trial is a multi-centre, randomized, open-label clinical trial conducted in a non-endemic area, where re-infection can be excluded. There are nine participating sites, in three European countries: Italy, Spain, and the United Kingdom. The Centre for Tropical Diseases in Negrar, Verona, Italy is the coordinating site. The aim of the trial is to compare the efficacy of two regimens of ivermectin for the treatment of strongyloidiasis: a single dose of 200 µg/kg versus 4 doses (200 µg/kg for 2 consecutive days, repeated two weeks apart). Baseline evaluation of the eligible patients included serology, fecal culture and/or PCR. The diagnostic criteria for inclusion were: either positive serology at "high titers" (according to pre-defined cutoff values) irrespective of the results of the fecal tests, or positive serology (irrespective of the value) and positive fecal tests. The enrolled patients were randomized into the two arms and followed up 6 and 12 months after treatment, with serology and fecal tests (the latter were repeated in case of positivity at baseline). Enrollment ceased in May 2017, with 311 patients included. An interim analysis on 194 patients with completed 12-month follow up showed high efficacy of both drug regimens, with no significant difference between the study arms. Final results are still pending (they will be presented at the annual meeting), but according to our projections they are most likely to confirm the data obtained from the interim analysis.

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HOOKWORM INFECTION IN CHILDREN IS ASSOCIATED WITH NEGATIVE HEALTH OUTCOMES IN RURAL ALABAMA

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Hookworm infection affects 430 million people, including 156 million children, worldwide and has been associated with anemia, iron deficiency, impaired cognitive development, and stunting in children. Hookworm, specifically *Necator americanus*, was known to be endemic in the southern United States before improvement of sewage disposal systems and eradication programs in more resource-poor areas. A recent study using modern molecular diagnostics revealed that, in high-risk environments with open sewage disposal systems in Lowndes County, Alabama, 34% of inhabitants tested positive for *Necator americanus*, with a low parasite burden. In addition, 60% of children in this study population also tested positive for hookworm infection. Given the preliminary findings, the lack of studies on low burden hookworm infection on health outcomes, a more in-depth study to evaluate prevalence and health impact of hookworm infection among children in high-risk, resource poor areas in the United States is desperately needed. A larger follow-up study is underway to determine the prevalence and disease burden of hookworm infection among children and soil in high-risk areas and the association of negative health impacts on children. Stool and blood samples will be collected from 334 children (2-12 years old) in high risk areas in Lowndes County, Alabama. The stool samples will be tested for hookworm using multi-parallel real-time quantitative PCR (qPCR) and blood samples will be tested for hemoglobin (Hb), iron, and markers of gastrointestinal injury (Intestinal fatty acid-binding protein [I-FABP]) and microbial translocation (soluble

CD14 [sCD14]). Soil near sewage run-off sites will be also be tested for evidence of hookworm by qPCR. This work is important in determining that soil-transmitted helminth infections are not only a problem in endemic countries, but in resource poor areas in the United States as well, and despite low burdens of infection, negative health outcomes could be affecting children within these resource-poor communities, prompting future study and intervention.

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CEREBROSPINAL FLUID NEOPTERIN AND CXCL13 ARE SUITABLE BIOMARKERS FOR STAGING AND DETECTION OF TREATMENT FAILURE IN A NON-HUMAN PRIMATE MODEL OF HUMAN AFRICAN TRYPANOSOMIASIS

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Follow-up of treated human African trypanosomiasis (HAT) patients has been a routine practice for confirming cure and early detection of treatment failure. The aim of this study was to verify whether the neopterin and CXCL13 biomarkers are suitable for determining the stage of disease, and for monitoring the efficacy of treatment in a vervet monkey model of HAT. Six monkeys were infected with Trypanosoma brucei. Late stage disease was induced by sub-curative treatment with diminazene aceturate (DA) from 28 days' post-infection (dpi). When relapses occurred, the animals were treated curatively with melarsoprol (Mel B) from 82 dpi. Blood and cerebrospinal fluid (CSF) samples were collected at weekly intervals and assessed for parasitosis, as well as tested for neopterin and CXCL13 by ELISA, for a period of 39 weeks. The concentration of neopterin in serum increased rapidly after infection in early disease (stage 1), peaking at 14 dpi. Levels then dropped rapidly to pre-infection levels by 35 dpi. In contrast, there was a marginal increase in CSF neopterin during early infection, with a minor peak at 21 dpi. Serum CXCL13 increased rapidly from 7 dpi, peaking at 28 dpi, after which the levels dropped upon sub-curative treatment with DA. The concentration of CXCL13 in CSF also increased gradually in early stage disease and continued to rise after sub-curative treatment with DA. Curative treatment with Mel B resulted in its gradual decline, reaching pre-infection levels 105 days later. In conclusion, the changes in CSF neopterin and CXCL13 correspond to important time-points and stages of the disease. That peak levels coincided with time of relapses demonstrates the potential of these biomarkers in staging and detecting treatment failure. Moreover, the rapid fall in CSF neopterin after curative treatment confirms its great potential for use in development of a test of cure.

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ADDRESSING CHAGAS DISEASE AS PART OF PRIMARY CARE IN MASSACHUSETTS - THE STRONG HEARTS PROJECT

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In Massachusetts, there are over 3,000 estimated cases of Chagas disease (CD). Immigrant communities at risk of CD often seek primary care in community health centers (CHCs). The Strong Hearts (Corazones Fuertes/Corações Fortes) program is a model to establish CD screening as a component of primary care for high-risk patients and to implement referral to specialists in tertiary hospitals for evaluation and antiparasitic treatment. The program also aims to educate medical professionals and community members, and to address barriers to care. In this study, we aim to describe the results of one year of screening. Among the screening tests with results available, 78 were initially positive with the Hemagen test and of these, 19 cases of CD were confirmed by the Centers for Disease Control using two different types of T. cruzi serologic assays. 11 (58%) of these 19 cases were detected in women, 3 of whom were pregnant; the average age of confirmed positive patients was 41 years, and 18 (95%) were from El Salvador. All 19 patients with confirmed CD were referred for specialty care, 13 (68%) have been evaluated and 3 (16%) have been started on antitrypanosomal therapy. One patient had already been treated for Chagas disease prior to this screening program. Treatment was deferred for the 3 pregnant patients until after they given birth. Moreover, inter-institutional care algorithms are being developed to follow infants of these mothers to test for congenital infection and, if present, provide antitrypanosomal therapy. While there are substantial barriers to care for communities affected by CD, the Strong Hearts program demonstrates that this disease can be addressed within primary care settings in the U.S.

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EVALUATION OF SAFETY AND EFFICACY OF *LEISHMANIA MAJOR* CENTRIN DELETED (*LMCEN-/-*) LIVE ATTENUATED PARASITES AS A PROPHYLACTIC VACCINE AGAINST VISCERAL LEISHMANIASIS

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Leishmaniases are vector-borne parasitic diseases affecting millions of peoples worldwide. To date there is no licensed vaccine available against human leishmaniasis. It has been shown that dermatotrophic *Leishmania major* confers protection against cutaneous leishmaniasis as well as against visceral leishmaniasis. However, such a method of immunization is not practical because of the greater risk of infection in a naïve population. Therefore, genetically modified live attenuated parasites that are non-pathogenic might induce the same protective immunity as leishmanization. We have developed centrin-gene deficient *Leishmania major (LmCen-/-)* using CRISPR-Cas methodology and evaluated the safety, immunogenicity as well as protective efficacy against *L. donovani* challenge. Golden syrian hamsters were immunized intradermally with *LmCen-/-* parasites in the presence or absence of the adjuvant GLA-SE. Intradermal immunization of hamsters with *LmCen-/-* did not develop any detectable lesion after immunization. *LmCen-/-* parasites alone is sufficient to induce a host protective immune response compared to *LmWT* infection. Seven weeks post-immunization hamsters were infected with *L. donovani* by needle injection or by infected sand flies. In both sets of experiments, nine months post-challenge, non-immunized hamsters developed severe pathology of VL, while immunized hamsters showed significantly lower parasite burden in liver and spleen compared to non-immunized animals. We also evaluated the cellular immune response between immunized & non-immunized hamsters after challenge with wild type parasites. Spleen cells from *LmCen-/-* immunized and challenged hamsters produced significantly more Th1-associated cytokines including IFN-γ and TNF-α, and significantly reduced expression of the anti-inflammatory cytokines IL-10 and IL-21, compared to non-immunized and challenged animals. The enhanced proinflammatory immune response correlates with lower

parasite in immunized animals. Our studies demonstrate that the *LmCen-/-* mutant parasite is safe and has a potential to be an effective vaccine against VL.

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EVALUATION OF POINT-OF-CARE TESTS FOR VISCERAL LEISHMANIASIS DIAGNOSIS IN KENYA

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According to the Kenyan guidelines, the first approach for the laboratory diagnosis of visceral leishmaniasis (VL) is serology, testing VL suspects with rK39-based rapid diagnostic test (RDT) and direct agglutination test (DAT). Parasitological diagnosis, based on splenic aspiration microscopy (SAM), is invasive and is applied when antibody detection is useless: i) diagnosis of VL in seronegative suspects or in those with a previous VL episode, ii) test of cure and confirmation of relapses. Unfortunately rK39-RDTs have low sensitivity in eastern Africa, DAT is technically demanding and splenic aspiration presents the risk of bleeding, and requires technical expertise. This prompted us to assess whether an RDT based on the synthetic antigen rK28 (CTK Biotech, CA, USA) and a simple molecular test based on loop-mediated isothermal amplification (LAMP) to detect *Leishmania* DNA in blood (Eiken Chemical Co., Japan) could improve the current approach for VL diagnosis in Kenya. We recruited 133 VL suspects in two VL treatment centres in Baringo and West Pokot counties; 89 of them were classified as VL cases according to the clinical case definition: fever ≥ 2 weeks, splenomegaly or weight loss, and positive rK39-RDT, DAT or SAM. When blood and plasma from VL suspects were tested by rK39-RDT and rK28-RDT, both tests showed 100% specificity and similar sensitivity in blood (83.9% and 83.0% respectively), while rK28 RDT returned higher sensitivity (91.1%) than rK39-RDT (79.0%) in plasma samples. The most sensitive serological test was DAT using plasma (92.2%), with 100% specificity. SAM was more sensitive (82.9%) than LAMP (78.3%) and both were highly specific (100% and 96.9% respectively). The rK28-RDT was not superior to the current approach for serological diagnosis (rK39-RDT + DAT), but the sequential use of rK28-RDT in blood and, when this is negative, in plasma allows achieving a similar performance. LAMP did not perform better than SAM but this method may be preferred as it uses peripheral blood, and would allow reduction of the number of patients requiring splenic aspiration for parasitological confirmation.

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CHARACTERIZATION OF PREGNANT WOMEN WITH CHAGAS ASSISTING THE LABOR HOSPITAL OF PERCY BOLAND IN SANTA CRUZ, BOLIVIA

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Chagas disease represents a serious public health problem due to its magnitude, transcendence, impact and challenges in disease control. The known area of dispersion of the main vector of Chagas disease in Bolivia covers approximately 60% of the territory, in geographical areas between 300 and 3000 meters above sea level. In different studies conducted between 2000 and 2017 in Bolivia, rates have been found between 19 and 29%. From May 2016 to March 2018, with the support of John Hopkins University and PRISMA, a prospective study was performed to screen for Chagas' in mothers and follow up their newborns. Mothers were given prior informed consent, InBios rapid tests for Chagas and HAI, the results of which could be available in under one hour. In addition, sociodemographic data and blood samples for ELISA were collected. Of the 5,420 mothers recruited, 19.2% had Chagas' disease. 67.3% were born in Santa Cruz, 16.5% came from Chuquisaca, 6.7% Cochabamba, 3.8% Potosí, 2.8% Tarija and 2.9% from other departments. The 37.1% were born in Andrés Ibáñez, 8.9% Cordillera, 3.2% Warnes, 2.2% Vallegrande and 1.75% Ichilo. In the study, 805 patients lived in urban areas with good living conditions, including in homes made with good materials, and not those commonly known to harbor triatomine. 11.43% of these patients had Chagas' disease. Among patients living in urban areas with good living conditions, 10.10% of those without mothers with Chagas' disease had Chagas. 23.17% of patients with Chagas-positive mothers living in urban areas with good living conditions also tested positive for Chagas. The odds of Chagas disease were over two and a half times higher in those with a Chagas-positive mother versus no Chagas-positive mother (OR: 2.69, 95%CI: 1.52-4.74, $p < 0.001$). In conclusion, the incorporation of the rapid test made it possible to speed up the detection of mothers with Chagas and subsequent follow up with their newborns. The women of Chuquisaca contributed a significant percentage of cases. It is important to ask pregnant women if their mother had Chagas, which shows a significant increased risk of exposure.

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DIAGNOSTIC PERFORMANCE OF A RECOMBINANT POLYMERASE AMPLIFICATION TEST LATERAL FLOW (RPA-LF) FOR CUTANEOUS LEISHMANIASIS IN COLOMBIA: A PILOT STUDY

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Current diagnostic methods for cutaneous leishmaniasis (CL) are limited by the required technical expertise and invasive sampling, which hinders access to diagnosis in rural remote areas. Non-invasive sampling coupled to molecular parasite detection can improve access if they can be implemented in primary care facilities. This pilot study evaluated the performance of a Recombinant Polymerase Amplification Test - Lateral flow (RPA-LF) coupled to non-invasive sampling in Colombian patients. Patients ≥18 years of age with cutaneous lesions (>15 days duration) suggestive of CL were eligible. Samples were obtained from ulcerated skin lesions using the FTA Whatman paper. The sensitivity of the test was estimated against two gold standards: conventional diagnostic methods (smear/culture) and qPCR targeting the 18S rDNA. Twenty patients from the Colombian Pacific Coast with suspected CL were included. Age ranged from 22 to 62 years (Median: 35 years old). Among them, 17 were confirmed cases by conventional methods and qPCR. Median duration of disease was 2 months (range: <1-6 months since the appearance of the first lesion). *Leishmania* species were identified in 65% (11/17) of the confirmed cases: 73% were *L.V. panamensis* (8/11) and 27% *L. V. braziliensis* (3/11). The sensitivity of the RPA-LF test compared to standard diagnostic methods (smear and/or culture) and qPCR was 100% (95% CI: 80.5 - 100). The RPA-LF isothermal amplification test coupled to non-invasive sampling could facilitate access to diagnosis in remote settings where CL occurs. These results provide bases for a larger study to assess field performance and scale-up of this diagnostic method.

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THE REALITIES OF DIAGNOSING VISCERAL LEISHMANIASIS (VL) IN ENDEMIC AREAS OF GADARIF, SUDAN: A MIXED METHODS STUDY

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Visceral leishmaniasis (VL) is a lethal parasitic disease caused by Leishmania sp parasites, leading to 20,000 - 30,000 deaths annually. Correctly diagnosing VL is crucial, as its signs and symptoms L are not specific enough to differentiate the condition from other diseases causing persistent fever. Ideal VL test is highly sensitive, as VL is potentially fatal, and it should also be specific, as empirical treatment needs toxic, injectable drugs. Current invasive microscopic methods are unsuitable for settings with limited resources. Since the rollout of Rapid Diagnostic Test (RDT) in the Sudan leishmaniasis control program, their real value in clinical practice was never evaluated. The clinicians' adherence to current guidelines of clinical case definition and RDT in VL is critical, yet it never been documented. We aim to understand factors affecting adherence to diagnostic guidelines in Gadarif state, the VL hotspot in Sudan. We will conduct a mixed method study in purposively sampled four public hospitals. We collect qualitative data from in-depth interviews using semi-structured questionnaire, to study the perspective of health workers in the public and private sector on the diagnostic process for VL (awareness, opinions on feasibility, user-friendliness, relevance). We will document the adherence of health workers in the public and private sector to the current diagnostic guidelines, in particular to the clinical case definition and the use of RDT. We will analyze routinely collected data (lab register, patient registration, and medical records) in the study sites combined with exit interview for VL patients already discharged. The data collection will start in May 2018, and the results are expected to be ready by August 2018. Our results would help inform policy-makers and stakeholders on how best to adapt the diagnostic strategy in VL control in their diverse settings.

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POPULATION PHARMACOKINETIC OF BENZNIDAZOLE, A DRUG FOR CHAGAS DISEASE, IN AN ACUTE INFECTION MURINE MODEL

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Chagas disease, caused by Trypanosoma cruzi, affects an estimated of 8 million people in the world. Benznidazole, one of the only two drugs with proven efficacy, lacks important information on metabolism, specifically pharmacokinetics in both humans and animal models.The objective of this study was to evaluate the pharmacokinetics of benznidazole in a murine model of Chagas disease. A total of 28 male, 2 months old, BALB/C mice (16 infected, 12 healthy controls) were administered a single dose of 100 mg/kg benznidazole by gavage and blood was sampled by submandibular puncture at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours. Mice were infected (N=16) by intraperitoneal inoculation with 500 parasites, VD strain, and treated when parasites were detected in blood circulation (15 days post infection). Blood was obtained from every animal (infected and healthy) at least twice and in some cases three times. Blood samples were centrifuged, and serum was precipitated with acetonitrile (1:1) and conserved at -70C until measurement by UHPLC-MS/MS. A population pharmacokinetics model was implemented with Monolix software (Lixsoft). Population pharmacockinetics followed a one compartment model. The maximum observed concentration was 67.7 µg/ml (Tmax 0.5 h). Absorption was fast (absorption constant ka $3.3h^{-1}$), volume of distribution was 29.9 ml and clearance 16.2ml/h (half life 2hs). No significant differences between infected and healthy animals were observed. We observed conserved pharmacokinetics behavior of benznidazole in infected animals compared to healthy mice, providing reassurance that concentrations obtained in this model reflect those required for drug effectiveness. Elimination of the drug was fast, suggesting that once daily dosing may be insufficient to sustain tissue concentrations required to sterilize the animals (but also confirming the lasting impact of benznidazole on the parasite, as beznidazole concentrations are expected to be low during most of the day with daily dosing).

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COMPREHENSIVE CONTROL OF CHAGAS DISEASE IN ENDEMIC RURAL AREAS FROM THE ARGENTINEAN CHACO - IMPACT ON DIAGNOSIS AND TREATMENT COVERAGE

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The Municipality of Quitilipi (Chaco, Argentina) is located in the Gran Chaco Ecoregion, recognized as a hotspot for Chagas Disease (ChD) and other Neglected Tropical Diseases (NTDs). It is composed of 2,188 rural houses inhabited by 17,982 people, most of them creoles although there are some residents that belong to the Qom ethnicity. Up to 2014, diagnosis of ChD was performed either by demand or by family antecedents by the local health teams. This strategy allowed, during that same year, the study of 105 individuals under the age of 20 of which 26 were seropositive for ChD and 19 received etiological treatment. In 2015, entomological control actions with pyrethroids were initiated in 2,045 houses (93.4% coverage), with a global infestation rate of 13.9%, range of 3.9% to 44.4% between the different localities. Subsequently, community meetings and serological screening were performed in school and health posts. A total of 1,015 individuals were screened in 2015, 894 individuals in 2016 and 842 individuals in 2017 with seroprevalences of 26.1%, 12.4% and 12.9%, respectively. Starting in 2015, residents with positive serology were included in a treatment program conducted by medical teams in the rural area. In 2015, 108 individuals under the age of 20 were treated, 118 in 2016 and 65 in 2017, thus clearly increasing the number of treatments conducted in 2014 (n=19). Finally, interviews were held with community leaders and health professionals from two rural localities, La Pampita and La Matanza, in order to understand the success of the program. The articulation of the institutions with the community showed to awaken and sustain greater interest in the inhabitants while the discontinuity of the field actions by the different institutions generated less community interest and increases de disbelief of future actions. These results show that control actions carried out with institutional and community support, coupled with a comprehensive approach that integrates entomological and epidemiological aspects, tend to improve the coverage of diagnosis and treatment of ChD in an efficient and sustainable manner.

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IDENTIFICATION OF NOVEL ANTI-LEISHMANIAL COMPOUNDS AND THEIR EFFICACY ACROSS NINE *LEISHMANIA* STRAINS

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Leishmaniasis is a neglected vector-borne tropical disease caused by a protozoan of the genus *Leishmania.* The clinical spectrum of leishmaniasis ranges from self-healing cutaneous lesions (papules or ulcers) to irreparable damage of the soft and cartilaginous tissues and even to fatal systemic illness. Most of the drugs available today are difficult to use, have significant side effects, and have variable efficacy. Consequently, there is a search for safer, less expensive and more effective drugs. Known anti-leishmanial compounds such as Miltefosine have species-specific efficacy, while drugs such as Ambisome have potent efficacy across a wide range of *Leishmania* species. It is important to screen anti-leishmanial hits against a wide panel of leishmanial species early in a drug discovery program. In an effort to find novel anti-leishmanial drugs, a high throughput screen on a wide range *Leishmania* species is needed to evaluate IC_{50} values on intracellular amastigotes. In this study, a pLEXSY-hyg based vector was used to generate *Leishmania* parasites that constitutively express the firefly luciferase gene, to allow for rapid, luminescence-based readout. Nine transgenic species (*L. major, L. guyanensis, L. infantum, L. mexicana, L. panamensis, L. donovani, L. amazonensis and L. peruviana*) were evaluated in comparison to their wild type counterparts in terms of fitness and characterized for their utility for use in a high throughput screening format. A screen was performed using a collection of 219 antibacterial compounds in which seventeen candidates were identified to have potent activity across the 9 different *Leishmania* species.

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CHARACTERIZATION OF *TRYPANOSOMA BRUCEI* 20S PROTEASOME INHIBITOR

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Phenotypic screening resulted in identification of triazolopyrimidine (TP) class of inhibitors that are active against all kinetoplastids. Resistant *Trypanosoma cruzi* parasites were generated against this compound class resulted in identification of F24L and I29M mutations β4 subunit of 20S proteasome responsible for this phenotype. These residues (F24 and I29) are conserved in all three kinetoplastids but not in humans, and ectopic expression of F24L or β4 I29M mutants alone in Trypanosoma brucei resulted in significant shift against the TP series of compounds. We have developed a homology model by using human 20S proteasome as template (PDB: 4R67). Using this homology model, additional contacts with b5 subunits were identified such as Y113 and G129. The model greatly facilitates the structural guided drug design to improve physchem properties while preserving on-target activity. Further investigation using genetics, biochemical and structural means are being pursued.

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CELLULAR AND HUMORAL IMMUNE RESPONSES TO *TRYPANOSOMA CRUZI* AFTER COMPLETE OR INCOMPLETE TREATMENT SCHEDULES WITH BENZNIDAZOLE

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Chagas disease is the main cause of infectious myocarditis in the world. Benznidazole treatment for the chronic phase of Chagas disease is suspended in 17-30 percent of the cases mainly due to the appearance of severe dermatitis. We have previously shown that conversion to negative serology can be achieved after incomplete schedule with benznidazole raising the question of whether incomplete treatment may still be effective. The aim of this study was to perform a close monitoring of the changes of *T. cruzi* specific antibodies by a Luminex-based multiplex assay and the polyfunctional capacity of specific T cells by multiparameter flow cytometry after shortened treatment schedules with benznidazole. Multiplex assay was conducted in 11 patients with incomplete treatment schedule (7-25 days of treatment) and compared with 14 patients with full dose schedule with at least 36 months of follow up. Both patient groups had significantly decreased the mean fluorescence intensity of at least one protein at 36 months post treatment and had similar percentages of declining antibodies at 72 months of follow up (i.e., 9 out of 11 patients with incomplete and 14 out of 14 patients with complete schedule). An enrichment of IL-2-producing cells indicating a cessation of antigen stimulation was observed in both groups with complete and incomplete treatment schedules. Likewise, phenotyping analysis of IL-7R, CCR7, CD62L and activation marker HLAD-R in CD4+ and CD8+ T cells showed similar changes after complete or incomplete treatment. These findings strengthen our previous results showing that even shorter schedules of treatment with benznidazole can reduce antigen-induced alterations in T-cells.

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SURFACE GLYCOCONJUGATES FROM *LEISHMANIA MAJOR* AND *L. TROPICA* AS TARGETS FOR HUMAN HOST IMMUNE RESPONSE

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Surface glycans have wide ranging importance in cell biology, including in recognition of invading pathogens by the human host. The dense glycocalyx of the *Leishmania* parasite protects the it from destruction by host factors, as well as presenting targets for antibodies to bind. During human infection, anti-glycan antibody levels increase, specifically those that can recognise terminal α-galactosyl residues. The structures responsible for this increase are not known, although a likely candidate is a family of glycoinositolphospholipid (GIPLs), which are one of the most abundant *Leishmania* glycoconjugates. As the glycan profile of GIPLs varies across *Leishmania* species, the possibility of selecting species-specific glycan-based biomarkers increases, which would improve diagnosis and aid the selection of appropriate chemotherapies. We have previously reported the identification of biomarkers of infection for Old World Cutaneous Leishmaniasis using a library of synthetic neoglycoproteins, which are recognised by sera from patients infected with either *L. major*

or *L. tropica*. Building on this work, we have now used a 1) structural glycobiology approach (i.e. thin layer chromatography (TLC) and mass spectrometry analyses of *Leishmania* glycolipids) and 2) screening novel glycan microarrays using sera from infected patients, to identify novel and species-specific α-galactosylated structures. Furthermore, initial imaging results indicate that α-galactosylated glycoconjugates may be secreted by *L. major* amastigotes during development within macrophages. Subsequent immunofluorescent imaging will investigate further the localisation of antigenic glycoconjugates throughout the parasite lifecycle.

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INVOLVEMENT OF THE NON-INFLAMMASOME FORMING NUCLEOTIDE-BINDING DOMAIN LEUCINE-RICH REPEAT PROTEIN 12 (NLRP12) IN MURINE VISCERAL LEISHMANIASIS (VL)

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Leishmania infantum (Li) [MEW1] causes VL, with suppression of type 1 immune responses. The NLR proteins include >20 cytosolic proteins that regulate inflammation and immunity. Activation of three NLRs can cause assembly of an inflammasome leading to IL-1β and IL-18 release. Functions of non-inflammasome forming NLRs are not as well understood. We hypothesized that NLR proteins influence the course of VL by modifying the localized inflammatory response to *Li*. We screened for NLR effects by infecting NLR pathway gene knockout or wild type (WT) mice with *Li* coexpressing luciferase and mCherry. Progressive parasite expansion was monitored by *in vivo* imaging, qPCR and Luciferase assay. The screens suggested involvement of the non-inflammasome forming Nlrp12 in progression of VL. Parasite loads expanded early but were controlled in WT mice, whereas *Li* continued to expand and were 2-fold higher than WT on day 56 of *Nlrp12*$^{-/-}$ infection. Consistently, liver-derived infiltrating cells from *Nlrp12*$^{-/-}$ mice released less antigen-induced IFN-γ than WT cells on infection day 56. Flow Cytometry showed infl. monocytes expanded on day 28 in WT but not *Nlrp12-/-* mice, preceding clearance from WT. Instead, resident Mø expanded in *Nlrp12-/-* mice in parallel with the late expanding parasite load (day 56). The kinetics of monocyte derived DC (MNDC) recruitment paralleled parasite load, with recruitment at 28 days in WT but recruitment at 56 days in *Nlrp12-/-* mice. Both *in vitro* and *in vivo* migration studies showed an intrinsic DC migration defect in NLRP12 KO cells, while the gene expression for chemokines CCL-21, CXCL-12 and CCL-19 remains at same level as WT spleens. Surface expression analysis showed that DCs nlrp12-/- have reduced CXCR4 and CCR7 expression, which could explain the diminished migratory capacity. These data suggest that Nlrp12 plays a protective role in VL, associated with recruitment of both inflammatory monocytes and MNDCs at the time of peak parasite growth, followed by parasite clearance. DC infiltration in lymphoid tissue is impaired in the absence of Nlrp12, leading to delayed MNDC influx and impaired IFN-γ which permit parasite growth.

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IMMUNE IMPACT OF TICK-BORNE CO-INFECTIONS ON CANINE LEISHMANIASIS

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Visceral leishmaniasis (VL), a zoonotic protozoal disease, kills an estimated 20,000 people annually (WHO, 2012). Dogs are the primary reservoir for parasite transmission to humans and clinical status correlates with infectiousness to sand fly vectors. Like in humans, progression from asymptomatic to symptomatic canine visceral leishmaniasis (CanL) is accompanied by loss of CD4$^+$ T cell responsiveness to *Leishmania* antigen. The molecular signals driving exhaustion in either species are poorly understood. Intriguingly, within a naturally infected cohort of 174 dogs with CanL, asymptomatic dogs were 9.9x more likely to progress to clinical leishmaniosis if seropositive for ≥2 tick-borne co-infections (TBCs) over a six-month study period (p=0.0071). In Brazil, where dogs are a predominant reservoir for human VL, 80% of dogs were found to be seropositive for at least one TBC. We hypothesize that TBCs cause acute systemic inflammation in *Leishmania*-infected asymptomatic dogs, which promotes T cell exhaustion and symptomatic CanL. To evaluate this hypothesis, Type 1 immunity (circulating CD4$^+$ and CD8$^+$ T cell proliferation, cytokine production, and inhibitory receptor expression in response to *Leishmania* antigen stimulation *ex vivo* and peripheral NK cell phenotypes) and clinical progression of CanL (positive blood PCR or serology and clinical signs of CanL) will be compared in *Leishmania infantum*-infected dogs seronegative or seropositive for tick-borne pathogens: *Anaplasma*, *Ehrlichia*, and/or *Borrelia spp*. These data may provide new targets to prevent disease progression in dogs or people already infected with *Leishmania* and decrease parasite transmission to people by decreasing canine reservoir host parasitemia.

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CYTOTOXIC ACTIVITY BY NK CELLS CONTRIBUTE TO PARASITE KILLING AND IMMUNOPATHOLOGY IN CUTANEOUS LEISHMANIASIS

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Skin lesions in patients with *Leishmania braziliensis* infection has been associated with inflammation induced by CD8+ T cells cytotoxicity. Moreover, cytotoxicity by these cells does not contribute to parasite killing, but instead, mediates pathology. Meanwhile, the role of cytotoxicity by NK cells in cutaneous leishmaniasis (CL) is still poorly understood. Here, we observed that NK cells frequency was increased in peripheral blood from CL patients when compared to healthy subjects, and that NK cell subsets express more IFN-γ, TNF, Granzyme B and Perforin than CD8+ T cells. We also found that most of the cytotoxicity activity in CL lesions is performed by NK cells. Also, in CL lesions, Granzyme B and Perforin were associated with lesion size. Lastly, we observed that NK cells contribute to destruction of *L. braziliensis* parasites. Our data suggest that NK cells contribute to the control of parasite multiplication, but also induce inflammation and tissue damage.

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NEW BIOMARKERS FOR HUMAN ASYMPTOMATIC *LEISHMANIA INFANTUM* INFECTION

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In our previous study assessing the prevalence of asymptomatic visceral leishmaniasis (AVL) in 200 Operation Iraqi Freedom (OIF) deployers during 2002-11, we showed that 20% of deployed subjects were infected, using molecular and immunological assays. New biomarkers capable of identifying asymptomatic *Leishmania infantum* infected individuals are necessary for pinpointing persons at risk for VL activation. Samples were obtained from 27 interferon gamma release assay (IGRA)+, 8 ELISA +, 2 polymerase chain reaction (PCR)+, 8 uninfected OIF deployers, and 9 never-traveled subjects in the AVL study. Soluble Leishmania antigen (SLA)-stimulated supernatants of peripheral blood cells were used to assess cytokine levels by Luminex™ assay (Cytokine 25-Plex Human Panel, Invitrogen). Serum parasite load was determined using a repeat region (REPL) target *Leishmania* PCR; as well C-reactive protein (CRP) levels were

tested using sera obtained both at time of enrollment (2016-17) and banked within 6 months post-deployment. Moreover, we tested the sera reactivity against SLA using Western Blot. Our results show that mean CRP levels (mg/ml) were 0.037 in AVL IGRA+, 0.038 in AVL ELISA+, and 0.054 in non-exposed control subjects (p=0.14). One of the 200 deployers had 5 parasites/ml measured with serum *L. infantum* PCR. MCP-1, CXCL9, and Interleukin (IL)-8 were detected at high levels. IL-8 levels were higher in AVL IGRA+ compared to AVL ELISA+ and control subjects (56, 0.6 and 0.35 mg/ml, p=0.0001). IL-8 was not detected in the AVL PCR+ group. We also identified antibodies directed against a 70 kDa antigen in some AVL ELISA+ participants. In conclusion, elevated CRP was not associated with AVL, some limited serum PCR and western blot observations should be corroborated with a larger sample size. These results show that IL-8 could be a robust candidate biomarker of immunity for assessing AVL; differentiating between AVL IGRA+ versus AVL ELISA+, AVL PCR+. IL-8 has a role in the recruitment and activation of immune cells during visceral leishmaniasis which is a likely mechanism underlying our findings.

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IMMUNOGENICITY OF CYTOMEGALOVIRUS VECTORED VACCINES FOR CHAGAS DISEASE

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Chagas disease, caused by infection with the protozoan parasite *Trypanosoma cruzi*, affects 6 to 7 million people worldwide. Chronic Chagasic Cardiomyopathy develops in up to 30% of chronically infected individuals and it is the leading cause of non-ischemic cardiomyopathy in Latin America. Anti-parasitic treatment during chronic infection has poor efficacy and does not prevent cardiac death, thus adjunctive therapies are urgently needed. In chronically infected individuals, robust parasite specific T_H1 immune responses correlate with reduced or absent cardiac disease, thus vaccines that induce robust T_H1 immune responses are an attractive option for adjunctive therapy. Amastigote surface protein 2 (ASP-2) is an efficacious vaccine antigen when delivered as cDNA or adenoviral vectored vaccines in mice. Cytomegalovirus-vectored vaccines induce robust T_H1 directed immune responses in mouse and primate models. The goal of this study was to evaluate immunogenicity of cytomegalovirus-vectored Chagas vaccines in mice. Naïve or chronically infected female C57BL/6 mice were vaccinated once with either wild type or replication deficient murine cytomegalovirus (MCMV) constructs containing ASP-2 antigen. Antigen specific IFNγ responses were measured from splenocytes by ELISpot and flow cytometry 3 months after vaccination. Both constructs induced robust antigen specific IFNγ producing cells in naïve mice. Vaccination of chronically infected mice with the wild type MCMV vector containing the ASP-2 antigen induced a significant increase in total antigen specific CD8+ cells. Further both the wild type and replication deficient MCMV vectors containing the ASP-2 antigen induced significantly increased antigen specific CD8+ IFNγ+ cells. These data indicate that CMV-vectored vaccines induce robust antigen specific T_H1 directed immune responses and can boost the immune response in the presence of chronic *T. cruzi* infection. This may translate to therapeutic efficacy against Chagas disease. Future studies will evaluate the efficacy of therapeutic vaccination with MCMV vectored vaccines against Chronic Chagasic cardiomyopathy.

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EFFECTS OF INSULIN-LIKE GROWTH FACTOR-I AND IL-4 ON *LEISHMANIA (L.) INFANTUM*-INFECTED HUMAN MACROPHAGES

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In *Leishmania* infection non-specific factors influence the development of the infection. We have shown previously the role of insulin-like growth factor-I (IGF-I) as an effector element of IL-4 response *L. (L.) major*-infected murine macrophages. In the absence of IGF-I the parasite growth promoting effect of IL-4 was nullified. In *Leishmania infantum* infection responsible for visceral leishmaniasis the role of IL-4 is controversial and it is not clearly related to susceptibility. Thus we aimed to evaluate the role of IL-4 and its relationship with IGF-I in *L. (L.) infantum* infection using human macrophages. Human monocyte cell line THP-1 ($5x10^5$ cells) was infected with stationary phase *L. (L.) infantum* promastigotes (8 parasites/cell) under stimulation with recombinant IL-4 (20 ng/mL) and recombinant IGF-I (50 ng/ml) that were maintained for 24 and 48 hours. The parasitism was evaluated by light microscopy, the *Igf-I* mRNA expression was quantified by Real Time (qPCR), and nitric oxide (NO) by Griess method and, arginase (*Arg1*) by qPCR and arginase activation by urea production. The parasitism did not show any increase in the parasite number after 24 and 48 hours under IL-4 and IGF-I stimuli in contradiction to what is seen in *L. major* infection. NO production and arginase activity remained constant during experimental period. The *Arg1* mRNA expression and *Igf-I* mRNA expression increased during the infection but IL-4 and IGF-I stimuli had not changed these expressions. Knowing from the previous study that IGF-I is directly related to expression and activation of arginase mainly on *Leishmania* leading to increase in the parasitism and decrease in NO production our present findings with *L. infantum*-infected human macrophages suggest that neither cell nor parasite arginase are critical to parasite development. Further NO production present during experimental period is seemingly not harmful to the parasite. In *L. infantum* infection, the parasite growth promoting effect of IGF-I, different from that observed in other cutaneous species of *Leishmania*, may explain any evident participation of IL-4 in *L. infantum* infection development.

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EXPRESSION OF COSTIMULATORY MOLECULES DURING VISCERAL LEISHMANIASIS AND AFTER CLINICAL CURE

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Visceral leishmaniasis (VL) is a parasitic disease caused mostly by *Leishmania infantum* or *L. donovani*. The clinical outcome of the infection is in part related to the immune response elicited by the pathogen. Costimulatory molecules provide the second signal necessary to initiate the immune response and can be used by the parasite leading to an anti-inflammatory profile. The aim of this work was to evaluate costimulators and ligands present in cells of people exposed to *L. infantum*. Principal Component Analysis (PCA) was performed based on flow cytometry data for costimulatory molecules expressed in lymphocytes and CD14+ monocytes, in whole blood samples, obtained from subjects with symptomatic VL (n=12), recovered VL (n=12) and healthy subjects from the Leishmania endemic area (n=8), in *ex vivo*, unstimulated or after soluble Leishmania antigen (SLA) stimulation. The first two components explained more than 50% of the variance (PC1 - 38% and PC2 - 18%). Variables CD4/CD28/OX40/ICOS (loading=-0.510) and CD8/CTLA4/OX40/ICOS (loading=-0.583) were the most important within PC1, whereas variables CD4/CTLA4/OX40/ICOS (loading=-0.510) and CD14/HLADR (loading=0.504) were the most important within PC2. The groups showed different profiles with regard the T-cell populations expressing multiple costimulatory molecules (*i.e.* CD4/CD28/OX40/ICOS; CD4/CTLA4/OX40/

ICOS; CD8/CD28/OX40/ICOS; CD8/CTLA4/OX40/ICOS), in *ex vivo* and *in vitro* conditions, and these variables were different among the groups (p=0.001). However, CD4 and CD8 lymphocytes, *ex vivo*, expressing single costimulators (CD28, CTLA4, OX40 and ICOS) showed similar profiles (p>0.05). The same was observed to monocytes CD14+ expressing CD80, ICOSL, CD40, HLADR and CD80/ICOSL simultaneously. Whereas, *in vitro* condition, CD28, CTLA4 and ICOS (in CD4 and CD8) from symptomatic VL showed a distinct profile of the other groups. Only CD14/CD80/ICOSL showed different profile between the groups in the same condition. In summary, T-cell populations expressing multiple co-stimulatory molecules are those that most contribute on explaining variability across groups evaluated.

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DROMEDARY CAMELS: GROWING ZOONOTIC DISEASE RISK AT THE HUMAN-LIVESTOCK-WILDLIFE INTERFACE

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Dromedary, or one-humped, camels (*Camelus dromedarius*) are an almost exclusively domesticated species that are common in arid areas as production animals for meat and milk. There are approximately 13 million dromedary camels in the world today, with heavy population concentrations in Africa and the Middle East. As a food animal and beast of burden, the hardiness of camels in arid regions means humans are often heavily dependent on camels as a stable protein source. Like other livestock, camels also carry and may transmit pathogens to humans. Such zoonotic disease transmission is a concern due to growing human populations, increasing demands for meat, lack of biosafety and biosecurity protocols in abattoirs and milking facilities, and increased interface with wildlife as camel herds become sympatric with these non-domestic species. Our review describes the major zoonotic diseases present in camels, their risk to humans, and recommendations to minimize spillover events. We conducted a literature review of camel-borne zoonotic diseases and found that the majority of journal articles focused on Middle Eastern respiratory syndrome (MERS), brucellosis, *Echinococcus granulosus*, and Rift Valley fever. The outbreaks of MERS in 2012-2016 and high case fatality have elicited an aggressive response from the research community in MERS related research. However, other camel-borne diseases such as *Yersinia pestis*, *Coxiella burnetii*, and Crimean-Congo hemorrhagic fever are just as important to include in surveillance efforts. Camel populations, particularly in sub-Saharan Africa, are increasing exponentially in number in response to prolonged droughts. Using Laikipia County in Kenya, where camel populations increased 835% over a 30-year period, we will show how increased camel numbers and subsequent land use change, coupled with an increased interface with dense, but declining wildlife populations, has created a hot spot for the risk of emerging infectious disease spillover.

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A GENERALIZABLE ONE HEALTH PATHWAY FOR THE CONTROL OF ZOONOTIC DISEASES

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Effectively controlling zoonotic diseases requires a One Health strategy that cross-cuts the disciplines of human, animal (both domestic and wildlife) and environmental health. In many countries, however, coordinating mechanisms that facilitate multi-sectoral collaboration are inadequate or lacking, which can severely limit country capacity to prevent and control zoonoses. Here we describe a framework that takes a One Health approach and is generalizable in application to the control of any zoonotic disease. Specifically, the framework includes three components. The first is a pathway that outlines a stepwise approach to managing zoonoses. This pathway includes four stages that guide a country through the process of zoonotic disease management: 1) establishing a list of prioritized diseases; 2) evaluating country capacity; 3) developing plans, protocols, and procedures to address priority diseases; and lastly, 4) monitoring and evaluating progress. The second component is a toolkit that compiles existing guidance documents and resources matched to the elements of the generalized pathway. The final component are vignettes of five frequently prioritized zoonoses (anthrax, brucellosis, zoonotic influenza viruses, rabies, and viral hemorrhagic fevers), which use schematics to illustrate recommended multi-sectoral surveillance and response strategies. This approach highlights the commonalities of following a One Health model to zoonotic disease management, while also providing specific recommendations for common zoonoses. Importantly, in compiling and arranging existing yet disparate guidance documents, this framework provides operational guidance, which enhances country-level capacity to prevent, detect, and respond to zoonotic disease threats.

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CYSTINET-AFRICA: A ONE-HEALTH NETWORK FIGHTING TAENIASIS/CYSTICERCOSIS IN SUB-SAHARAN AFRICA

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Taenia solium is the most important food-borne parasite globally. Sub-Saharan Africa is among the regions highly affected by this parasite, leading to significant human morbidities and agricultural losses. Cysticercosis Network of Sub-Saharan Africa (CYSTINET-Africa) is a one-health network fighting cysticercosis and taeniasis caused by *T. solium* (TSCT) in Sub-Saharan Africa through North-South and South-South collaboration in research and capacity building. The network consists of a multi-disciplinary team of six institutions, four in Africa (Sokoine University of Agriculture, Tanzania; National Institute for Medical Research, Tanzania; University of Zambia, Zambia; Eduardo Mondlane University, Mozambique) and two in Germany (both at the Technical University of Munich). This project started officially in October 2016 and will run for five years. The project aims at (1) Estimation of prevalence and co-infections of TSCT/neurocysticercosis (NCC) in large-scale community based studies in Tanzania, Mozambique and Zambia, (2) Evaluation of the pathomechanisms involved in the development of symptomatic NCC in immunocompetent and immunocompromised individuals as well as pathomechanisms involved in different treatment responses in people with symptomatic NCC on standard treatment in longitudinal studies in Tanzania and Mozambique, (3) Establishment of an experimental pig model for *T. solium* cysticercosis for human and veterinary research needs as well as investigation of pig breed susceptibility to *T. solium* in Zambia and (4) Development, evaluation and implementation of a locally adapted health education intervention package for the prevention and control of TSCT in Tanzania. Progress towards achieving the project objectives will be presented. CYSTINET-Africa will create impact by linking human, animal and community health with respect to zoonotic diseases at various levels in sub-Saharan Africa. A rapid expansion of this network and its activities as well as its linkage with similar initiatives are envisaged.

1291

SEROCONVERSION OF BATS IN NORTHEAST INDIA TO FILOVIRUSES AND HENIPAVIRUSES

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Bushmeat is an integral part of protein acquisition of communities worldwide. This practice increases the wildlife-human interface and provides opportunities for spillover of zoonotic pathogens into human populations. In Northeast India, there are several groups that hunt wildlife and in the state of Nagaland, bats are a prized not only for their protein, but also for their medicinal value. Bats are important vertebrate reservoirs for several medically-important families of viruses, including the filoviruses (ebolaviruses and marburgviruses) and henipaviruses (Nipah virus and Hendra virus). Here we screen sera from three species of pteropodid bats (*Cynopterus brachyotis*, *Eonycteris spelaea*, and *Rousettus leschenaultii*) that were harvested in Nagaland. We assessed seroreactivity using a multiplex bead-based assay that has glycoproteins in their native state for eight filoviruses (the five species in the genus Ebolaviruses, Marburg virus, Ravn virus, and Lloviu virus) and for three paramyxoviruses (Nipah virus, Hendra virus and Kumasi virus). Our findings indicate that all three species of bats had individuals that were seropositive for the ebolaviruses, but not Reston virus or Marburg virus. Individuals were often cross-reactive for multiple filovirus glycoproteins. There were individual bats that were seropositive to the glycoproteins of Ravn virus and Lloviu virus. One *E. spelaea* bat was seropositive to Kumasi virus and all three species had individuals that appear to be seropositive to henipaviruses, often cross-reactive to Nipah virus and Hendra virus. Pools of kidney, spleen and lung were screened for filoviruses with a nested RT-PCR, but all were negative. These results corroborate with a serology study in bats from Singapore and with the discovery of novel filoviruses in China from *Rousettus* and *Eonycteris* bats. It is likely there is much undiscovered filovirus and paramyxovirus genetic diversity in bats and expands the current geographic range of filoviruses.

1292

DETECTING SPECIES-SPECIFIC FECAL CONTAMINATION USING MICROBIAL SOURCE TRACKING MARKERS: A VALIDATION STUDY IN THE PERUVIAN AMAZON

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Fecal contamination and exposure to enteric pathogens within public and domestic domains is a well-known source infection and associated diarrheal disease. Traditional microbiologic methods are not able to discern human from non-human fecal contamination. However, the use of Microbial Source Tracking (MST) offer us a possibility to do so. However, the discriminatory power of these markers is site specific, requiring a performance evaluation to determine the sensitivity and specificity of each marker. The functional capacity of the LA35, Pig2Bac and BacCan MST primers and probes in laboratory conditions will be tested using real time PCR. We will use gBlocks as synthetic controls for each assay (IDT). Standard curves will be developed using serial dilutions (3×10^1 - 3×10^8) of gene copies/mL of the gBlock. We will test cross-reactivity by examining fresh feces from humans, chickens, ducks, parakeets, pigeons, goats, buffalos, pigs, dogs, and cats (10 of each species). We will calculate the sensitivity and specificity of the markers, as well as the positive and negative predictive values, as well as calculate the mean and standard deviation of log10 gene/copies per gram of feces. We are currently analyzing data. Results will be ready for the 2018 ASTMH conference.

1293

PREVALENCE AND GENOTYPING OF *LEPTOSPIRA* CARRIAGE OF RODENTS IN SOUTHERN AND NORTHEASTERN THAILAND

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We report data confirming that rodents serve as the primary natural reservoirs of pathogenic *Leptospira* causing leptospirosis. A total of 1,075 rodents collected from two regions known to have high incidences of human leptospirosis cases: Ranong in Southern and Sisaket in Northeast of Thailand. Rodent trapping was carried out in residential and around farming areas close to human habitation. *Leptospira* carriage was identified directly on kidney specimens from the trapped rodents using *lipL*32 real-time PCR and 16S rRNA sequence analysis. *Leptospira* isolation and genotyping were also performed for these rodent specimens. The *Leptospira* prevalence rate in rodents was 10 % (50/490) in Ranong and 1.7% (10/585) in Sisaket. We found rodents carrying *Leptospira interrogans* in both regions in the same proportion at 50% (25/50 in the South versus 5/10 in the Northeast). The proportion of *Leptospira borgpetersenii* carriage among rodents in the Northeast (5/10, 50%) is higher comparing to the South (18/50, 36%). The twelve strains isolated from rodents in Ranong were *L. interrogans*. The novel sequence types for all isolates were found by MLST analysis and separated into two characters. The first of eight isolates (66 %) exhibited a new combination of known alleles; while the other four additional isolates (33 %) were composed of putative novel allele sequences. *Rattus rattus* was the dominant carrier (80%) in both regions.

1294

BRUCELLOSIS AMONG PYREXIA OF UNKNOWN ORIGIN CASES IN AMSING JORABAT SUB-CENTRE, KAMRUP METRO, ASSAM, INDIA

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Globally, around 500,000 human brucellosis cases are reported every year. In India, burden of human brucellosis is likely underestimated because of limited laboratory capacity. We investigated to estimate brucellosis burden among pyrexia of unknown origin (PUO) patients attending Amsing Jorabat Sub-centre in Kamrup Metro, Assam, an area with 10% prevalence of animal brucellosis. We reviewed records of PUO patients attending Amsing Jorabat sub-centre from January 1 to October 31, 2014. We defined a case as brucella positive if Rose Bengal Plate Test (RBPT) or Serum Agglutination Test (SAT) or both were positive along with IgM or IgG positive. We reviewed records of routine animal surveillance by Assam Agriculture University to see if cases had brucella positive animals in their farms. We analysed data using EpiInfo 7.1. Among 46 PUO patients in 2014, 25 (54%) were Brucella positive. Median age of brucellosis patients was 33 years (range: 18-67 years). Among the 25 cases, 88% were males and 80% were dairy farmers. All 25 case-patients owned dairy farms or worked as farm attendants. Major presenting symptoms were arthralgia (68%), fever (48%) and night sweats (48%). Patients became ill mostly during March (28%) and July (24%). Most positive patients (68%) were from Shillongia village. All 25 case-patients were engaged in milking and 19 (76%) were engaged in handling abortus during birthing. Assam Agriculture University found at least one animal positive for Brucella spp in each farm with a case. We found over half of PUO cases were brucellosis

cases among dairy farm workers who had brucellosis positive animals in their farms. We recommend multisector coordination involving health and veterinary departments to improve surveillance and control of brucellosis.

1295

USING BEHAVIORAL AND SENTINEL SITE SURVEILLANCE TO ESTIMATE ACUTE LEPTOSPIROSIS PERIOD PREVALENCE IN NORTHERN TANZANIA

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Our understanding of the burden of human leptospirosis is hampered by few robust estimates of incidence and crude means of modelling data from sentinel sites to other locations. We sought to explore the use of cross-sectional community behavioural surveillance to estimate leptospirosis occurrence based on exposure to locally relevant risk factors. We previously identified leptospirosis risk factors through a case-control study among patients who attended hospital with fever at a sentinel site in Moshi, Tanzania. We also conducted a cross-sectional study among randomly selected livestock-owning community members from six districts in northern Tanzania: Hai, Longido, Monduli, Moshi Municipal, Moshi Rural and Rombo. We administered a standardised questionnaire to participants asking about leptospirosis risk factors and the presence of fever within the previous two weeks. We analysed results by district. We estimated the probability of leptospirosis within the previous two weeks by applying coefficients from the multivariable model of risk factors for leptospirosis derived from our sentinel site to cross-sectional study participants who had experienced fever. We recruited 286 community participants. The mean predicted probability (95% confidence interval) of leptospirosis among livestock owners in each district was: Hai District 0.8% (0.0-1.8%), Longido District 1.6% (1.2-2.1%), Monduli District 1.6% (1.2-2.0%), Moshi Rural District 0.4% (0.1-0.8%), Moshi Municipal District 0.6% (0.4-0.8%), and Rombo District 0.9% (0.0-1.9%). Assuming the risk factors for leptospirosis that we established among febrile patients in Moshi convey similar risk in different districts, the two-week period prevalence among livestock owners, and therefore leptospirosis incidence is likely to be higher in Monduli District and Longido District than in MoshiMunicipal District or Moshi Rural District. Given the challenges of identifying confirmed cases of human leptospirosis, this approach may provide a useful way of estimating human leptospirosis risk in communities where surveillance of cases is challenging.

1296

HABITAT FRAGMENTATION AND LAND-USE CHANGE AS DRIVERS OF YELLOW FEVER OUTBREAKS IN SOUTH AMERICA

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Yellow fever (YF) is an arbovirus of the family flaviviridae found in Africa and South America. In South America YF is transmitted in two cycles: a jungle cycle between non-human primates and sylvatic *Haemogogus* and *Sabethes* mosquitoes, and an urban cycle propagated by *Aedes aegypti*. Since 1942, nearly all YF cases in South America have been due to the sylvatic cycle. Habitat fragmentation and land use change have been shown to alter zoonotic disease transmission dynamics, though the link to YF has only been postulated. Here we investigate the role of land-use change and habitat fragmentation on the inter-annual occurrence of YF. We fitted a series of random forest and logistic regression models to YF occurrence at the first administrative division to time-series data (2003-2012) and averaged values over this period of a number of covariates measuring land-use, habitat fragmentation and climate. The best performing models were combined to produce a weighted time-series and static model. These were evaluated using a spatial block bootstrapping method. Both time-series and static weighted models had a high out-of-sample predictive ability (AUC = 0.92 and 0.94). The most influential covariates were habitat fragmentation, cropland, the change in cropland over time and forest cover. Cropland, along with forest cover, appeared to be protective against YF reports - however habitat fragmentation and increases in cropland from previous years heightened the risk. This highlights a transition period where the risk of sylvatic spillover is greatest, and suggests that increased habitat conversion and fragmentation will increase the risk of YF cases. These findings may help to explain Brazil's ongoing largest YF outbreak since records began which has occurred outside the traditional endemic zone, but in areas with increasing cropland and deforestation. Applications of these findings may guide proactive vaccination or surveillance in areas where the risk of YF will increase with land conversion. By guarding against the expansion of YF, this could reduce the risk of large scale outbreaks such as those seen recently in Brazil, Angola and Nigeria.

1297

SEROPREVALENCE OF BRUCELLOSIS AND ASSOCIATED RISK FACTORS IN HUMANS AND LIVESTOCK IN HOIMA, UGANDA

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Zoonoses such as brucellosis may be significant contributors to non-malarial febrile illness in sub-Saharan Africa. Persons presenting with an acute febrile illness are often empirically treated for malaria, and many cases never reach medical attention. We described the seroprevalence of brucellosis and associated risk factors for seropositivity among people and livestock in western, rural Uganda. This was a cross-sectional study to assess seroprevalence of *Brucella* in Hoima, Uganda. We used random sampling to select households within two distinct communities. We tested participants' serum for *Brucella* IgG by ELISA; in livestock we used Rose Bengal Test.We administered surveys to collect potential demographic risk factors. We enrolled 286 adults from 194 households and tested 77 cattle and 284 goats. Seroprevalence of *Brucella* among households was 6.7% (13/194; 95%CI: 3.6-11.2%); when equivocal results were included, this increased to 19.1% (37/194; 95%CI: 13.7-25.3%).). Seroprevalence in cattle was 7.8% (6/77). No goats tested positive. Of the 37 households with positive/equivocal results, 49% (18/37) reported fever in the prior year. Of those with prior fever, 44% reported diagnosis of malaria and 31% reported never having a final diagnosis. None of the behavioral risk factors, including farming practice or consumption of dairy products, were statistically significant. Interestingly, 90% of households denied consumption of local dairy products. *Brucella* seroprevalence in humans was comparable to other studies estimating 8-14% and correlated with cattle seroprevalence. Despite frequent reports of fevers, many never received a diagnosis or were diagnosed with malaria. We found no statistically significant risk factors associated with *Brucella* seropositivity; however, there were relatively low numbers of reported behaviors traditionally linked to brucellosis. Our findings suggest that brucellosis may

contribute to non-malarial febrile illness in Uganda and should remain on the differential for febrile illness, even if patients deny risk factors typically associated with *Brucella* transmission.

1298

ACUTE RESPIRATORY INFECTION SURVEILLANCE IN A PHASE 2 PROSPECTIVE, COMMUNITY-BASED COHORT IN VIENTIANE, LAOS

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Emerging and re-emerging infectious disease pathogens are a bane to public health in both developed and underdeveloped nations. Respiratory infections caused by emerging and re-emerging viruses are among the leading causes of morbidity across the globe. A prospective, community-based cohort study in Laos to elucidate Acute Respiratory Infections across 25 villages in Vientiane Province has been ongoing since 2013. Enrollment criteria include participants ages >6 months who have lived in the same village for at least six months. Active case detection for influenza-like (ILI) symptoms is obtained via weekly phone calls to participants' homes. Those reporting symptoms were visited at home during which demographics and symptoms were recorded and nasal pharyngeal swabs and throat swabs taken. Specimens were analyzed using Real-Time PCR detection for 24 respiratory pathogens. To date, a total of 1,070 households have been enrolled, 973 cases of ILI reported, and 543 visits performed. A majority of the samples (61.6%) were from female participants. Of 540 PCR results, 22 different disease pathogens were detected. The majority of pathogens detected were FluA (22.04%), followed by rhinovirus (20.19%), human metapneumovirus (13.33%), and *S. pneumoniae* (18.7%). Results of this study help characterize the incidence and burden of respiratory pathogens among villagers in Laos in a country that normally has a tropical environment. The disease trends reported in this study are difficult to attribute to seasonal changes in Vientiane Province as FluA was more common from July to October. ILI symptom significance in this population and location provide impetus to continue monitoring viral pathogens in Southeast Asia.

1299

USE OF PULSE OXIMETRY FOR DIAGNOSIS OF HYPOXEMIA AND MONITORING OF CHILDREN WITH PNEUMONIA: A DESCRIPTIVE STUDY FROM ETHIOPIA

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Pneumonia is a leading cause of mortality among children under-five in Ethiopia and globally. Hypoxemia, low oxygen concentration in the blood is a fatal condition associated with pneumonia and a strong predictor of mortality. Pulse oximetry is recommended for detecting hypoxemia though the extent of its use is unknown in Ethiopia. We conducted a study to assess how often pulse oximetry was used among children diagnosed with pneumonia in 14 Ethiopian public hospitals. The primary outcome of interest was documentation of an arterial blood oxygen saturation measurement at diagnosis and during routine patient monitoring among children admitted to the pediatric ward. Medical records of 443 children aged 0-59 months with a primary diagnosis of pneumonia and seen at the hospitals between February 1, 2016 and April 30, 2017 were randomly selected for review. Data collectors consisted of three individuals with post-graduate degrees and who are familiar with clinical services and documentation practices in the hospitals. A structured checklist was employed to gather data on the use of pulse oximetry. Data was entered using Epi Info and analyzed using SPSS. Out of the 443 charts reviewed, 245 (55.3%) were diagnosed with pneumonia while the remaining 198 (44.7%) had severe pneumonia. Forty four (10.2%) children had documented oxygen saturation measurements at diagnosis. None of the children seen at primary hospitals had pulse oximetry assessments at diagnosis. Thirty one (14.8%) and 14 (12.6%) children seen at general and referral hospitals, respectively, had oxygen saturation measurements at diagnosis. Thirty eight children (19.2%) with a diagnosis of severe pneumonia had pulse oximetry measured at diagnosis compared to 7 (2.9%) children with a diagnosis of non-severe pneumonia. Out of 196 children admitted to the pediatric wards, 87 (43.9%) had measurements of pulse oximetry for monitoring purposes. There is limited use of pulse oximetry for diagnosis of hypoxemia and patient monitoring in hospital settings in Ethiopia. Efforts to increase consistent use of pulse oximetry by health workers will be important to improve child survival in Ethiopia.

1300

CLINICAL CHARACTERISTICS OF PATIENTS ADMITTED TO PNEUMONIC PLAGUE TREATMENT CENTERS DURING THE 2017 MADAGASCAR PNEUMONIC PLAGUE OUTBREAK: A PROSPECTIVE OBSERVATIONAL STUDY

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In August 2017, an urban pneumonic plague outbreak began in Madagascar. Pneumonic plague is caused by inhalation of *Yersinia pestis*, a gram-negative bacterium. It is classically described as presenting with abrupt onset of fever and rapid, fulminant, progression of respiratory symptoms. The clinical data on pneumonic plague is limited however, and predominantly based on retrospective case reports and small case series. Early in the outbreak there were reports of apparent atypical presentations, including patients presenting with prolonged duration of symptoms prior to admission. There were several potential reasons for this, including a more diverse clinical spectrum than that described in the literature, the use of antibiotics in the community, co-infection with other pathogens, and poor specificity of the F1 rapid antigen test (RAT) on sputum for diagnosing pneumonic plague. The main objectives of this prospective study therefore were to characterise the clinical presentation of patients admitted to pneumonic plague treatment centres, and identify factors associated with atypical presentations. All patients admitted to pneumonic plague treatment centres with a diagnosis of suspected pneumonic plague were eligible for inclusion. Clinical data was collected at admission and daily until discharge. The following laboratory tests were performed at admission: sputum culture; blood culture; sputum PCR and RAT for *Y.pestis*; blood PCR for *Y.pestis*; nasopharyngeal swab PCR for viral pathogens; urine and serum antibiotic levels (septrin, gentamicin, ciprofloxacin); CBC and CRP; *Y.pestis* serology (and at discharge). Patient recruitment started on the 2nd November 2017 and ended on the 31st of December 2017. Data from the study will be presented.

1301

PREDICTORS OF *STREPTOCOCCUS PNEUMONIAE* CARRIAGE AND ESTIMATES OF PCV10 VACCINE EFFECTIVENESS IN RURAL PAKISTAN

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Background and aimsKnowing predictors of S. pneumococcus (SP) carriage in developing settings is important for introduction of PCV. Further estimates of vaccine effectiveness against carriage in such settings is unknown. MethodsAn ongoing NP carriage survey near Karachi, Pakistan randomly selects 60 infants per month and gathers information on illness indicators, socioeconomic status, and household demographics. Multiple logistic regression models were fit to explore potential predictors of vaccine-type (VT) carriage as well as to estimate the vaccine efficacy in this setting. ResultsVT carriage was positively associated with having runny nose in the previous two weeks (OR: 1.893; 95% CI: 1.41-2.55). Factors negatively associated with VT carriage were: parental education >6 years and fever in the previous two weeks (OR: 0.348; 95% CI: 0.19-0.64, and 0.686; 95% CI: 0.51-0.93, respectively). Three doses of PCV10 showed a significant vaccine efficacy for VT carriage of 0.386 (95% CI: 0.08-0.59) adjusted for various demographic factors. ConclusionsPCV10 demonstrated efficacy for NP carriage in our model in rural Pakistan. As in similar settings, increased parental education was negatively associated with SP carriage, while symptoms (runny nose in the previous 2 weeks) was positively associated with carriage.

1302

ESTIMATION OF A PREDICTIVE FUNCTION FOR MMR VACCINATION BEHAVIOR AS A FUNCTION OF YEAR AND AGE

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Measles is a highly contagious viral disease that can potentially cause pneumonia, encephalitis, and even death. Measles transmission is currently on the rise globally and is recently facing situations of lower rates of vaccination. If such low rates persist, the increasing number of unvaccinated individuals will lead to worsening outbreaks and could possibly even lead to the re-establishment of endemic measles if vaccine coverage deteriorates to a large enough extent. We developed a model designed to predict the probability an individual has received at least one dose of MMR vaccine as a function to their age, the current year, and their demographic characteristics. We formulated this model as a bivariate B-spline hazard function in two continuous dimensions: year and age. Refinements of this model that depend on demographic characteristics were explored, with particular demographic features used in the model being chosen on the basis of model selection with the Akaike information criterion. The model was fitted to three datasets published by the Centers for Disease Control and Prevention that reflect surveys carried out in a subset of the population for infants, toddlers, and teenagers, mostly at the state level, and stratified as a function of demographic characteristics. The model's geographical detail provided insight about which parts of the country might be more susceptible to outbreaks and in need of higher vaccination coverage. The model we developed has implications for estimation of vaccination coverage for measles and helps extract nuanced information about vaccination behavior from publically available data sources.

1303

BACTERIAL AETIOLOGY, ANTIBIOTIC SUSCEPTIBILITY, AND OUTCOMES AMONG HOSPITALIZED UGANDAN CHILDREN DIAGNOSED WITH PNEUMONIA

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Pneumonia accounts for about 10-30% of childhood deaths in Uganda. Emerging resistance to antibiotics commonly used for treatment of bacterial pneumonia in Uganda is of concern. We report on the bacterial aetiology and their susceptibility profile, and mortality rates among children hospitalized with pneumonia. In 2016, an Acute Febrile Illness (AFI) sentinel surveillance programme started in the Paediatrics wards of six reference hospitals in Uganda including Jinja, Arua, Mubende, Kabale, Tororo, and Apac. All children ≤14 years with a history of fever or temperature ≥37.5˚C were tested for malaria by microscopy or rapid diagnostic test. Those with a negative malaria test result got a blood draw for culture. Samples were incubated in BACTEC machines at the hospitals. Positive samples were sent to a core laboratory at Makerere University for sub-culturing. Antimicrobial Susceptibility Testing (AST) was done using Kirby-Bauer disk diffusion method. Pneumonia was diagnosed using the Integrated Management of Childhood Illness (IMCI) guidelines. Between July 2016 and January 2018, 21,207 children were hospitalized. 4,429 (20.9%) of them had pneumonia as the primary diagnosis. 50% of children with a diagnosis of pneumonia were between 1 and 4 years of age, while 40% were below 1 year. Blood cultures were performed on 1,272 patients with pneumonia and 22 (1.7%) yielded a pathogen. Staphylococcus aureus (6, 27.3%), Salmonella species (5, 22.7%), and Streptococcus pneumoniae (3,13.6%) were the commonest bacteria associated with pneumonia cases. ceftriaxone (18.2%), gentamicin (40.8%), and ampicillin (22.3%) were the most commonly prescribed antibiotics for treatment of pneumonia. 66.7% of S. aureus organisms were sensitive to both oxacillin and gentamicin, whereas 33.3% of S. pneumoniae were sensitive to ceftriaxone. Salmonella spp were 80% sensitive to Ceftriaxone.The case fatality rate was 5.6%. A proportion of the bacteria showed resistance to commonly prescribed antibiotics. There is need to continue surveillance of the bacterial etiology of pneumonia and susceptibility patterns to commonly used antibiotics.

1304

EVALUATING CHANGES IN PNEUMONIA INCIDENCE AFTER TWENTY YEARS IN CHILDREN AGED 2-59 MONTHS IN OSHIKHANDASS, PAKISTAN: A COMMUNITY-BASED STUDY

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Pneumonia and diarrhea were the main causes of childhood mortality from 1989-1991 in Oshikhandass, a rural village in the Karakorum mountains of northeast Pakistan. Research Workers (RWs) trained in pneumonia management did weekly surveillance of children 2-59 months from 1992-1996 and again from 2012-2014 to evaluate changes in pneumonia incidence. RWs classified pneumonia cases by severity according to WHO Integrated Management of Childhood Illness criteria, gave treatment or referred, and followed-up until recovery. In the first study, 1252 children were followed for 2180 child-years (CY); 42.9% had at least one episode, and 7 children died from pneumonia. In the second study, 979 children were followed for 953 CY; 19.7% had at least one episode, and none died from pneumonia. Between the two study periods, the incidence of pneumonia decreased from 0.5 to 0.3 episodes/CY (RR: 0.6 second v. first study, 95% CI: 0.5-0.6), and prevalence decreased from 2.5 to 1.6 days of pneumonia/CY (RR:0.6, 95% CI: 0.6-0.7). The mean duration of illness before seeking care was consistent between the two studies at 2.4 days. From 1992-1996, 3.7% of pneumonia cases were severe and 1% were very severe (i.e. had danger signs per WHO criteria). In the second study, 3.6% were severe and no cases were very severe. Incidence was highest in children 2-5 months of age in the first study (0.9/CY, 95% CI: 0.7-1.0), and in children 6-11 months of age in the second study (0.5/CY, 95% CI: 0.3-0.6). In the first study 8.4% of children with pneumonia were reported to have prior use of antibiotics, with 5.5% of cases referred for additional care, 2.7% hospitalized, and 99% cured. In the second study 52.6% of children were reported to have prior antibiotic use, with 2.4% of cases referred and hospitalized, and 99.6% cured (0.4% lost to follow-up). In both studies the highest rates of pneumonia occurred in the winter months from November to March. Over the 20-year period, cure rates remained high, while incidence, prevalence and mortality decreased substantially between the two studies. Reasons for the decrease are being explored and could include introduction of HIB vaccine and improved SES.

1305

MODELING MEASLES IMPORTATION PATTERNS INTO THE UNITED STATES USING PUBLIC HEALTH SURVEILLANCE AND AIRLINE TRAVEL DATA

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Despite the declared elimination of endemic measles in the United States in 2000, the number of measles cases and outbreaks in the United States has begun to rise in recent years, as measles-mumps-rubella (MMR) vaccination rates in the US are declining and transmission internationally is on the rise. Measles is a highly infectious illness that can cause symptoms such as high fever, acute rash, and even death in susceptible individuals. Cases imported to the US often result in outbreaks that affect vulnerable populations, including children who are too young to be vaccinated. The majority of these imported cases reach the US via international passenger air travel through major metropolitan hubs. Our goal was to use air travel data in conjunction with measles data reported by the World Health Organization at the country-level worldwide and state-level data on imported measles cases in the US to develop an explanatory model for patterns of measles importation into the US. We focused on using maximum likelihood to estimate numerical values for quantitative parameters that scale estimated risk of measles among international travelers arriving in the US to numbers of observed cases imported into the US. With these parameter estimates, we used our fitted model to establish country-specific importation probabilities and to document fluctuations in the relative probability of importation into the US from different countries over time. These estimates were found to have sufficient predictive capability to enable nowcasting of possible measles importation into the US on the basis of updated measles surveillance data from the WHO and to inform models of measles outbreak dynamics in the US.

1306

ACUTE RESPIRATORY VIRAL INFECTIONS: AN IMPORTANT CAUSE OF ADMISSIONS FOR ACUTE FEBRILE ILLNESS IN THE SOUTHERN PROVINCE, SRI LANKA

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Respiratory virus contribution to acute febrile illness (AFI) varies globally. The objective of this study was to determine the prevalence, etiology, and seasonality of viral respiratory illness among hospitalized AFI patients in Sri Lanka. We enrolled consecutive AFI patients ≥1 year admitted to the largest hospital in the Southern Province from Jun 2012-Oct 2014. We collected epidemiologic and clinical data and a nasal/nasopharyngeal sample and tested alternate samples using multiplex polymerase chain reaction (Luminex NxTAG Respiratory Pathogen Panel; 19 viruses, 3 bacteria). We determined correlation between weather data and the monthly proportion of respiratory viral AFI using the Spearman correlation. We assessed seasonality of respiratory viruses using a Program for Appropriate Technology definition (monthly positive cases divided by annual positive cases; 'peak' defined as monthly proportion ≥10% for two consecutive years). A total of 964 patients were enrolled; median age was 26.2 years (IQR 14.6- 39.9 years) and 646 (67.0%) were male. Approximately one-fifth (205, 21.3%) tested positive for a respiratory pathogen. The most common pathogen detected was influenza (134, 13.9% of all patients), with 9.1% having influenza A. Other viruses identified included human enterovirus/ rhinovirus (13, 1.4% of all patients), parainfluenza virus (13, 1.4%), respiratory syncytial virus (11, 1.1%), human metapneumovirus (11, 1.1%), and bocavirus (0.7%). Two patients had a bacterial respiratory pathogen (1 *Mycoplasma pneumoniae*, 1 *Chlamydophila pneumoniae*) and 7 (0.7%) had respiratory viral co-infections. The proportion of monthly respiratory viral cases was associated with maximum daily temperature (p=0.03) but not with minimum daily temperature, rainfall, or daytime or nighttime humidity. Respiratory virus and influenza positivity peaked in February- June of each year (excluding March). We identified respiratory viruses in a large proportion of patients admitted with AFI in southern Sri Lanka, with clear seasonality. Improved preventative efforts through targeted influenza vaccination may help reduce burden of illness.

1307

THE USE OF SMOKE BASED MOSQUITO PREVENTION METHODS: A RISK FACTOR FOR ACUTE RESPIRATORY INFECTIONS IN CHILDREN?

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Globally, Acute Respiratory Infections (ARI) are a leading cause of morbidity and mortality among children <5 years of age. The majority of the ARI disease burden exists in the low income countries, with particularly high

mortality rates in sub-Saharan Africa. Exposure to indoor air pollution (IAP) is an established risk factor for ARI. Most research on IAP and respiratory health has focused on environmental tobacco smoke and cooking indoors with biofuel. The use of smoke-based mosquito prevention methods (ex. coils) has received little attention, despite the estimate that 40-50 billion mosquito coils are used annually by 2 billion people worldwide. To our knowledge, no studies investigating the association between mosquito prevention methods and respiratory health have been conducted in sub-Saharan Africa. Using data from a cross sectional survey conducted in high (>1500 m) and low (<1500 m) altitude sites in rural western Kenya, we investigate the relationship between smoke-based mosquito control measures (ex. mosquito coils) and ARI in children <12 years of age. Households completed detailed surveys on knowledge, attitudes and practices around malaria as well as illness prevalence among household members. The study sample consisted of 1,217 households with 1,669 children <12 years of age. ARI, defined in this study as the presence of a cough within 2 weeks of the survey, was present in 33% percent of children <12. Nine (9) percent of households reported using smoke-based mosquito control methods, 88% reported use of other methods (ex. bednets), and 2% reported using no methods. Significantly more lowland households used smoke-based methods than highland households (16% vs. 4%). There were no significant differences in ARI among children <12 between high and low altitude sites. Preliminary analyses suggest no significant difference in ARI between children in households using smoke based vs all other mosquito prevention methods (including none). However, prevalence of ARI was quite high compared with national averages, illuminating the need for further evaluations of potential risk factors associated with ARI in this study sample.

1308

SENSITIVITY AND SPECIFICITY OF POINT-OF-CARE CIRCULATING CATHODIC ANTIGEN TEST BEFORE AND AFTER PRAZIQUANTEL TREATMENT IN DIAGNOSING *SCHISTOSOMA MANSONI* INFECTION IN ADULT POPULATION CO-INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS-1, NORTHWESTERN TANZANIA

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The effect of HIV-1 on $CD4^+$ Th_2 cells is hypothesized to affect parasitological diagnosis of *Schistosoma mansoni* using Kato Katz technique. Thus, the use of more sensitive technique such as Point-of-Care Circulating Cathodic Antigen test is recommended. The present study assessed the sensitivity and specificity of the point-of-care Circulating Cathodic Antigen test in diagnosing *S. mansoni* infection before and after praziquantel treatment in adult population co-infected with HIV-1. A prospective longitudinal study was conducted among individuals aged 15-55 years at Igalagala village, north-western Tanzania. At baseline and four weeks after treatment, a single stool and urine samples were collected from each participants. Kato Katz (KK) technique and Point-of-Care Circulating Cathodic Antigen tests (POC-CCA) were used for diagnosis of *Schistosoma mansoni.* At baseline, based on KK and POC-CCA, the prevalence of *S. mansoni* was 57.8% (95%CI: 52.9 – 62.4) and 87.5% (95%CI: 83.9 – 90.4). The prevalence of HIV-1 infection was 6.9% (95%CI: 4.8 – 9.8). Based on KK technique and POC-CCA test, 3.6% and 5.7% of the study participants were co-infected with *S. mansoni* and HIV-1. Four weeks after treatment, based on KK technique and POC-CCA, the prevalence of *S. mansoni* was 17.6% and 28.7%. At baseline the sensitivity and specificity of POC-CCA were 96.3% (95%CI: 93.1-98.3) and 24.6% (95%CI: 18.4-31.6). In the HIV-1 seropositive group, the sensitivity and specificity of POC-CCA were 93.3% (95%CI: 68.1-99.8) and 28.6% (95%CI: 8.4 – 58.1). Four weeks after treatment, the sensitivity and specificity of POC-CCA were 47.8% (95%CI: 26.8 – 69.4) and 74.7% (95%CI: 67.9 – 80.8). In conclusion, the sensitivity of POC-CCA in diagnosing *S. mansoni* infection is high than KK technique in adult individuals likely to have low infection intensity and co-infected with HIV-1. However, its sensitivity decreases following praziquantel treatment but remained higher then Kato Katz technique. If the goal of the post-treatment is to identify uncured individuals, then POC-CCA test offers the best choice.

1309

REPEATED DOSES OF PRAZIQUANTEL IN SCHISTOSOMIASIS TREATMENT (REPST) - SINGLE VERSUS MULTIPLE PRAZIQUANTEL TREATMENTS IN SCHOOL-AGE CHILDREN IN CÔTE D'IVOIRE: A STUDY PROTOCOL FOR AN OPEN-LABEL, RANDOMIZED CONTROLLED TRIAL

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Large scale administration of the anthelminthic drug praziquantel (PZQ) to at-risk populations is the cornerstone of schistosomiasis control, although persisting high prevalence of infections in some areas and growing concerns of PZQ resistance have revealed the limitations of this strategy. Most studies assessing PZQ efficacy have used relatively insensitive parasitological diagnostics, such as the KatoKatz (KK) and urine-filtration (UF) methods, thereby overestimating cure rates (CRs). This study aims to determine the efficacy of repeated PZQ treatments against *Schistosoma mansoni* infection in school-age children in Côte d'Ivoire using the traditional KK thick smear technique, as well as more sensitive antigen- and DNA detection methods. To determine the efficacy of multiple PZQ treatments, an open-label, randomized controlled trial will be conducted in the region of Taabo in Côte d'Ivoire, an area endemic for *S. mansoni*. This 8-week trial includes four two-weekly standard doses of PZQ in the "intense treatment" intervention group and one standard dose of PZQ in the "standard treatment" control group. The efficacy of PZQ will be evaluated in stool samples using the KK technique and real-time PCR as well as in urine using the point-of-care circulating cathodic antigen (POC-CCA) test and the up-converting phosphor, lateral flow, circulating anodic antigen (UCP-LF CAA) assay. The primary outcome of the study will be the difference in CR of intense versus standard treatment with PZQ on individuals previously positive for *S. mansoni* measured by KK. Secondary outcomes include the difference in CR and intensity reduction rate (IRR) between the intense and standard treatment groups as measured by the other diagnostic tests, as well as the accuracy of the different diagnostic tests, and the safety of PZQ. This study will provide data on the efficacy of intense PZQ treatment on the clearance of S. mansoni, measured with several diagnostic techniques. These findings will inform future mass drug administration (MDA) policy and shed light on position of novel diagnostic tools to evaluate schistosomiasis control strategies.

1310

ULTRASONOGRAPHIC EVIDENCE OF HEPATIC DISEASE DUE TO *SCHISTOSOMA MANSONI* IN CHILDREN IN THE MAROLAMBO DISTRICT, MADAGASCAR

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We present a follow-up study investigating schistosomiasis-related, hepatic morbidity in children in Madagascar (Marolambo District) previously reported to have a high prevalence of *Schistosoma mansoni* infection. Circulating cathodic antigen (CCA) and Kato-Katz stool analysis were used to test 298 children (5-14 years old) for *S. mansoni*. Abdominal ultrasound (US) was performed and interpreted based on the Niamey Protocol. In CCA analysis, 97.7% of children (291/298) were infected with *S. mansoni*. Kato-Katz analysis revealed heavy infections in 18.1% (54/298) of the children. Preliminary US data show that an estimated 5 % of children have early signs of liver fibrosis. Persistent high prevalence of childhood intestinal schistosomiasis in this area of Madagascar appears associated with hepatic complications in a small, but clinically significant minority.

1311

BILHARZIOSIS IN MIGRATION MEDICINE - AN UNDERESTIMATED PROBLEM?

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Bilharziosis is one of the most common infectious diseases in the world, with a prevalence of about 200 million people. Up to 200k people die annually, mainly as a result of late complications, such as Liver cirrhosis, hepatocellular or bladder carcinoma.The question of whether an intestinal invasive schistosomiasis with liver or pulmonary involvement is present, has significant consequences for the person concerned with regard to long-term health.Patients with confirmed intestinal schistosomiasis (proven by positive stool microscopy) were assigned and examined to explore hepatic and / or pulmonary manifestations. Lower intestinoscopy was done in every patient, addtionally, abdominal and cardiac ultrasound were performed. In case of suspect findings, liver biopsy was also done. The results from these tests were compiled to assess the extent of invasive infection. Of 23 patients treated, 22 (95.7%) were male and one female (4.3%). Schistosoma mansonii was detected in 22 patients and *Schistosoma haematobium* in one patient (excluded from further analysis). In all patients, schistosomalantibodies were detected in the blood, 20 patients (95.2%) had signs endoscopic of intestinal schistosomiasis, histological evidence was proven in 15 patients (71.4%). Sonographic abnormalities of the liver were seen in 15 patients (68.2%), with increased echogenic liver parenchyma (45.5%) or signs of pericholangiolar fibrosis (22.7%). Histologically, hepatic involvement was confirmed in 15 patients (83.3%). Elevated liver enzymes were detected in only one patient, cholestasis parameters in five (21.7%). Furthermore, 17 patients (90%) had marked increased immunoglobulin E was increased, more than half of the patients presented with blood eosinophilia (52.2%). All patients were successfully treated with praziquantel (stool microscopy negative four weeks after treatment).The high level of invasive infections in the cohort presented is striking. Obviously, strategies for the prevention and early detection of late complications have to be implemented in the care of patients with bilharziosis.

1312

OPTIMIZING STRATEGIES TO DIAGNOSE AND CONTROL INTESTINAL SCHISTOSOMIASIS IN A LOW AND MODERATE ENDEMICITY AREA OF BRAZIL

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Current diagnostic methods for intestinal schistosomiasis (detection of eggs in stool by the Kato-Katz (KK) method) are limited and may be particularly unreliable at low infection intensities, as would be expected after several rounds of treatment or in regions where transmission is low. In order to overcome some of the pitfalls of the KK method, new and more sensitive tests for the diagnosis of schistosomiasis have been developed. A point-of-care test for detecting schistosome circulating cathodic antigen (POC-CCA) has been documented to be a sensitive and specific alternative to KK through direct comparisons in several endemicity settings. However specific data comparing multiple KK over several days with antigen detection tests in low prevalence settings is currently lacking. The primary aim of this project is to compare the performance of POC-CCA with that of the three days of duplicate KK in addition to PCR, in an area with moderate *Schisosoma mansoni* infection in Sergipe State and low endemicity area of Minas Gerais State, in Brazil. The present study aims to enhance knowledge of the spatial distribution of schistosomiasis in these two states of Brazil, and generate data for a better planning of interventions and strategies within the National Brazilian Schistosomiasis Control Program (PCE), especially in areas with low endemicity and/or low parasite loads.

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PERFORMANCE OF POC-CCA AND KATO-KATZ TO DIAGNOSE *SCHISTOSOMIASIS MANSONI* IN LOW AND MODERATE ENDEMICITY AREAS OF BRAZIL

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Current diagnostic methods for intestinal schistosomiasis (detection of eggs in stool by Kato-Katz (KK)) are limited and may be particularly unreliable at low levels of infection, as would be expected after several rounds of treatment or in regions where transmission is low. In order to overcome some of the pitfalls of the KK method, there has been interest in developing more sensitive tests for the diagnosis of schistosomiasis. The Point-Of-Care (POC) Circulating Cathodic Antigen (CCA) urine assay (POC-CCA) has been documented to be a sensitive and specific alternative to KK in several moderate and high endemicity settings. Specific data comparing the performance of microscopy and antigen detection tests in low Schistosomiasis prevalence settings, however, is currently lacking. In this context, the aim of this work was to compare the performance of POC-CCA and KK in the urine and stool samples of school-aged children (SAC) (5-16 years-old) from low endemic area of Minas Gerais State, and in a moderate endemic area of Sergipe State Brazil. For the KK assay, three stool samples were collected, and 2 KK slides were prepared per sample. Up to the present moment, of the 1,223 SAC surveyed in the state of Minas Gerais, 31 (2.5%) of the samples were positive for KK (intensity mean, 20 eggs mg/stool). However, when evaluated the results of the POC-CCA test, 222 (18.5%) samples were positive in Minas Gerais state. Moreover, of the 542 SAC surveyed until the moment in Sergipe state, 188 (35%) samples were positive by the POC-CCA test, considering trace as

positive. The frequency of POC-CCA positives in both areas, considering trace as negative were 96 (7,9%) and 114 (26,5%) respectively. KK results from Sergipe state are still being analysed. Taken together our results from Minas Gerais state indicate that POC-CCA is a more sensitive test than Kato-Katz in areas of low endemicity, even considering trace results as negative. Urine, stools and sera from all the individuals will be tested for other based tests (qPCR and UCP-CAA) for the calculation of sensitivity, specificity, and ultimately, to define the relationship between POC-CCA and KK at low endemicity areas.

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FIRST MAPPING OF HUMAN SCHISTOSOMIASIS IN BENIN: EVIDENCE OF COUNTRYWIDE *SCHISTOSOMA HAEMATOBIUM* PREDOMINANCE

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National mapping of schistosomiasis infections was conducted for the first time in national scale from 2013 to 2015. This mapping aimed to provide basic epidemiological data essential for the implementation of the national strategy against the neglected tropical diseases in the context of achieving the WHO target of controlling these infections by 2020. Parasitological surveys were conducted from 2013 to 2015 in three hundred eighty-five selected sampling units for baseline mapping in all districts (seventy-seven districts): Fifty school children (25 females and 25males) aged 8-14 years were randomly selected per site. A total of 19,000 samples (urine and stool) were examined using haematuria dipsticks, parasite-egg filtration for urine samples and the Kato-Katz technique for stool specimens. Two species of human schistomiasis were observed with intra- and inter-specific variations in the prevalence and the intensity of these parasites. Urinary schistosomiasis was widely distributed and present in 75/77 districts with an average prevalence of 17.56% (95° CI:17.02%-18.10%) at the national level compared to *Schistosoma mansoni* focused and found in 28/77 districts with 2.45% (95° CI:2.23%-2.67%) as national prevalence. The cumulative prevalence was 19.78% (95% CI:19.21%-20.34%) and 45/77 of the districts required a preventive chemotherapy treatment according to World Health Organization recommendations for schistosomiasis control. In several districts where the two species are endemic with prevalence more than 10%, *S. haematobium* were most prevalent parasites. Boys are significantly more infected than girls (18.29% v 16.82%, p=0.007). Most infestations were light except in the districts located in the department of Atacora where children were highly infected for *S. haematobium* (more than 50 eggs/10mL). In all districts surveyed, children are suffering more for diseases related to the lack of sanitation and hygiene than water related disease. This mapping study provided a global view of the epidemiological pattern of schistosomiasis, and was essential for the implementation of a control strategy and the establishment of sentinel sites.

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LOWER CURE RATE AND EGG REDUCTION RATES FOR *SCHISTOSOMA JAPONICUM* USING PRAZIQUANTEL AT 40 MG/KG DOSE

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Historically, Praziquantel dosing for *Schistosoma japonicum* (60 mg/kg) had been higher than doses recommended for *S. mansoni* and *S. haematobium* (both 40 mg kg). The higher dosing for *S. japonicum* was based on early efficacy and cure rates in studies conducted among Filipino adults. Recently, the World Health Organization changed their dosing recommendations to 40 mg/kg in response to a meta-analysis of studies examining cure rates (CR) and egg reduction rates (ERR) at both doses. Of note, only 4 of 55 studies used in the meta-analysis were conducted in *S. japonicum*endemic areas. In recent studies, we noted higher treatment failure rates at the lower dose than we had with 60 mg/kg dosing. The objectives of this study were to assess CR and ERR using 40 mg/kg of Praziquanel for *S. japonicum*, and compare these to a) WHO recommended targets and b) CR and ERR found in the same study area using 60 mg/kg in 2002. In this study, 301*S. japonicum* infected subjects ages 8-30 were enrolled and treated with 40 mg/kg Praziquantel. A Kato-Katz examination of stool (three stools each in duplicate) were conducted 4-5 weeks later. Overall CR at 40 mg/kg was 79.6% and the ERR was 87.6% (95%CI: 80.7, 94.5). Individuals with moderate or high intensity infection (N=33) had lower cure rates at 72.7% as compared to those with light intensity infection at 80.6%. ERR among those with moderate or high intensity infections were 99.1% (95%CI: 98.5-99.8) and 86% (95%CI: 78.2-93.9) among those with low intensity infections. There were no significant differences in ERR or CR by gender or age. In 2002, CR and ERR utilizing 60 mg/kg were 93.2% and 92.2 (95%CI 86.1-98.3) respectively, using similar methodology. The ERR found in recent studies using 40 mg/kg falls below the WHO recommended target of 90% and is lower than that found using 60 mg/kg dosing in the same study area, raising the concern that 40 mg/kg may be insufficient for the treatment of *S. japonicum.*

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EVALUATION OF SENSITIVITY AND SPECIFICITY OF CIRCULATING CATHODIC ANTIGEN IN THE CONTEXT OF *SCHISTOSOMA JAPONICUM* AT USING LATENT CLASS ANALYSIS

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The Kato Katz (KK) method of evaluating stool samples to quantify eggs remains the diagnostic approach for diagnosing *Schistosoma japonicum*. This method is costly, labor intensive, and demonstrates low sensitivity, particularly if fewer than three separate stools are evaluated. A point-of-care circulating cathodic antigen (CCA) test of urine has been shown to be more sensitive than KK in the context of *S. mansoni* and *S. haematobium*. Less is known about the test characteristics of CCA for *S. japonicum*. The use of latent class analysis (LCA) approaches has aided the evaluation of CCA, as this analytic tool is better able to evaluate sensitivity and specificity in the absence of a gold standard. In *S. japonicum* endemic villages in Leyte, The Philippines, 741 subjects were screened for the presence of *S. japonicum* by evaluating three separate stools, each in duplicate, by KK. Within one week of KK assessments, a CCA test was performed on a single urine sample. Of these 741, 291 subjects who were found to have *S. japonicum* were enrolled into a longitudinal study and treated with 40 mg/kg of Praziquantel, and both KK and CCA assessments were repeated 4 weeks after treatment. Prior to treatment, we found the prevalence of infection as assessed by KK was 30.6%. The prevalence as assessed by CCA was 77.3%. Using Bayesian LCA, the sensitivity and specificity of CCA were 94.5% [90.0-99.5] and 84.1% [42.1-98.5] and the sensitivity and specificity of three KK were 32.3% [27.9-36.4] and 76.4% [67.8-83.9]. Four weeks after treatment, by LCA, the sensitivity and specificity of CCA were 88.0% [79.1 - 98.9] and 71.0 [31.4 - 97.1], respectively and the sensitivity and specificity of three KK were 25.2 [19.2 - 36.8] and 88.8 [76.5 - 97.5] respectively. Overall, CCA was much more sensitive that KK in the diagnosis of *S. japonicum*. This point of care test may be used in conjunction with mass drug administration to reduce patient refusal related to lack of knowledge of infection status, in community surveys to more rapidly assess prevalence, and in areas approaching elimination where more sensitive tests are able to detect low intensity infections.

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COMPARISON OF THE EFFICACY OF TWO ANTIPARASITIC TREATMENT FOR SHEEP INFECTION WITH *FASCIOLA HEPATICA*

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Fasciolosis, a parasitic infection caused by *Fasciola hepatica*, mainly affects ruminants in endemic areas causing economic losses among affected population. Triclabendazole (TBZ) has been used as the first line treatment for controlling the disease; however, due to the increasing resistance, other antiparasites such as closantel (CST) has been used as an alternative. Efficacy of CST against adult stage has been assessed, but its efficacy against young stages is still be discussing. The current study aims to estimate the long-term efficacy of CST against TBZ. We randomly assigned 79 naturally infected sheep from endemic areas into two groups: 41 for of TBZ 10 mg/kg (Zolinex Dorado®, Biomont Lab) and 38 for CST 10 mg/kg (Prosantel®, Montana Lab), both administrated as a unique dose. The primary outcome was the cure rate at week 14th. , defined as the complete absence of eggs in stools samples and young/adults stages in the liver of necropsied sheep. Coproparasitological assessment was performed using the sedimentation technique. Egg count per gram of feces was calculated using the McMaster method. Detection of young/adults trematodes in liver was obtained by in situ observation during necropsy. Cure rate at week 14th in CST group (89.5%) was significantly higher than TBZ group (31.7%) (IRR = 2.8; CI 95% 1.7 to 4.5; $p < 0.001$). Mean (SD) number of trematodes in TBZ group was 31.6 (23.5) in comparison with CST group which has no one trematode in all sheep. Furthermore, despite that CST group has zero epg at week 4th, there were some eggs at week 8th that increased for the week 14th. Closantel and Triclabendazol showed a clear adulticidal action effect; however, the appearing of eggs after 8 weeks should be an indicator of poor effect among young stage.

1318

DIFFERENTIAL IMPACT OF MASS AND TARGETED DEWORMING CAMPAIGNS FOR SCHISTOSOMIASIS CONTROL IN CHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Chronic infections with *Schistosoma* species lead to significant morbidity among the world's poorest populations, including anaemia, malnutrition, and poor physical and cognitive development. Less frequently they can cause fatal complications. Regular delivery of praziquantel to school-aged children (SAC) is the principal strategy for schistosomiasis control. Recent studies suggest that if chemotherapy programs are delivered using a community-based approach they will achieve a higher coverage of SAC. Moreover, if they are expanded to treat entire communities, they may have an additional impact on transmission and eventually lead to elimination. This systematic review and meta-analysis aimed to compare the impact of community-wide and child-targeted administration of praziquantel on schistosomiasis prevalence in school-aged children. Studies reporting prevalence before and after child-targeted or community-wide treatment were identified searching MEDLINE, EMBASE, and Web of Science. Data extracted included *Schistosoma* species, drug administration strategy, drug dose, number of rounds of chemotherapy, treatment coverage, diagnostic method, follow-up interval, and schistosomiasis prevalence before and after treatment. Inverse variance weighted generalised linear models were used to examine the impact of community-wide vs child-targeted chemotherapy on prevalence reduction in school-aged children. We will present results of the generalised linear models, used to test the hypothesis that similar to a recently published meta-analysis comparing community-wide versus child-targeted deworming for control of soil-transmitted helminths, children will show a significantly greater prevalence reduction when praziquantel is administered to entire communities, compared to when it is administered to children only. The results of this meta-analysis may provide additional evidence to optimize current guidelines by expanding chemotherapy for schistosomiasis from child-targeted to community-wide treatment.

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DETECTING AND DISRUPTING ENVIRONMENTAL CONTAMINATION OF *SCHISTOSOMA MANSONI*

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For the estimated 240 million people across the tropics and sub-tropics infected with schistosomiasis, preventative chemotherapy with praziquantel is the primary control measure. Despite repeated mass drug administration with this tremocidal drug, schistosomiasis persists in highly endemic communities. Supplementary control measures to improve sanitation and/or access to cercariae-free water, could interrupt the free-living lifecycle stages and reduce this parasitic fluke's transmission. Our overall aim is to assess the potential for local, targeted engineering interventions to reduce environmental contamination by *Schistosoma mansoni.* To this end, our first objective was to develop a molecular assay to detect and quantify *S. mansoni* DNA in soil. Existing quantitative polymerase chain reaction (qPCR) assays were adapted to detect and quantify *S. mansoni* DNA from soil samples. To standardise the qPCR technique and determine the sensitivity of environmental DNA (eDNA) detection, *S. mansoni* egg-spiked soil samples of varying density were used. Thus, the lower limits of eDNA detection were ascertained enabling design of a field-based survey of environmental contamination. The field applicability of the qPCR assays were assessed in a high *S. mansoni* endemic area, proximal to Lake Victoria in Mayuge District, Uganda using environmental soil samples taken at increasing distances from open defecation and pit latrine sites. I will present the methods and preliminary results of this eDNA assay and their implications for local sanitation solutions and *S. mansoni* transmission.

1320

UNRAVELLING INTERACTIONS BETWEEN SCHISTOSOMES, THE MICROBIOME AND ANTI-HELMINTHIC DRUGS

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Schistosomiasis is a neglected tropical disease that infects millions of people globally mainly in developing countries. In Mayuge District, Uganda, *Schistosoma mansoni* infection remains prevalent and has been increasing in some communities despite over a decade of annual mass drug administration interventions. The influence the microbiome may have on health and disease has become increasingly apparent in recent years, with correlations being observed between gut bacterial composition and intestinal helminth infection status both in natural and laboratory environments. This research aims to quantify associations between *S. mansoni* infection intensity, gut microbiome community structure and efficacy of anti-helminthic drug treatment: hypothesizing that gut microbiome diversity will reflect schistosome infection intensity

and affect treatment efficacy. Initially, stool samples were collected from three individuals and stored raw, in RNAlater, and in ethanol to compare how the preservation method in a remote field setting may influence the 16S rRNA microbiome sequencing profiles obtained. These samples were then frozen at the time of collection (0 h) and at doubling incremental time points from 1 – 32 h to explore the impact of time taken to freeze on microbial diversity and abundance. A cohort of 200 school-aged children were then enrolled in a longitudinal study. Children provided stool samples for microbiome analysis pre-treatment and at 24 h, 3 wk and 6 mth post-treatment with praziquantel and albendazole. At each time interval children were also tested for schistosomiasis and soil-transmitted helminthiasis, and 24 h dietary recall surveys were undertaken to control for dietary variation. Initial pilot study data optimizing storage and analysis for microbiome will be presented. Preliminary findings from the longitudinal study, controlling for individual demography and co-infections, will then be discussed. This study has important implications for understanding individual health outcomes and informing how individual variation could influence onward transmission in a community highly endemic for *S. mansoni*.

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ECO-EPIDEMIOLOGICAL SURVEY OF URINARY SCHISTOSOMIASIS IN TOGO'S OGOU DISTRICT

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Schistosoma blood flukes are found on three continents, yet roughly 85% of the estimated 700 million people at risk of infection live in Africa. While preventative chemotherapy (PC) control strategies have proven largely successful, in 2016, two of Togo's 40 health districts experienced a significant rise in urinary schistosomiasis cases despite six years of mass administered PC. To ascertain the cause of this anomaly, several aspects were examined, notably: PC drug delivery, public health awareness, and prevalence of parasite-infected snails in freshwater sources. We classified 3,280 field-collected snails between April and November 2017, and conducted qPCR diagnostics on those belonging to the *Bulinus* genus, a known intermediate host of schistosomiasis. We will present the latest data resulting from public health surveys, drug distribution evaluations, and snail infection load. These indicators will offer programmatic insights to aid the Togolese Ministry of Health's efforts to address infection control through the continuation of PC programs and the targeted use of molluscicides in affected waterways.

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PERSISTENCE OF SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS IN MALI DESPITE HIGH COVERAGE RATES: EXCLUDING LOW COVERAGE RATES AND COMMUNITY HEALTH WORKERS MOTIVATION AS CAUSE

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In Mali, schistosomiasis and soil-transmitted helminth (STH) control activities have been in place since several decades. Despite these efforts, not only has schistosomiasis and STH persisted in the Bankass Health District (BHD), but there also was reported a very low coverage rate in 2016 of 64.8%. This study was initiated to assess the 2017 MDA coverage in Bankass Health District and the factors underlying the low coverage. A cross-sectional study was conducted in 19 health areas in BHD. We surveyed school age children (5-14 years) to determine the MDA coverage. A random cluster sampling was adopted to select households where questionnaires were administered to all volunteers aged from 5 to 14. The questionnaire was designed to assess the level of motivation of community health workers. We surveyed 2128 school age children and 52 health workers that participated in the 2017 MDA campaign. The epidemiological coverage rate for praziquantel was 93.51% (1990/2128); 95% CI [92.38% - 94.52%] and for albendazole 95.25% (2027/2128); 95% CI [94.26% -96.12%]. Adverse events (AEs) were reported in 399 respondents. Of the 399 AEs, 76.94% (307/399) were classified as minor whereas 23.06% (92/399) were considered moderate. No serious AEs were reported. Among the 98 (4.6%) children who did not receive medications as part of the MDA activities, the main reasons stated included a lack of awareness of the MDA in 31.63% (31/98) and an inability to access the centers where the drug distribution was taking place (26/98). Most of the health workers surveyed stated that they were motivated to participate in MDA campaigns in the future, and they suggested that increased incentives during the MDA campaigns would make them even more successful. The extraordinarily high coverage rate during this campaign in 2017 suggests that the low coverage rates reported in the prior MDA campaign may have been due to under reporting. In conclusion, the current data suggest that the reported low MDA coverage in BHD was likely due to underreporting, though this does not explain the persistence of schistosomiasis and STH in this health district.

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FACTORS ASSOCIATED WITH URINARY SCHISTOSOMIASIS AMONG IRRIGATION FARMERS IN MARKE COMMUNITY, JIGAWA STATE, NIGERIA

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Urinary schistosomiasis is water borne parasitic infection. Globally, over 200 million people are infected and 20 million have severe disease. Nigeria had about 29 million cases of schistosomiasis in 2008. We conducted this study to determine the prevalence, intensity and factors associated with urinary schistosomiasis among irrigation farmers in Marke community of Jigawa state. A cross-sectional survey was conducted, 430 irrigation farmers aged 15 years and above from Marke community were recruited using systematic random sampling technique. Urine samples were collected from the participants and examined for ova of *Schistosoma haematobium* using filtration technique. Data on socio-demographic variables and exposure for schistosomiasis were collected using semi-structured questionnaire. We calculated adjusted odds ratios using logistic regression method to assess risk factors. Of the 430 respondents 429(99.8%) were male. Median age (range) was 37(15-80) years. A total of 171 (39.8%) respondents were infected with urinary schistosomiasis. Median egg count (range) was 8(2-298) eggs/10ml of urine and 159 (93%) of the infected respondents had low intensity of infection (excreting less than 50 eggs/10ml of urine). Age less than 25 years (AOR 4.05; 95% CI: 1.4-8.3), being single (AOR 3.2; 95% CI: 1.8-5.5) and frequent washing of clothes (≥2 times per week) in open water bodies (AOR 1.8; 95% CI: 1.2-2.7) were found to be independent risk factors for urinary schistosomiasis among irrigation farmers in Marke community. The prevalence of urinary schistosomiasis among irrigation farmers in Marke community was moderate while the intensity of infection was found to be low. Age less than 25 year and frequent washing of clothes (≥2 times per week) in open water bodies are independent risk factors for the disease. We gave health education on the risk of transmission of the disease to the entire community and recommended mass drug administration to the high risk group.

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GEOGRAPHIC SPREAD OF AN AVIAN EYEFLUKE WITH ZOONOTIC POTENTIAL

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Philophthalmus are ocular parasites utilizing snail intermediate and avian definitive hosts in a complex life cycle. Since the last review on the genus published in 1995 by Nollen and Kanev, at least 60 articles have improved our understanding of these parasites. Furthermore, while *Philophthalmus* has long been known to be globally widespread, *P. gralli* in particular appears to be a relatively new introduction to the Americas, where human modification of aquatic habitat may encourage proliferation and spread of invasive *P. gralli*. Given that this parasite has zoonotic potential, the continued spread of this parasite presents a threat to public health.

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NUTRITION AND *FASCIOLA HEPATICA* INFECTION AMONG CHILDREN IN THE ANTA PROVINCE OF CUSCO, PERU

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Fasciola hepatica infection disproportionately affects children from the highlands of Bolivia and Peru, where prevalence can reach 70%. Acute infection is often asymptomatic, however chronic infection can remain undiagnosed for years and has been associated with anemia and undernutrition. We conducted a cross-sectional study to evaluate the impact of fascioliasis among all children attending pre-school and school in 26 communities in the Anta province in the Cusco region of Peru. We conducted interviews at school and home to collect information on demographics, socioeconomics, medical history and nutrition. Blood samples were tested for complete cell count, FAS2 ELISA antibodies and transaminases Three stool samples were collected per patient and tested with Kato-Katz and Lumbreras rapid sedimentation for parasites. Children's height and weight were recorded and used to calculate body mass index and Z scores for weight and height for age. 2958 children were included in the analysis. 50% were male, and mean age was 9.7 years (±3.59). 111 (3.9%) reported prior treatment for malnutrition, and 389 (13.7%) for parasite infection. 262 (8.9%) had at least one positive test for *Fasciola* infection. The median HAZ was -1.41 (IQR: -2.03 to -0.82) and was similar in males and females. 768 (26.1%) met WHO criteria for stunting with HAZ < -2. Children with any positive *Fasciola* test had a lower median HAZ compared to children with negative *Fasciola* tests (-1.51 vs -1.40, p=0.023). Median BMI was higher in those with a positive *Fasciola* test as compared to those without a positive test (17 vs 17.5, p=0.002), though there were no differences in BMI for age z score (BAZ) (0.24 vs. 0.30). History of treatment for malnutrition was associated with lower HAZ in those who did not test positive for *Fasciola*, but not in those with a positive test for *Fasciola*. Among those with *Fasciola* eggs in stool, BMI and weight for age were similar in the group with a high egg burden (>100 eggs) as compared to those with a low egg burden. A positive serologic test for *Fasciola* was not associated with low HAZ or WAZ.

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ECOLOGY OF *SCHISTOSOMA MANSONI* TRANSMISSION: LEVERAGING SEASONAL VARIABILITY FOR CONTROL

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Schistosomiasis, caused by various trematodes of the genus *Schistosoma* is one of the five most prevalent parasitic infections worldwide. The transmission cycle of *Schistosoma mansoni* involves both humans and snails from the genus *Biomphalaria* as obligatory hosts. *S. mansoni* is endemic across much of sub-Saharan Africa and, as such, has been the subject of control measures including mass drug administration campaigns targeting the parasite in the human host and molluscicides targeting the snail population. Fluctuations in the aquatic environment and in snail population size or community composition may concentrate or dilute the free-living portions of the schistosome life cycle. Variation in these populations can impact the infection pressure experienced by both hosts and therefore presents an opportunity to potentially magnify the effect of control measures through seasonal treatment timing. To quantify seasonal variation in snail abundance, we collected absolute and relative snail density, snail community composition, and *S. mansoni* infection prevalence bimonthly from a perennial stream in the Kisumu, Kenya area from July 2015 - January 2018. Daily rainfall and temperature data was also obtained for January 2013 -January 2018. We used time-series analysis, to identify the conditions most predictive of host snail density and infection prevalence. There were substantial seasonal fluctuations in the infection prevalence. These fluctuations do not appear to be directly related to shifts in temperature and rainfall but instead to prior snail community composition, with a correlation between high *S. mansoni* prevalence and prior dominance of Biomphalaria in the snail assemblage. These data were then used to parameterize seasonal forcing of snail population size and infection prevalence in a dynamic model of schistosomiasis. This model allows us to identify the times of year when either mass drug administration or molluscicides alone, or a combination of these control strategies, results in the maximum reduction in the force of infection experienced by the human population.

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FIELD AND MODELING STUDIES OF THE INFLUENCES OF SNAIL AND TREMATODE BIODIVERSITY ON *SCHISTOSOMA MANSONI* TRANSMISSION IN AND AROUND LAKE VICTORIA

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Human infectious agents exist within a complex environmental milieu that influences the likelihood of transmission, and nowhere is this more evident than with parasites with multi-host life cycles. Among them is *Schistosoma mansoni*, which is transmitted primarily by three different species of *Biomphalaria* in western Kenya. We show that the force of infection of *S. mansoni* to people as estimated by the number cercariae-producing snail infections is significantly influenced by domestic cattle and wild vertebrate hosts because of their role in transmitting digenetic trematodes with larval stages that compete with and/or displace *S. mansoni* sporocysts in snails. Modeling efforts indicate the removal of one key trematode parasite, an amphistome species infecting cattle, would increase the number of *B. pfeifferi* shedding *S. mansoni* cercariae by 3-fold. Furthermore, the permanence of aquatic habitats influences the species composition of *Biomphalaria* and of other snails, again with indirect effects on trematode abundance and the likelihood that cercariae-producing infections of *S. mansoni* develop. Our results suggest that the predictable co-dominant exploitation of aquatic habitats by domestic animals and humans have enabled some trematodes to depend on and exploit *S. mansoni* for their transmission. Our results help expand a conceptual framework to better understand the many factors dictating the abundance of this and other endemic neglected tropical diseases.

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AN ECO-EVOLUTIONARY PERSPECTIVE ON SCHISTOSOMIASIS

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In areas where human schistosomiasis is endemic, infection prevalence, worm burden, and egg output are known to rise rapidly through childhood, reach a peak at 8-15 years of age, and decline thereafter. A similar peak ("overshoot") followed by return to equilibrium infection levels often occurs after mass drug administration. These patterns are assumed to be due to acquired immunity, which is induced by exposure, directed by the host's immune system, and develops slowly over the lifetime of the host. Here we offer four alternative explanations for these patterns and present evidence supporting or refuting each one: 1) differential exposure of hosts, 2) differential mortality of hosts, 3) progressive pathology, and 4) concomitant immunity and worm senescence. These alternative explanations are not mutually exclusive and do not rule out an immune response by the host. Additionally, the final explanation approaches schistosomiasis from an eco-evolutionary perspective, suggesting that acquired immunity is orchestrated by adult worms for their own benefit. We present a mathematical model showing that concomitant immunity, in combination with senescence of adult worms, can explain the age-intensity pattern observed in endemic areas, and we propose experiments to test this hypothesis. Our results have implications for the treatment of human schistosomiasis. Specifically, if acquired immunity is worm-directed, then treatment of long-standing infections should be avoided, as old worms with low fitness could protect hosts against new infections. Furthermore, our results could suggest promising new directions in the effort to control human schistosomiasis, as sterile adult worms, single-sexinfections, or a vaccine that mimics the immune-modulating activity of adult worms might protect the host from new infections, while minimizing pathological consequences. We emphasize the value of an eco-evolutionary perspective on host-parasite dynamics.

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COMPUTER VISION AND MACHINE LEARNING ENABLE ENVIRONMENTAL DIAGNOSTICS FOR TARGETING SCHISTOSOMIASIS CONTROL

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A capacity gap in the developing world hinders broad scale environmental monitoring of schistosomiasis risk. A major component of this gap is a shortage of people with expertise in snail and trematode identification in Africa and elsewhere in the developing world where schistosomiasis is endemic. Yet, with the rapid advance of new affordable computing technology, promising schistosome 'environmental diagnostics' based on computer vision and machine learning technologies might emerge to fill this capacity gap and help to successfully target control measures to high-risk environments. Focusing on a case study in northern Senegal, we demonstrate a proof of concept that computer vision can assist in snail and trematode cercarial identification, with accuracy rivaling that of well-trained human technicians. We outline steps for scaling this technology, enabling citizen science, and expanding the availability and access to environmental monitoring data for schistosomiasis control efforts. We conclude that computer vision and machine learning technologies can modernize environmental diagnostics for trematode parasites and their snail hosts, with promising implications for the World Health Assembly's stated goal to eliminate schistosomiasis.

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TOXICS (PB, CD) AND TRACE ELEMENTS (ZN, CU, MN) IN WOMEN DURING PREGNANCY AND AT DELIVERY, SOUTH BENIN, 2014-2015

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Fetal development can be hindered in case of abnormal maternal concentrations of toxic and trace elements. Few data are available in African countries. Our aim was to assess the body burden of lead (Pb), cadmium (Cd), manganese (Mn), zinc (Zn) and copper (Cu) in pregnant women in Benin. The study was carried out in Sô-Ava district, from November 2015 to April 2016. Sixty women were recruited from the RECIPAL pre-conceptional cohort study. In all women, blood samples were collected during the first trimester of pregnancy. Thirty-two women had additional maternal and cord blood samples collected at delivery. Blood samples were analyzed by inductively coupled plasma mass spectrometry. At delivery, Cd median (range) concentration in maternal blood was 0.34µg/L (0.18-1.63µg/L) in this non-smoking population. Pb median (range) concentration in maternal blood at delivery was 37.4 µg/L (6.3-110.6µg/L), with 31.3% of blood Pb levels above the recommended 50µg/L threshold. These pregnant women lived in lakeside villages, thus not in an urban area. Potential sources of Pb exposure during pregnancy identified were having water supply by drill pump and activities such as smoking fish by the woman and fishing by the household head. Pb, Cd, Mn and Cu blood concentrations were significantly higher at delivery than during the first trimester of pregnancy. Pb, Cd, Zn and Cu concentrations were significantly lower in cord blood than in maternal blood, contrary to Mn concentration, which was significantly higher in cord blood than in maternal blood at delivery. This exploratory study is the first one performed in Benin, which assessed blood concentrations of toxic (Pb and Cd) and trace elements (Zn, Mn and Cu) in pregnancy prospective mother-child cohort. These findings suggest relatively high levels of exposure to Pb and Cd during pregnancy in this population of pregnant women.

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LOCAL PERCEPTIONS OF SEASONALITY AND REPORTED WATER CONTACT BEHAVIOR IN THE SAHEL: IMPLICATIONS FOR SCHISTOSOMIASIS TRANSMISSION

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Across endemic areas of sub-Saharan Africa, hundreds of millions of people acquire infections of schistosomiasis by simply being in contact with water. This exposure is often tied to mundane tasks such as washing laundry, irrigating crops and bathing, especially in rural areas where improved water sources are scarce and agricultural livelihoods common. Environmentally, the schistosome life cycle thrives in the waters that support human food security because stagnant water is prime habitat for the aquatic intermediate host snails. This environmental risk for acquiring schistosomiasis often fluctuates with seasonal changes in temperature and rainfall. Seasonal changes in the human behaviors that lead to exposure are less well-understood. In this study, focus group discussions were used to examine how perceptions of local environmental characteristics and their seasonality affect how people interact with their freshwater resources in northern Senegal. Focus group participants associated the

risk for schistosomiasis with increased temperatures on both seasonal and daily bases. They also reported altering their water contact behavior based on their perceptions of water quality, which often deteriorates during the rainy season. When perceived water quality is low, people reduce certain water contact activities, such as bathing and washing. These perceived seasonal decreases in water quality, are explained locally by reduced need for irrigation and growth of aquatic vegetation during the rainy season. Despite the relatively sophisticated local understanding of schistosomiasis transmission and the adoption of potentially protective behaviors, participants also expressed resignation at the inevitability of exposure because of the pervasiveness of schistosomiasis in the environment. The timing and targeting of environmental interventions to control schistosomiasis in this hyper-endemic region may benefit from understanding how local perceptions and livelihoods influence water contact behaviors and interact with the known ecological determinants of schistosomiasis transmission.

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CHEMICAL AND MICROBIOLOGICAL CONTAMINATION OF DRINKING WATER IN PERUVIAN HOUSEHOLDS WITH INFANTS

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In June-July 2016 we collected water and stool specimens from 96 households with infants enrolled in a cohort study in Piura, Peru. At visit 1 we collected 15- and 100-ml water samples from the primary source of drinking water. At visit 2 (4-7 days later) we collected a stool sample from the study infant and recorded diarrhea symptoms. The 100 ml samples were tested for *E. coli* on the day of collection using the IDEXX Colilert method. The 15 ml water samples were analyzed using inductively-coupled plasma mass spectrometry to quantify arsenic with a detection limit of 1 µg/l. Stool samples were assayed for bacterial, viral, and parasitic enteropathogens using the Luminex Multiplex Gastrointestinal Pathogen Panel. Arsenic was detected in half of the water samples; 32% were positive for *E. coli*. 24 samples (25%) exceeded the World Health Organization arsenic limit of 10 µg/l. The maximum arsenic concentration detected was 15 µg/l. *E. coli* and arsenic were each found in samples from all types of primary drinking water sources (piped water inside the house, outside the house, or from a neighbor; protected and unprotected wells; public water basins; and water bought in bottles or from motorbikes/trucks). Of the 94 infants available for follow-up, 13 (14%) had diarrhea in the 4-7 days between visit 1 and visit 2 and 64 (68%) had an enteropathogen detected in stool at visit 2. Having *E. coli* in drinking water at visit 1 was not significantly associated with diarrhea or enteropathogen detection at visit 2. Infants from households in which the water sample had >10 µg/l of arsenic had higher odds of diarrhea at visit 2; the difference was not significant (OR: 3.00; 95% CI: 0.89-10.06). Eleven infants who were still breastfed did not receive drinking water in the week before visit 1. These infants had significantly lower odds of having an enteropathogen detected in stool at visit 2 compared to the infants who received drinking water in the week before visit 1 (OR = 0.22; 95% CI: 0.06-0.82), but lower odds of diarrhea were not observed. Drinking water contamination in a variety of water source types may pose a risk of acute and/or long-term adverse health outcomes in infants in Piura, Peru.

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EVALUATING THE EFFICACY OF POINT OF USE WATER FILTRATION UNITS IN VILLAGES THROUGHOUT FIJI

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Over 50% of the permanent population in the country of Fiji does not have regular access to clean drinking water. A water quality intervention strategy was developed for Fiji, which includes the installation of hollow fiber membrane filter bucket systems. Residents that received filtration systems were trained at the installation and at follow up visits. The residents were trained in both the proper use of filters and basic hygiene practices, in line with WHO standards. This strategy was recently adopted by the Fiji Ministry of Health, with a goal of country wide implementation by 2023. To date, five economic and health outcomes have been tracked in 511 households to evaluate the efficacy of this strategy. At the time of filter installation, all households were surveyed inquiring about the prior 14-28 day period, and then again between 19 and 224 days later (mean=66 days). When comparing installation to follow-up, diarrhea related medical costs reduced by an average of FJD$10.16/month, drinking water expenses reduced by FJD$1.82/month, and the average adult worked an additional 0.1 days/month. Among the 351 households with children under 18, 2-week diarrhea prevalence reduced from 13.0% at installation to 1.4% at follow-up. General linear and logistic mixed-effect models were used to adjust for seasonal and spatial variation, household size and time between installation and follow-up measurements. Changes in economic and health outcomes from installation to follow-up were highly statistically significant ($p<0.001$ in all cases), in both unadjusted and adjusted models. Future work entails evaluation in other countries and contexts, long-term health monitoring and comparison to alternative water quality interventions. These continued efforts will confirm comparative advantages, long-term health impact and economic sustainability of the intervention.

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WHAT'S IN THE WATER? AN OBSERVATIONAL AND MICROBIOLOGICAL ANALYSIS OF RESTAURANTS IN IQUITOS, PERU

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Foodborne illness is a global problem estimated to affect 1 in 10 people worldwide. This correlates to 600 million people per year, with 420,000 deaths annually. Previous studies conducted in restaurants in Iquitos, Peru on food preparation and hygienic practices of local restaurants demonstrated that enteric organisms contaminate the kitchens of some restaurants. In continuation of this longitudinal study, we hypothesized the sink as a potential source of contamination. Thirteen restaurants were identified, nine of which were surveyed during previous years. Kitchen surfaces with highest probability of contamination were measured by distance from the sink to the sampled surface. The presence of coliforms (*Escherichia coli* and *Klebsiella* spp.) and pathogenic bacteria (*Salmonella* spp., *Shigella* spp., and *Aeromonas* spp.) was assessed by microbiological culture. In addition, observational surveys were performed assessing hygienic standards and sanitation practices within three areas of the restaurant (kitchen, restroom, and dining space). Results were compiled via a numerical grading system with each area receiving an average score. While the majority of surfaces showed presence of coliforms (104/139 surfaces, 74.8%), there was no significant relation between the presence of coliforms and average minimum distance to the sink ($p = 0.916$). *Aeromonas* spp. was the most common bacterial isolate and was significantly more prevalent in restaurants with lower average kitchen

(p = 0.028) and overall restaurant (p = 0.038) sanitation scores. While not statistically significant, the presence of *Aeromonas* spp. also trended toward lower average minimum distances to the sinks (p = 0.061). *A. hydrophila* is a human pathogen found in water that produces virulence factors linked to gastroenteritis, cellulitis, and septicemia. Since humans may acquire *Aeromonas* spp. via ingestion of contaminated raw foods and water, it is possible that water from restaurant sinks could contribute to foodborne illness in this area. Future studies may assess prevalence of *A. hydrophila* associated with diarrheal disease in this region.

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THE ROLE OF PREVALENCE OF WATERBORNE INFECTIONS ON THE REGRESSIVE EFFECTS OF INCOME DISPARITY IN ECUADOR

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Approximately 2.1 billion people in our planet (29% of the world population) are still vulnerable to water-related diseases, commonly referred as diseases of poverty, with Latin America accounting disproportionately higher burden: 51% of the region does not have free from contamination water. This paper uses dynamic mathematical models incorporating network of water distributions in Ecuador such as natural sources, (e.g. rivers, lakes, etc.) and tankers, commonly used as direct sources of water in developing countries. The aim is to study how population income disparity are altered as changes in prevalence of water borne diseases. The modeling framework takes into account socio-demographic factors, economic characteristics (such as income inequality) of the population, distribution of water infections, transmission pathways and available information from national public surveys from Ecuador, to address the question. Results present a framework to construct alerts to recognize the increase in inequality due to changes in infections. Also, inequality increases with more infections but the rate of increase slows down with appropriate income interventions. The results from these models provide elements to construct policies to potentially decrease waterborne disease transmission in environments with economic stress and consequently, avoid widening the disparity gap.

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MICROBIOLOGICAL EVALUATION OF WATER QUALITY AND ANTIMICROBIAL RESISTANCE PROFILE IN RIVERS OF RURAL AND URBAN BAHIA, BRAZIL

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Natural water bodies are important contributors to the dissemination of antibiotic resistance. Multiple factors facilitate the selection of resistant bacteria in this environment and therefore represent a potential risk to human health. We evaluated the antibiotic resistance profile for *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumoniae* isolates from a rural river system and three urban lakes in the state capital Salvador, Bahia Brazil. Coliform counts were made by Coliscan® culture, and water was then screened for resistant *Enterobacteriacea* on MacCokey agar with cefotaxime or imipenem. Selected *Enterobacteriaceae* were identified by MALDI-TOF and antibiotic profiles by Vitek 2. Antimicrobial resistance genes were detected by PCR for the most frequent carbapenemases and β-lactamases. Mean total coliform count for Jenipapo was 203 CFU/mL. For the urban sites Dique do Cabrito, Dique do Tororó, Abaeté Lagoon had 331, 145 and 145 CFU/mL, respectively. All sites had enterobacteria resistant to the antibiotics screened. *E. cloacae* was the most frequent resistant enterobacteria found. By Vitek, resistance to ampicillin sulbactam was most in all locations. Carbapenem resistance in one *K. pneumoniae* isolate (Dique do Cabrito) and one *E. cloacae* (Dique do Tororo). One *E. coli* and one *K. pneumoniae* were resistant to ciprofloxacin (Dique do Cabrito) and two *E. cloacae* resistant to gentamicin (Abataé Lagoon). There was no resistance to amikacin or tigecycline in any location. By PCR, 63% of the selected *Enterobacteriaceae* presented with genes for carbapenemases and 20% produced beta-lactamases. ESBL *blaCTX-M* enterobacteria was found in all locations and *blaTEM* only in Dique do Cabrito. Enterobacteria with carbapenemases *blaOXA-48*, *blaVIM*, *blaSPN* from Dique do Cabrito and Jenipapo river system; *blaOXA-48*, *blaVIM*, *blaSPN*, *blaIMP and blaNDM* were found in Dique do Tororo and Lagoa do Abaete. Surface waters of both rural and urban areas pose a significant risk due to the degree of fecal contamination and abundance of enterobacteria resistant to essential antibiotics to treat human and animal infections.

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INSTITUTIONALIZING INFECTION PREVENTION AND CONTROL PRACTICES IN HEALTH FACILITIES IN LIBERIA FOLLOWING THE EBOLA EPIDEMIC

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Ebola virus disease (EVD) swept through Liberia in 2013-2015, infecting four percent of the health workforce and causing the death of nearly 200 health workers. Poor infection prevention and control (IPC) practices in health facilities and communities facilitated the rapid spread of EVD. The Ministry of Health (MOH) introduced IPC standards in 2015 to benchmark adherence to IPC practices and certify a health facility safe to provide routine care. Through OFDA, KOICA, and USAID funding, Jhpiego worked with the MOH to achieve and maintain adherence to IPC standards in over 200 health facilities through training, infrastructure upgrades, supply provision, and monthly supervision and mentoring visits from 2015 - 2017. Jhpiego assessed health facility adherence to IPC standards four times from March 2015 - Dececember 2017. Hospitals, health centers, and clinics were sampled from 8 of 15 counties using stratified random sampling. At each facility, trained clinicians and MOH supervisors used the 52-item checklist to verify IPC standards in: administrative controls, supply & equipment, staff health, screening, isolation, WASH, and utilization of personal protective equipment. Facility score was the average of all category scores; county score was average of all facility scores. In March 2015, average county score was 42% (range: 22% to 67%) By November 2015, average score increased to 80% or above for all eight counties, meeting the 80% national target. Health facilities maintained high adherence to IPC practices in 2016 and 2017, with average scores of 78% (in 4 of 8 sampled counties) and 82% (in 3 of 8 sampled counties), respectively. No health worker contracted EVD in supported facilities. These results show rapid gains in adherence to IPC guidelines and maintained compliance years after the immediate threat of EVD receded. Maintaining high adherence to IPC practices requires continued supervision and mentoring focusing on safe provision of health services, using a checklist to benchmark performance. This approach could be adapted to other countries to improve IPC practices and prevent the spread of infection in health facilities.

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EPIDEMIOLOGICAL PROFILE OF PEOPLE WITH CHOLERA ACCORDING TO THE CHARACTERISTICS OF HEALTH ZONES: DEMOCRATIC REPUBLIC OF THE CONGO

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Cholera is major public health problem because of it sever mortality on the affected persons ;in epidémic regions 80% of cases notify by OMS in the world plan comes from sub_sahara africa . in DRC, the profil of infected people with cholera is less known; and strategies to prevent it by vaccine campaign is wrongly done. To determine different profiles of people affected by cholera. A retrospective analytical study was conducted in 17 health zones of the city of Kinshasa Province. Active data collection of suspected cholera cases identified between 2000-2016 was made based on national registries available in the 17 selected areas. These data were organized in excel spreadsheets with variables year, week, health zone, age, sex, occupation for the comparison between the groups and proportion; the statistics of the tests (chi square and Fisher test) were carried out using the SPSS software version 21 for each evolutionary period. The proportions of each variable were calculated with their 95% fixed confidence interval. Traders, fishermen, pupils, students, farmers, travelers and drivers are the most affected in endemic areas with a proportion of 78.5% (95% CI: 75.9-80.8). The most affected age group is 5-14 years old with 30% (95% CI : 29,5-31,1) in endemic areas and 25.7% (95% CI : 22.9%-28.8%) in non-endemic areas. The proportion were respectively 28.7%(95% CI : 27.3-307), 20%(95% CI : 27.1-29.3) and 25.9%(11.5-44.7) in the evolutionary phases of lull, epidemic peak and restart endemic areas in the age group of 5-14 years old. The results of this study remain a challenge because the strategy will be applied in terms of age and other aspects. A multidisciplinary approach is essential to prevent cholera.

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DEADLY PIPES: A STUDY ON THE DIFFUSION OF CHOLERA TROUGH PIPED WATER SYSTEMS IN THE YOVI RIVER VALLEY, TANZANIA

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With more than 4000 cases yearly, cholera is hyperendemic in Tanzania and many cholera epidemics are reported every year in the resource-poorest areas of the country. We report a brief cholera epidemic in the Yovi river valley, Kilosa district, Morogoro region, Tanzania. This area is served by a piped water system, built as a European community project in 2014. We analyzed the geographical distribution and the timing of microbiologically confirmed cases admitted to local health centers (Msange and Kisange) to explore a possible role of the piped water system in the diffusion of cholera cases. A total 76 bacteriologically confirmed cases were admitted to the two wards, either with medium (68.4 %) or severe (31.5 %) dehydration. One patient (1.3%) died. The first cases of cholera were detected on November 3rd, 2016, in patients from Msowelo village, which is close to the river source. The last cases were detected on January 6th, 2017 in the Madizini village, about 20 miles from the source. Interestingly, the first group of patients was reported to drink and use the river water but the second wave of cases involved people using tap water in Msolwa, Madizini, and Kisange. The geographical distribution of the cases shows the spread from Msowelo to the other villages of the valley along the net of water distribution. In the summer of 2017 a study performed on the Yovi aqueduct and quality of piped water by the Department of Environmental Engineering, Trento University, Trento, Italy, showed a high contamination rate of the piped water by coliforms due to the close contact with latrines and waists. Our study confirms that systems for the distribution of piped water do not prevent the diffusion of cholera in rural areas if the water supply system is not maintained on a regular basis. In such settings, piped water could actually favor the epidemic.

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EVALUATION OF THE EFFECTIVENESS OF BUCKET CHLORINATION IN CHOLERA OUTBREAKS

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Bucket chlorination is a commonly implemented intervention for emergency water treatment employed during cholera outbreaks where agents stationed near a water source dose beneficiaries' water containers with chlorine. Guidelines provide two different recommendations for chlorine dosage: a fixed dosage (either 2 mg/L for low turbidity or 4 mg/L for high turbidity) or a variable dosage that provides a free chlorine residual (FCR) of 0.5 mg/L 30 minutes after dosing. To understand the effectiveness of these guidelines and the factors that impact their implementation, we are investigating bucket chlorination in both a laboratory study and field evaluations.In the laboratory, we are assessing the inactivation of *Vibrio cholerae* in the aqueous phase by treating with three different chlorine types at the recommended dosages. To challenge the recommendations, we will be testing in prepared waters of varying turbidity, total organic carbon concentration, and pH. Results, expected Summer 2018, include: 1) whether recommended dosages inactivate *V. cholerae;* 2) whether recommended dosages can maintain a 0.2 mg/L FCR 24 hours after treatment; and, 3) what dosages can achieve a 2 log reduction in *V. cholerae*. Field evaluation protocols for assessing bucket chlorination in cholera outbreaks have been developed to understand: the implementation modalities of response organizations, the effectiveness of bucket chlorination in meeting program goals, and the perceptions of the intervention by both program staff and beneficiaries. To accomplish this aim, a mixed methods protocol including key informant interviews, focus group discussions, and household surveys will be conducted. Additionally, drinking water samples will be collected for microbiological and FCR testing. Two field evaluations will occur in the Democratic Republic of Congo and Bangladesh in Summer 2018.This presentation will provide laboratory data on bucket chlorination efficacy against *Vibrio cholerae* and preliminary results from field evaluations. These results will provide the basis for the development of recommendations to existing guidelines.

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VALIDATION OF MOLECULAR ASSAYS FOR THE DETECTION OF *SALMONELLA TYPHI* AND *S. PARATYPHI* A IN ENVIRONMENTAL SAMPLES

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Enteric fever is a severe systemic infection caused by *Salmonella enterica* serovar Typhi (ST) and *Salmonella enterica* serovar Paratyphi A (SPT). In order to study risk of exposure to ST and SPT a large exposure assessment study in Kolkata, India. Quantitative microbial risk assessment (QMRA) requires quantitative estimates of exposure to the pathogens of interest. Here we present, results from methods validation studies to quantitatively detect both ST and SPT by PCR in a range of relevant environmental samples. Our goals were to evaluate sample concentration and DNA extraction methods in order to optimize the limit of detection of ST and SPT in environmental samples by real-time PCR. Briefly, 10^9 cells/mL from

bacterial culture in 10-fold serial dilution were used to spike drinking water, pond water, sewage influent, soil, cucumber and tomatoes. Three methods to concentrate ST and SPT from liquid samples were evaluated: membrane filtration (50-100mL), polyethylene glycol (PEG) precipitation (500 mL) and ultrafiltration (5 - 100 L) followed by PEG precipitation. After concentration, total DNA was extracted using Qiagen DNeasy extraction kit. Samples were tested by PCR in triplicate using a Taqman-based real-time PCR platform and primers described by Nga et al 2010. Extrapolated data using standard curves from membrane filtration experiments, suggested that for most of the pathways range of detection was 10^3 to 10^9 cells/mL. PEG precipitation of sewage samples gave better recovery than membrane filtration; as indicated by an average 6 Ct difference between the two methods. Positive Ct values (22-30) were obtained on 5 and 100L samples concentrated by ultrafiltration. As assays do not include an enrichment step, they can provide quantitative estimates of the target pathogens in relevant food and environmental samples and can be used to inform "dose" estimates in QMRA analyses. Next steps include testing environmental samples in Kolkata, and developing and validating concentration and detection methods for ST and SPT in street food, dairy products, and surface swabs from public toilets.

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WATER, SANITATION, AND HYGIENE MOBILE HEALTH (MHEALTH) MESSAGES AS AN INNOVATIVE TOOL TO FACILITATE BEHAVIOR CHANGE: RANDOMIZED CONTROLLED TRIAL OF THE CHOBI7 MHEALTH INTERVENTION

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Diarrhea is the second leading cause of death in young children globally. Furthermore, household members of diarrhea patients are at a much higher risk of developing diarrheal diseases (>100 times for cholera) than the general population during the one week period after the diarrhea patient presents at a health facility. This is likely because of poor WASH practices in the home. The time patients and their caregivers spend at a health facility for the treatment of severe diarrhea episodes presents the opportunity to deliver WASH interventions when perceived severity of diarrheal diseases and benefits of water treatment and handwashing with soap is likely the highest. Effective scalable WASH interventions are urgently needed to reduce diarrheal diseases globally. Our research group recently developed the Cholera-Hospital-Based-Intervention-for-7-Days (CHoBI7, "picture" in Bangla), a WASH intervention which promotes handwashing with soap and water treatment to diarrhea patients and their household members in a health facility setting. Our recent randomized controlled trial (RCT) in Bangladesh of CHoBI7 demonstrated this WASH intervention was effective in significantly reducing symptomatic cholera in diarrhea patient households, and led to sustained improvements in WASH practices over time. Building on this work, we are currently conducting a second RCT evaluating the impact of Mobile Health (mHealth) messages as a low cost scalable approach to reinforce the CHoBI7 WASH intervention delivered in a health facility setting to diarrhea patient households. This study is being conducted in collaboration with the Bangladesh Ministry of Health and Family Welfare. This is the first RCT to our knowledge evaluating the efficacy of sending mHealth messages to households to increase WASH behaviors. Our initial findings have shown that the CHoBI7 mHealth intervention is effective in significantly improving household water quality and increasing observed handwashing with soap at stool and food related events during the one week high risk period for diarrhea patient household members and 1 month after discharge from a health facility.

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VESICLE FORMATION AND ADP-RIBOSYLATION FACTORS ASSOCIATED WITH TICK-BORNE FLAVIVIRUS INFECTION OF *IXODES SCAPULARIS* (BLACK-LEGGED TICK) SALIVARY GLAND CULTURES

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The tick, *Ixodes scapularis*, transmits a number of pathogens, including *Borrelia burgdorferi* and tick-borne flaviviruses (TBFVs). TBFV infections cause thousands of human encephalitis cases worldwide each year. In the US, confirmed human infections with Powassan virus (POWV) have a fatality rate of 10-30% and are increasing. The attenuated Langat virus (LGTV) is often used as a convenient experimental TBFV model. The tick salivary glands (SGs) serve as the primary organ barrier to TBFV transmission. Recent optimization of TBFV growth in *ex vivo* tick organ cultures allows a deeper understanding of virus biology in viable organs. Information on host proteins involved in TBFV infection is limited. ADP-ribosylation factors (ARFs) are involved in vesicle formation and transport in mammalian cells. ARFs have a role in mosquito-borne flavivirus infection but their role is unknown in TBFV infection. The goals of this study were to identify (1) vesicle formation with TBFV-like particles in SG cultures from blood-fed and unfed ticks and (2) the association of ARF1 and ARF4 in TBFV infection in human cell, tick cell and SG cultures of the two different physiological states. Using transmission electron microscopy, vesicle formation with LGTV-like particles was only observed in SGs from unfed ticks. Using small molecule and siRNA/dsRNA-mediated transcript knockdown assays, our results indicated that human ARF1 and ARF4, and their predicted tick orthologs are critical for TBFV replication in *in vitro* human and tick cells. However, transcript knockdown impact on POWV replication differed between *ex vivo* SG cultures from blood-fed and unfed ticks. This data suggests a complex relationship between the tick's physiological state and TBFV biology, especially in an organ critical for TBFV transmission. Future research may include similar studies in TBFV-infected ticks to determine effect on TBFV infection and transmission. This research helps to identify host proteins as potential targets for countermeasure development against TBFV infection and transmission. This research was supported by the Intramural Research Program of the NIH, NIAID.

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LOW VACCINATION COVERAGE AND *AEDES ALBOPICTUS* AS POTENTIAL VECTOR OF TRANSMISSION OF YELLOW FEVER IN CAMEROON: RESULTS OF AN INVESTIGATION FOLLOWING POSITIVES CASES IN THREE HEALTH AREAS IN CAMEROON, 2017

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Because Cameroon is at risk of yellow fever (YF), national preventive vaccination campaigns are regularly conducted targeting people aged 9 months and older. Despite vaccine coverage of 85% during the last campaign in 2015, cases are continuously reported. In 2017, four cases were confirmed in three health areas. We investigated to confirm the epidemic, assess vaccination coverage, evaluate the surveillance system and identify the vector. We conducted active case finding in health facilities (HF) and the community using the standard YF case definition. Using cluster sampling, 321 households were selected and vaccination status of one adult per household was assessed. With structured questionnaires, we collected data on socio-demographics, and reasons for non-vaccination. Logistic regression identified factors associated with vaccination. An entomological survey in 173 randomly selected households identified the potential vectors involved. We also assessed surveillance system in 12 conveniently selected HF around the confirmed cases. No additional suspected case was found, and 3 of the 4 cases were traced with case fatality rate of 33.3%. One case was vaccinated. Among the 271 adults interviewed, vaccine coverage was 53% [143/271, 95% CI: 46.3-58.4]. Lack of information about the vaccination campaign (65%,51/79), absence during vaccination campaigns (22%,17/79) were the main reasons for non-vaccination. International trips [OR=3.9, 95% CI: 1.5-10.5], knowing how to guard against YF [OR=1.81, 95% CI: 1.03-3.36] and where to receive vaccination [OR=2.1, 95% CI: 1.2-3.7] were independent predictors of vaccination. *Aedes albopictus* was found in 99% of the households with a mean pupae index of 1.61. No other known YF's vector was found. Abandoned containers were the most productive breeding sites (65%, 11/17). The mean acceptability of the surveillance system was 82.5%. With low vaccination coverage, the risk of outbreak remains. We recommend mass education on YF prevention, and on the risk related to waste accumulation around homes. Otherwise, further studies to identify if *Aedes albopictus* could transmit YF should be conducted.

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CYTOKINE PROFILE OF WEST NILE VIRUS CASES DEVELOPING CHRONIC KIDNEY DISEASE

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West Nile Virus (WNV) is endemic in the United States and the sequelae resulting from infection are of considerable public health concern. With national estimates of over 3 million prevalent cases, greater understanding of related complications is an imperative facet of clinical care. In our cohort of WNV infected participants in Houston, TX, there was a marked increase in the development of chronic kidney disease (CKD). Of 91 enrolled participants, 21 (23%) were found to have a clinical diagnosis of CKD according to Kidney Disease Outcomes Quality Initiative (KDOQI) criteria. To begin to understand how WNV relates to the development of CKD, we evaluated expression profiles for 26 different chemokine and cytokine markers in our WNV positive participants with and without CKD. Our study indicated that those with a diagnosis of CKD had a statistically significant increase in 3 distinct cytokines: INFα2, IP-10, and MIP1a. These findings are consistent with renal pathogenesis associated with infection by other RNA viruses. Importantly, these cytokine profiles are distinct from profiles in non-infectious causes of renal injury, and are suggestive of specific cell type involvement in inducing a prolonged localized inflammatory response. These results provide an important snapshot of the relationship between WNV and CKD and could serve as the basis for future studies to better predict these outcomes in a patient population.

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THE ROLE OF SUBSTANCE P AND NEUROKININ-1 IN TREATING WEST NILE VIRUS DISEASE

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Since its introduction to the US in 1999, West Nile virus (WNV) has led to greater than 43,000 reported cases and 1,700 deaths; estimates indicate infections exceed 3 million. In survivors, up to 40% of patients will develop long-term, severe neurological complications. With no licensed vaccines and only supportive care, it is of public health importance to identify viable therapeutic options to prevent prolonged morbidity. Substance P (SP) and its receptor neurokinin-1(NK1R) have been identified as key players in neuroinflammation and we believe they may play a valuable role in the progression of WNV infection. We conducted a study in our wild-type BL6 mouse model of WNV neuroinvasive disease using a highly selective NK1R antagonist, CH123001. Mice were infected with WNV-NY99 strain at a lethal dose and monitored for signs of disease. At the onset of symptoms, mice were treated with CH123001 or mock treated with PBS. Kaplan-Meier survival curves were used to evaluate survival and time to death. Approximate viral loads and levels of SP RNA were evaluated in each group. Uninfected, age-matched controls were included to establish normal levels of SP. Treatment with CH123001 at the time of disease onset led to increased survival and prolonged time to death. While our study suggests treatment with CH123001 successfully lowered levels of SP RNA in brains, it did not appear to alter levels of WNV RNA. Nonetheless, improved outcomes were observed and may indicate a robust role of the immune response in neuroinvasive disease and death from WNV infection. This is the first example of the role of SP in WNV infection. With many NK1R antagonists already FDA approved for human use to treat nausea, this study provides evidence that this class of medication could be applied to improve outcomes during WNV infection.

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TISSUE EXPANSION INDUCED BASAL LAMINA MICRO-PERFORATIONS FACILITATE ARBOVIRUS SPREAD IN *AEDES SPP*. MOSQUITOES

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The recent Zika virus (ZIKV) and chikungunya virus (CHIKV) epidemics highlight the explosive nature of arthropod-borne (arbo) viruses transmitted by *Aedes aegypti* mosquitoes. Understanding how these viruses are able to infect, replicate, and navigate mosquito tissues en route to transmission is paramount to modeling disease risk and developing novel control strategies. Mosquito tissues including the midgut and ovaries are encased in a proteoglycan matrix termed the basal lamina. Previous studies have demonstrated that this structure is impenetrable to virus particles, yet arboviruses are still able to bypass this matrix and infect new tissue types. We recently demonstrated that administration of a second non-infectious bloodmeal significantly shortens the extrinsic incubation period of ZIKV-infected *Ae. aegypti* by enhancing virus escape from the mosquito midgut. The mechanisms facilitating this early escape are unknown but could be due to changes in the integrity of the basal lamina after bloodmeal acquisition. To explore this hypothesis further, we used scanning electron microscopy to examine the integrity of the basal lamina of both the midgut and ovaries of recently bloodfed individuals and non-bloodfed controls. Basal lamina micro-perforations were observed in both tissue types in engorged individuals compared to the controls. Subsequently, we performed a retrograde infection assay in which ZIKV was intra-thoracically inoculated and viral replication was assayed in midguts and ovaries of bloodfed and non-fed individuals.

Immunofluorescence revealed ZIKV *Foci* in the midgut and ovaries of fed mosquitoes, but not in the controls. These findings were confirmed by RT-qPCR. Together these data suggest that the formation of micro-perforations in the basal lamina as a result of mechanical distention during bloodmeal acquisition and oogenesis facilitates arbovirus spread within mosquitoes. This study provides a mechanistic basis for enhanced arbovirus escape from the midgut and infection of the ovaries after sequential blood meals.

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CHARACTERIZING THE IMMUNE RESPONSE TO RIFT VALLEY FEVER VIRUS IN MULTIPLE SPECIES AFTER NATURAL EXPOSURE OR VACCINATION WITH CHADOX1-RVF

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Rift Valley fever virus (RVFV) primarily infects ruminants, resulting in high levels of mortality and abortion. Humans can acquire infection through infectious mosquito bites or contact with virus-contaminated tissues and fluid. Vaccines are considered the most effective tool to limit infection. However, no licensed human vaccines are available, and current livestock vaccines have significant safety concerns. We recently developed ChAdOx1-RVF, a highly efficacious viral vectored vaccine expressing the RVFV envelope glycoproteins. To help inform vaccine strategies, we have characterised the quality of the immune response to ChAdOx1-RVF vaccination in all target species. In addition, we have characterised the immune response after natural exposure which is known to illicit lifelong protection. Sheep, goats, cattle and camels were vaccinated with ChAdOx1-RVF. Sera were sampled pre-vaccination and at various time points thereafter. Human and livestock sera from natural RVFV infection were obtained in Kenya. Samples were tested using RVFV neutralising antibody assays and ELISAs detecting total IgG, IgG subclasses and IgG avidity against the RVFV envelope glycoproteins. Vaccinating livestock with ChAdOx1-RVF elicits protective antibody responses within the range of natural human RVFV infection. Total IgG titres towards either glycoprotein strongly correlated with neutralising titre in all species. The avidity of these IgG was a poor predictor of neutralising titre. The naturally acquired human antibody response is dominated by IgG1, while livestock vaccination induced both IgG1 and IgG2. Analysis is ongoing and we plan to probe the cellular response in both livestock and human trials scheduled later this year.

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GOT MILK? DEFINING THE LINK BETWEEN MILK AND RIFT VALLEY FEVER

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Rift Valley fever virus (RVFV) is a zoonotic phlebovirus that primarily infects humans and livestock. RVFV is currently endemic in sub-Saharan Africa, as well as parts of the Middle East, with imported cases more recently extending into Europe and China. RVFV infection severity varies dramatically among individuals, ranging from asymptomatic cases to severe sequelae of hemorrhagic fever, multiple organ dysfunction, and meningoencephalitis. RVFV is transmitted by mosquitoes, or by direct contact with contaminated bodily fluids and tissues. Transmission method may contribute to disease severity and progression, as exposure through contact with contaminated fluids may initiate infection with a higher viral load, as opposed to that of a mosquito bite. While previous studies have included milk exposures in their analyses, their primary focus on livestock exposures has been on animal handling, breeding, and slaughter. We analyzed data from multiple field surveys in Kenya with the aim of associating RVFV exposure to raw milk exposures from common animal species. Of those with evidence of previous RVFV infection by serology (n=267), 81.7% engaged in milking livestock compared to 36.8% for 4020 co-local seronegatives ($p<0.001$, OR 2.3, CI_{95} 1.5-3.5), 86.8% seropositives consumed raw milk compared to 33.8% seronegatives ($p<0.001$, OR 10.9, CI_{95} 6.7-18.8). Individuals who milked and also consumed raw milk had greater odds of RVFV exposure than individuals who only milked animals but did not consume raw milk. Regardless of exposure type, increased risks were associated with exposure to milk sourced from cows ($p<0.001$), sheep ($p<0.001$), and goats ($p<0.001$), but not camels ($p=0.98$ for consuming, $p=0.21$ for milking). Our data suggest that exposure to raw milk may contribute to a significant number of cases of RVFV, especially during outbreaks and in endemic areas, and that some animal species may be associated with a higher risk for RVFV exposure. Livestock trade is regulated to limit RVFV spread from endemic areas, yet further interventions should be designed to educate around the risk of RVFV exposure from raw milk.

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WHAT IS THE BEST IMMUNIZATION STRATEGY FOR PROTECTING AFRICAN CHILDREN AGAINST INVASIVE *SALMONELLA* DISEASE?

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The first typhoid conjugate vaccine (TCV) to achieve WHO-prequalification, Typbar-TCV®, stimulates high titers of long-lived serum IgG-Vi antibodies when administered as a single-dose to infants as young as age six months and offers flexibility for inclusion at different points in the Expanded Program on Immunization schedules. Epidemiological evidence to guide optimal scheduling is required. International Vaccine Institute conducted a multi country study, Typhoid Fever Surveillance in Africa Program (TSAP), in 10 countries, Burkina Faso, Ethiopia, Ghana, Guinea Bissau, Kenya, Madagascar, Senegal, South Africa, Sudan and Tanzania, to establish

the burden of Typhoid fever (*Salmonella enterica* serovar Typhi (TF)) and invasive non-typhoidal *Salmonella* (iNTS). In this secondary analysis, incidences from in infants, stratified by age and region were calculated. A total of 30.1% (37/123) TF infections and 78.9% (71/90) iNTS infections were observed in children aged <5 years. No TF and 8.9% (8/90) iNTS infections were identified in infants <9 months of age. The annual TF incidence calculated for children aged <1 and 1 to <2 years was 5 and 39 per 100,000 person-years of observation (PYO), respectively, with the highest incidence of 304/100,000 PYO observed in children aged 4 to <5 years. The annual iNTS disease incidence for the same age groups were 81, 233, and 46.2 per 100,000 PYO. Geographically, higher incidences of both TF and iNTS disease infections were observed in West African TSAP sites. The cumulative incidence of typhoid in those aged <5 years was 279/100,000 in West Africa, compared to 154/100,000 in East Africa. The iNTS incidences in individuals aged <5 years were 828/100,000 and 48/100,000 in West and East Africa, respectively. A high TF burden in children <5 years of age supports the introduction of TCV in infants at nine months of age in Africa; for iNTS disease younger age-groups should be targeted. In the future, additional vaccines to comprehensively address the burden of invasive *Salmonella* infections observed in children will potentially be required.

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INCREASING ANTIBIOTIC RESISTANCE IN *SALMONELLA ENTERICA* AND *SHIGELLA* SPP. ISOLATES FROM CHILDREN UNDER-FIVE IN WESTERN KENYA, 1997-2010: INSIGHTS INTO SOURCES OF SELECTION PRESSURE FROM SURVEILLANCE IN A LOW-INCOME, RURAL SETTING

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While antibiotic resistance (AR) is a threat to public health throughout the world, longitudinal population-level AR surveillance is limited, especially in low-income settings with high burdens of enteric infections. Since 1997, the Centers for Disease Control and Prevention has collaborated with the Kenya Medical Research Institute to conduct antimicrobial susceptibility testing on 331 *Shigella* spp. and 369 non-typhoidal *Salmonella enterica* isolates from children &lt 5 years old presenting with diarrhea to sentinel clinics in rural, western Kenya. In this population from 1997-2010, we observed high *Shigella* spp. resistance to amoxicillin-clavulanic acid (AMC, 42% of isolates), ampicillin (AMP, 68%), chloramphenicol (CHL, 49%), streptomycin (STR, 91%), sulfisoxazole (SULF, 96%), sulfamethoxazole-trimethoprim (SXT, 97%), and tetracycline (TET, 88%). *S. flexneri*, was most commonly isolated (200/331), and had greater odds of AR than other *Shigella* species. For *Salmonella enterica*, resistance to AMC (32%), AMP (66%), CHL (53%), STR (67%), SULF (84%), and SXT (67%) was high. Serogroup B *Salmonella enterica* was most commonly isolated (236/369), followed by serogroup D (57/369). Both had comparatively higher odds of AR than other serogroups detected. Odds of SXT resistance in isolates of both pathogens increased with the child's age, and increased significantly over the 13-year period. *Salmonella enterica* resistance to CHL, STR, and SULF also increased significantly during this time. Despite prevalent *Salmonella enterica* and *Shigella* spp. infections resistant to AMP or SXT, patients with these infections were given AMP (or related drugs) 16% and SXT 17% of the time. Overall, despite high initial levels of resistance in 1997, population-level resistance to core drugs (such as SXT) increased over the 13-year period, especially among the most prevalent species/serogroups of *Shigella* and *Salmonella enterica* isolated from young children in a rural, low-income setting. These results highlight the potential for sustained increases in AR driven by antibiotic selection pressures in high-burden settings.

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SPATIAL AND GENOMIC DATA TO IDENTIFY TRANSMISSION ROUTES OF TYPHOID FEVER IN BLANTYRE, MALAWI

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Queen Elizabeth Hospital (QECH) in Blantyre, Malawi, experienced a sharp increase in cases of typhoid fever starting in 2011, the majority being multi-drug resistant. Despite ongoing transmission today, the dominant mechanisms of persistence remain unknown, posing a challenge for effective control interventions. Spatial patterns of genetic relatedness of clinically isolated *Salmonella* Typhi may offer insight into the mechanisms of transmission occurring, and we aimed to quantify and understand these trends in Blantyre. Beginning in 2015, *S.* Typhi isolated from cases presenting to QECH were whole-genome sequenced, and households were geo-located. Variable regions of the sequences (i.e. highly recombinant and prophage regions) were excluded, leaving 400 informative single nucleotide polymorphisms (SNPs) across isolates. Resulting pairwise SNP distances were converted into informative variables for statistical modeling using multidimensional scaling. Genetic patterns of *S.* Typhi isolates across Blantyre showed a heterogenous distribution of genotypes, with spatial correlation occurring among isolates of cases living up to 2 kilometers apart. Risk factor analyses have previously identified cooking and cleaning with river water as a potential exposure pathway for S. Typhi in Blantyre, so we then evaluated the ability of river catchment to explain the spatial patterns using a linear model with a spatial random effect, implemented in R statistical software. Results from the analysis indicate that the spatial genetic patterns seen across the city are influenced by three primary components: river catchment, close neighbors (living a maximum of 200 meters apart), and household units. Results from this study highlight the value of genomics in better understanding the complex transmission mechanisms of typhoid fever, and support control approaches targeting multiple scales of transmission, such as pairing vaccines with targeted water and sanitation interventions.

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COST-EFFECTIVENESS OF TYPHOID CONJUGATE VACCINE STRATEGIES IN 54 GAVI-ELIGIBLE COUNTRIES

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The World Health Organization recently recommended the programmatic use of typhoid conjugate vaccine (TCV) in endemic countries, and Gavi has pledged support for vaccine introduction. While TCV strategies are likely cost-effective in medium- to high-incidence settings, country-specific evaluations are warranted. We compared four strategies: no vaccination; routine immunisation at 9 months; or routine immunisation at 9 months with catch-up campaigns to either age 5 or 15 years. For each of 54 Gavi-eligible countries, output from an age-structured transmission dynamic model was combined with country-specific treatment and vaccine-related costs, treatment outcomes, and disability weights to estimate disability-adjusted life-years (DALYs) to identify: (1) the preferred strategy, based on the highest average net benefit for a range of willingness-to-pay

(WTP) values; (2) how certain we are about the preferred strategy; and (3) the minimum economic and epidemiological conditions under which vaccination is preferred. At a vaccine price of US$1.50 per dose and a WTP value of US$1,000 per DALY averted, routine vaccination including a catch-up campaign to 15 years of age is the preferred strategy in 46 countries. However, for half of these countries, the probability that this strategy results in the highest net benefit is <60%. Vaccination is increasingly likely to be preferred at higher rates of typhoid incidence and mortality, and at higher WTP values. While routine TCV immunisation with a catch-up campaign is preferred over no vaccination in most Gavi-eligible countries, investing in obtaining improved estimates of typhoid age-specific incidence and mortality may be valuable for many countries.

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ADVERSE EVENTS FOLLOWING IMMUNIZATION WITH TYPHOID CONJUGATE VACCINE: MASS IMMUNIZATION AGAINST CEFTRIAXONE RESISTANCE TYPHOID FEVER IN HYDERABAD, PAKISTAN

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Pakistan is facing the largest outbreak of extensive drug resistant typhoid. The outbreak started from Hyderabad and predominantly affected children less than 10 years of age. The Aga Khan University in collaboration with department of health, Government of Sindh, procured the WHO pre-qualified Typhoid conjugate vaccine (TCV), to initiate a mass immunization campaign in Hyderabad as a response to the outbreak. Door to door immunization of children 6months to 5 years of age was started in January 2018. In order to ascertain the adverse events following immunization (AEFI), a subset of children was actively followed on day 7 and 14 following the vaccination. Information about any illness, disability, hospitalization or death was collected by trained staff through a standard AEFI form translated in local language. In case of hospitalization or visit to a general physician, information on admissions, discharge, and prescriptions were obtained. Caretakers of all the vaccinees were also provided 24hrs emergency contact number to report any illness. A total of 4070 vaccinated children; 655 aged 6-12months, 851 aged 1-2 yrs, 911 aged 2-3, 836 aged 3-4 and 823 aged 4-5 yrs were actively followed and only 21, 13, 24, 16 and 22 children respectively in each of the age groups had some kind of AEFI. All of the AEFIs identified actively were mild in nature; 69/4076(1.7%) fever, 15(0.4%) diarrhea, 5(0.1%) vomiting, 3(0.07%) abdominal pain, 2(0.05%) chest infection, cough and swelling on injection site was 1(0.02%) each. Besides, out of total 32,215 doses administered there were 09(0.03%) of AEFI observed either immediately within 30 minutes of vaccination or passively reported through emergency contact number. 4/32215(0.01%) developed high grade fever, 2(0.006%) developed a rash on the whole body and severe vomiting, seizure, and swelling at the injection site were 1(0.003%) each. Typbar-TCV vaccine was found to be safe for administering in emergency situation during mass immunization campaign in Hyderabad city of Pakistan. Mild fever and diarrhea are the most frequent AEFIs. Seizure is extremely rare (1 in 32000 doses).

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ASSESSMENT OF IMMUNE AVIDITY TO DETERMINE THE COURSE OF DISEASE IN ACUTE AND CHRONIC STATE OF NATURAL *SALMONELLA TYPHI* INFECTION

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An assessment of the duration of immune response to natural typhoid (TF) infection was performed as a part of Severe Typhoid in Africa (SETA) study. Immune avidity assay was used for testing serum samples of acute cases and asymptomatic controls during one year follow-up. The avidity assay is based on Vi-ELISA assay, involving antigen-antibody binding in the presence of chaotropic agent (Gu HCl). Antigen-antibody complexes present in serum of chronic infection bind strongly to Vi antigen and dissociate weakly in presence of chaotropic agent, whereas in recent infection cases, antibodies dissociate easily. This assay will help identify carriers during follow-up visits and, thus, reduce risk of unrecognized TF transmission. The Vi-avidity assay was established and internally qualified. Using avidity assay we tested serum samples from febrile cases from Ghana sites of West Africa for Vi IgG and IgM ELISA & avidity. Addition to febrile cases we also tested neighborhood healthy controls, healthy household contacts. Initial data suggests all blood culture positive and several negative samples having high anti Vi-IgM titers and anti Vi-IgG titers of >100 ELISA units. High titers were also detected in healthy neighborhood controls and house hold contacts. Interestingly many of this subjects with high Vi- antibody titers also have high antibody avidity suggesting probability of repeat infection or persistent chronic state. Samples with high IgG avidity (80-100 avidity index) may be consider as repeat infection. Samples with high IgM avidity (80-100 avidity index) may be consider as recent infection with repeat exposure. Sample with both high IgG and IgM avidity may be consider as chronic state with persistence of *S typhi*. The avidity cut off estimates were done on basis of other reports on pneumococcal and cholera natural infection studies. Further additional analysis are needed to better understand the cutoffs. Bactericidal antibodies were also tested in blood culture positive patients. High bactericidal antibody titers were detected in this subjects, suggesting antibodies generated by natural infection have functional role in killing *S typhi*.

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UNPRECEDENTED PNEUMONIC PLAGUE EPIDEMIC IN MADAGASCAR, 2017: CAN WE MAKE PLAGUE HISTORY?

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Yersinia pestis continues to threaten the Malagasy population with around 300 reported cases/year during September - April. In 2017, Madagascar experienced a large epidemic of Pneumonic Plague (PP). We present the epidemiological analyses of plague cases based on the official national database from the Central Laboratory for Plague (CLP, a WHO Collaborating Center) at the Institut Pasteur de Madagascar (IPM), which combined case notification forms with results from biological analyses. Cases were classified as confirmed/probable based on positive culture and/or positive qPCR (*pla, caf* 1 and *inv* genes) and/or positive rapid diagnostic tests (RDT, locally-produced by IPM) performed by the CLP. On September 11, successive deaths of unknown cause triggered an alert in a plague-free area. Plague was diagnosed by RDT in one deceased case in Antananarivo (the capital city). Subsequent investigation led to identification of 5 unnoticed suspected plague deaths that occurred during the 2 weeks before the alert, including the index case. The risk of geographic extension of the PP epidemic triggered a national and international response. Between August 1 and November 26, 2,414 clinically-suspected plague cases were reported, of which 1,878 (78%) were PP cases and 395 (16%) were Bubonic Plague (BP). Confirmed/probable cases represented 22% (418/1,878) of PP and 35% (139/395) of BP cases. Confirmed/probable cases were reported from 41/114 (36%) administrative districts. PP hotspots were mainly identified in Antananarivo and Toamasina, the main seaport. The case fatality rate was 13% for confirmed/probable and 8% for suspected cases. Rapid confirmation of cases and identification of contacts was difficult due to the urban and highly populated setting

of the epidemic and the difficulties encountered during sputum sample analyses. This epidemic led to implementation of additional molecular diagnostic tools and strengthened the country's capacities for multisectoral responses. Lessons learnt will help to improve responses for the upcoming plague seasons and highlight the need to strengthen research on plague to eliminate the disease in humans.

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A REVIEW OF CONTROLLED HUMAN MALARIA INFECTION TRIALS AT THE UNIVERSITY OF MARYLAND

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Eradication of malaria will not likely succeed without an effective vaccine. Controlled human malaria infection (CHMI) is a powerful tool to evaluate potential vaccine candidates, allowing for down-selection of vaccine candidates before conducting expensive field trials. Investigators at the University of Maryland School of Medicine (UM SOM) pioneered the technique in the 1970s and continue to advance CHMI research. These advances include alternate routes of sporozoite delivery, ultrasensitive PCR diagnostics, exclusive outpatient study conduct, and heterologous CHMI. We reviewed records of 357 malaria-naïve volunteers who underwent CHMI with either the NF54 or 7G8 strain of *Plasmodium falciparum (Pf)* at our institution from 1973 until 2017. These 357 malaria-naïve volunteers experienced 406 unique CHMI events, including 60 via parenteral administration. No volunteer suffered an unplanned hospitalization or required invasive intervention related to CHMI. Among non-vaccinated volunteers, the median prepatency period (8 days) did not differ between *Pf* strains (NF54 vs 7G8) but time-to-event curves differed (p=0.033, log rank test). Real time quantitative PCR detected *Pf* infection 3.0 days earlier in non-vaccinated participants than thick smears (p &lt 0.0001). With dose optimization, administration via direct venous inoculation resulted in a similar prepatent period to mosquito bite challenge of non-vaccinated volunteers (log-rank test, p=0.17). Historical studies with NF54 showed a shorter median prepatency period of 10.8 days as compared with more recent studies (median 11.0 days, p=0.043 by log-rank test) in those who did not undergo vaccination, despite similar study conditions and mean salivary gland scores (where available). The 46-year experience of CHMI at UM SOM has advanced this model as an effective tool to winnow and optimize candidate vaccines, enabling better resource allocation, and could provide supporting data for vaccine licensure.

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PRELIMINARY DATA FROM A BIOMARKER EVALUATION TRIAL AMONG NON-SEVERE FEBRILE PATIENTS IN A MALARIA ENDEMIC SETTING IN MALAWI

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Although malaria incidence has been decreasing in low- and middle-income countries (LMICs) over the past two decades, acute febrile illnesses are still a major cause of mortality and morbidity. A lack of simple, cheap rapid tests for febrile illnesses other than malaria is resulting in overtreatment with antibiotics for those who test negative for malaria, contributing to the global rise in antimicrobial resistance. Host biomarkers appear as promising tools to differentiate bacterial from non-bacterial infections in febrile patients. However, very few evaluation studies have been performed in the overall non-severe febrile population in LMICs, resulting in poor knowledge on the performance of these biomarkers in a context of high prevalence of diseases such as malaria, HIV and arbovirus infections. To address this knowledge gap, FIND is conducting a multi-center study to evaluate the performance of candidate biomarkers to distinguish bacterial from non-bacterial infections in the adult and children febrile population presenting at outpatient departments. Since April 2017, the first study site, the Malawi Epidemiology & Intervention Research Unit in Northern rural Malawi, has recruited 1000 patients. A wide range of reference tests has been performed to inform the cause of fever. Further, clinical and microbiological data of all patients with an undetermined diagnosis will be reviewed by a clinical panel to classify each patient based on the cause of fever. Complete data for the first 406 patients (57% female; 35% 2-4y; 38% 5-17y; 27% 18-65y) and corresponding preliminary results on the performance of six biomarker tests (C-reactive protein (CRP), Procalcitonin, Human neutrophil lipocalin, Myxovirus resistance protein + CRP, Heparin binding protein, Chitinase 3-like 1) are available. This study will give a preliminary overview of the performance characteristics of multiple promising biomarkers in sub-Saharan Africa when used in non-severe febrile patients. This dataset will inform subsequent product development efforts and implementation efforts to help improve non-malarial fever management and reduce overuse of antibiotics.

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ECONOMIC COSTS OF *PLASMODIUM VIVAX* EPISODES: A MULTI-COUNTRY COMPARATIVE ANALYSIS USING PRIMARY TRIAL DATA

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A better understanding of the costs of *P. vivax* is needed to optimize the allocation of resources for malaria elimination. We elucidated the costs associated with vivax malaria across a range of endemic settings. Cost data were collected in the context of a multi-centered clinical trial comparing 7 and 14-day primaquine regimens in Afghanistan, Ethiopia, Indonesia and Vietnam. Patients were surveyed on days 0 and 14 to determine the costs of treatment and transportation, and the duration that they and any caregiver were unable to perform usual activities. These productivity losses were valued at one gross domestic product per capita per day. Micro-costing was used to collect the additional costs required to diagnose and treat vivax malaria. The mean (SD) total households costs ranged from US$8.4 (US$4.2) in Afghanistan to US$68.6 (US$ 66.8) in Indonesia. Across all countries, the productivity costs were the largest cost component with a median (range) of 2.5 (1-4) days for patients and 1 (0.5 - 3) day for carers. The cost of administering a G6PD RDT ranged from US$0.9 to US$13.9 and this was consistently lower than the costs of the widely-used Fluorescent Spot Test (FST), which ranged from US$13.7 to US$17.9. The cost of a Haemocue™ test to monitor for anemia ranged from US$0.2 in Ethiopia to US$1.6 in Indonesia. Screening for G6PD deficiency with an RDT was less costly than testing with FST. An episode of vivax malaria results in substantial costs to households. These costs are primarily due to productivity losses, which are often excluded from economic evaluations.

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USE OF MOBILE TECHNOLOGY-BASED PARTICIPATORY MAPPING APPROACHES TO GEOLOCATE HEALTH FACILITY ATTENDEES FOR DISEASE SURVEILLANCE IN LOW RESOURCE SETTINGS

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Identifying fine-scale spatial patterns of disease is essential for effective disease control and elimination programmes. In low resource areas without formal addresses, novel strategies are needed to locate residences of individuals attending health facilities in order to efficiently map disease patterns. We aimed to assess the use of Android tablet-based applications containing high resolution maps to geolocate individual residences, whilst comparing the functionality, usability and cost of three software packages designed to collect spatial information. Using Open Data Kit GeoODK, we designed and piloted an electronic questionnaire for rolling cross sectional surveys of health facility attendees as part of a malaria elimination campaign in two predominantly rural sites in the Rizal, Palawan, the Philippines and Kulon Progo Regency, Yogyakarta, Indonesia. The majority of health workers were able to use the tablets effectively, including locating participant households on electronic maps. For all households sampled (n=603), health facility workers were able to retrospectively find the participant household using the Global Positioning System (GPS) coordinates and data collected by tablet computers. Median distance between actual house locations and points collected on the tablet was 116m (IQR: 42 - 368) in Rizal and 493m (IQR: 258 - 886) in Kulon Progo Regency. Accuracy varied between health facilities and decreased in less populated areas with fewer prominent landmarks. Results demonstrate the utility of this approach to develop real-time high-resolution maps of disease in resource-poor environments. This method provides an attractive approach for quickly obtaining spatial information on individuals presenting at health facilities in resource poor areas where formal addresses are unavailable and internet connectivity is limited. Further research is needed on how to integrate these with other health data management systems and implement in a wider operational context.

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MALARIA AND RICE IN AFRICA: STILL A PADDIES PARADOX?

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Due to the growing popularity of rice as a staple crop in Africa and how rice fields increase mosquito abundance, the effect of rice on malaria needs to be revisited. Previously, this relationship has been disregarded due to a phenomenon found in numerous studies called the paddies paradox, where rice does not lead to increased malaria risk despite increased quantities of malaria vectors due to better socioeconomic conditions and higher bed-net and antimalarial drug usage in rice farmers. However, since these studies, the malaria situation across Africa has changed dramatically and one can no longer assume this disproportionate distribution in malaria protective measures. Therefore, through a comprehensive literature review, we aim to reveal the true effect of rice on malaria. A search for studies that assessed the effect of rice on malaria vector quantities and/or malaria transmission was performed, and 41 studies and 3 reviews were found. As anticipated, higher malaria vector abundances, of up to 500-fold, were observed in rice-growing communities. This was seen particularly in the dominant and highly efficient vectors *Anopheles gambiae* sensu lato. Preceding the amplification of malaria interventions across sub-Saharan Africa in the early 2000s, most studies were subjected to the paddies paradox. Various hypotheses attempted to explain the paradox, including wealth creation and increased bed-net usage due to greater nuisance from higher mosquito abundance in rice communities as well as lowered anthropophily of malaria vectors due to decreased longevity and increased zoophily in rice fields. However, as the malaria situation has changed, there is some evidence that the paddies paradox no longer applies and that rice actually results in an increase in malaria prevalence. Whilst an alternative option is to advocate for intermittent irrigation, many outstanding questions remain unanswered. Nonetheless, this intimation of a recent malaria increase in rice-growing areas is a cause for concern and suggests it takes a lot more to properly eliminate malaria in sub-Saharan Africa, considering its upcoming intensification in rice.

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IMPROVING PROCUREMENT AND REDEPLOYMENT OF DISTRICT LEVEL MALARIA COMMODITIES USING SHORT MESSAGE SERVICE (SMS) AND WEB MAPPING IN MADAGASCAR

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Malaria remains a public health problem in Madagascar. In 2016, it was the fourth leading cause of morbidity in health centers and the fourth leading cause of hospital mortality. One issue at health facilities is stock out of malaria commodities. However, the reporting system does not allow for oversight of the monthly stock situation in health facilities. The USAID Maternal and Child Survival Program provided a simple, low-cost tool to map the monthly stock levels of key malaria commodities through structured short message service (SMS) communications by health providers in facilities. Health providers sent a text message about seven essential health commodities, containing a maximum of 30 characters including spaces. The SMSs sent by health providers were registered directly into a database host on a web server and the web-based map was updated immediately with stock-out rates. This mapping was accessible via web and viewable at district, regional and national levels with Internet connections. Mapping facilitates decision-making at all levels to develop and implement commodity recovery plans, either by replenishing at the district level or by redeploying surplus stocks from neighboring public sector health facilities. The usual wait time of 2 months to receive new stocks can be decreased by requesting commodities from neighboring centers that have overstocks of the required items. Results for the first month of implementation (January 2018) from 107 facilities in 48 districts sending SMSs demonstrated that 51% of health facilities reported a stock-out of injectable artesunate, 4% of artemisinin combination therapy and 67% of insecticide treated bednets. These results have been shared with supply chain actors to alert them of the stock-out. This intervention is expected to contribute to improved commodity availability by recommending update of commodities management procedures and manuals to prioritize the closest resources first, and prompt supply chain actors to complete the normal process to replenish stocks.

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INTEGRATED DRUG EFFICACY SURVEILLANCE (IDES): THE FEASIBILITY OF USING ROUTINE CASE MANAGEMENT AND FOLLOW-UP ACTIVITIES TO MONITOR DRUG EFFICACY AND RESISTANCE IN THAILAND

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Therapeutic efficacy studies (TES) are the World Health Organization's (WHO) recommended approach for national malaria programs to monitor the efficacy of antimalarial drugs. With declining malaria transmission, TES case enrollment has become challenging. In 2017, WHO and the Thai Ministry of Public Health recommended piloting integrated Drug Efficacy Surveillance (iDES) to ensure complete treatment of all cases in the context of malaria elimination. iDES integrates patient follow-up for all species with microscopy and dried blood spots (for molecular testing) as part of routine case surveillance to ensure compliance and adequate treatment outcomes. The approach included supervised radical treatment according to the national guidelines (dihydroartemisinin-piperaquine + primaquine for *Plasmodium falciparum* and mixed infection; chloroquine + primaquine for *P. vivax* and monitoring of parasite clearance during follow-up visits. Blood films were confirmed by an expert microscopist via the routine laboratory quality assurance system. Dried blood spots were collected at the time of diagnosis for detection of drug resistance markers and during each follow-up for confirmation of positive microscopic results. From May-December 2017, a total of 270 malaria cases were registered. Approximately 25% of the 88 migrant cases completed three or more follow-up visits. Among the 182 Thai cases, 78% of *25 Pf* cases (78% on day 7, 87% on day 28, 65% on day 42, and 74% on day 60) and 63% of the 156 *Pv* cases (72% on day 14, 63% on day 28, 63% on day 60, and 62% on day 90) completed three or more follow-up visits. None of the patients exhibited delayed parasite clearance. While we are currently assessing what infrastructure and resources are needed to implement iDES in a malaria elimination context, as compared to TES, our results suggest that iDES can be used to monitor radical cure of cases and drug resistance through adequate routine case management.

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RISK OF EXPOSURE TO FECAL CONTAMINATION FOR ADULTS AND CHILDREN IN SLUM, NON-SLUM, AND HIGH-INCOME NEIGHBORHOODS ACROSS DHAKA, BANGLADESH USING THE SANIPATH EXPOSURE ASSESSMENT TOOL

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Dhaka, Bangladesh has experienced rapid population growth and challenges meeting sanitation service demands. Poor sanitation and fecal sludge management can lead to fecal contamination in the urban environment. To understand risk of exposure to fecal contamination, an assessment of 10 environmental pathways was conducted using the SaniPath Tool. Data were collected from 10 neighborhoods (6 low-income, 2 mixed-income, and 2 high-income) throughout Dhaka between April-June 2017. 1000 environmental samples were collected from shared latrines, public play areas, produce in markets, street food, open drains, flood waters, surface waters, bathing water, municipal water, and non-municipal water. Samples were analyzed using IDEXX-Colilert-24® Quanti-Tray/2000 to determine most probable number (MPN) of *E. coli*. Behavior surveys were conducted with households, school children, and community groups to understand interaction with the environment. Using Bayesian methods, the behavioral and microbiological data were used to estimate the percentage of the population exposed and mean dose of fecal exposure (*E. coli*) for each environmental pathway in each neighbourhood. For adults, consumption of raw produce was the dominant exposure pathway (i.e. makes greatest contribution to total exposure) in 6 of the 10 neighborhoods. The percentage of adults exposed to fecal contamination through ingestion of raw produce ranged from 78-99% and the mean dose ranged from $10^{4.8}$ to $10^{7.5}$ MPN of *E. coli* ingested/month. The most common dominant pathway of exposure amongst children (8/10 neighborhoods) was accidental ingestion of drain water from playing in, or passing through, open drains (up to 94% exposed; mean dose of $10^{7.8}$ MPN of *E. coli* ingested/month). Ingestion of fecal contamination via produce and street food was a high risk throughout Dhaka, while exposure to fecal contamination via municipal drinking water was mainly greater risk for those living in the south of the city. These results can guide city-wide WASH programming to prioritize interventions aimed at dominant exposure pathways and increase the potential for public health impact.

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SAND BARRIERS AROUND PIT LATRINES REDUCE FECAL BACTERIAL LEACHING I0NTO SHALLOW AQUIFER: A RANDOMIZED CONTROLLED TRIAL IN COASTAL BANGLADESH

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Pit latrines are used worldwide but present a risk to human health if fecal pathogens leach into the groundwater. We evaluated the effect of installing a sand barrier around pits to reduce the leaching of fecal indicator bacteria (FIB) into the shallow aquifer. We constructed 68 new off-set single pit latrines in Galachipa sub-district of Patuakhali district of coastal Bangladesh. We randomly assigned 34 latrines to have a 50-cm thick sand barrier under and around the pit and 34 received no sand barrier. Four monitoring wells of 20 feet depth were constructed around each pit, 1 meter from the pit edge to collect water samples at baseline and subsequent nine follow-up visits over 24 months. Samples were tested using the IDEXX Colilert method to enumerate the most probable number (MPN) of *E. coli* and fecal coliforms. We determined the difference in mean $\log_{10}$MPN of FIB counts/100ml water between intervention and control latrine well samples using multilevel linear models with random intercepts for each latrine and monitoring well and reported cluster robust

standard error. In total, 1209 water samples were tested for *E. coli* and fecal coliforms. The mean $\log_{10}$MPN counts/100ml water for *E. coli* was 1.43 for the control and 1.05 for the intervention latrines, and for fecal coliforms were 1.63 for the control and 1.25 for the intervention latrines. Compared to the control latrines' monitoring wells, the water samples of the intervention latrines' monitoring wells had a 0.38 (95% CI: 0.16, 0.59; p = 0.001) mean $\log_{10}$MPN reduction in *E. coli* and a 0.38 (95% CI: 0.14, 0.62; p = 0.002) mean $\log_{10}$MPN reduction in fecal coliforms suggesting a reduction of 27% *E. coli* and 24% fecal coliforms. Our data suggest that a sand barrier around and under the pit reduced groundwater fecal contamination in context of shallow aquifers.

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THE EFFECT OF SOCIAL COHESION ON DIARRHEAL DISEASE OVER TIME IN RURAL ECUADOR

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Though social organization and connectedness are critical to the functioning of communities and disease reduction because of behavior change, infectious disease transmission studies typically use social networks as maps of direct contact to model risk. Prior cross-sectional analyses in Ecuador have suggested a greater density of social ties between individuals in remote communities may lead to the spread of sanitation practices, both individual and collective, that help reduce the transmission of diarrheal disease. With social network data from 2007-2013 collected from the same cohort of villages in Ecuador, we evaluate the association between social cohesion and acute gastrointestinal illness (AGI) over time. Using different measures of social cohesion: individual trust in a community, individual belongingness to community social organizations, and social network structure, we examine the effect of social cohesion on AGI in 20 communities among persons >13 years of age. We determine social network structure from two different types of networks: who an individual passes time with and who an individual goes to for important matters. From 2007-2013, there is an 18% increase in AGI (10.6%-12.5%), a 36% reduction in trust, and a 44% reduction in the number of social organizations an individual belongs to. Using a Bayesian hierarchical model with an informative prior, we find an individual's trust in her community becomes more protective against AGI over time (2013: OR 0.64(0.54,0.75)). Individuals who are socially deviant, have a greater number of ties than community average, have increased risk of AGI over time in a passing time network (2007: OR 0.71 (0.66,0.77); 2013: OR 1.20 (1.11,1.30)). On the other hand, individuals who live in communities with a greater average of ties in an important matters network become more protected against AGI over time (2007: OR 0.89 (0.74,1.06); 2013: OR 0.74 (0.63,0.87)). Measures of social cohesion from different network structures, passing time and important matters, exhibit opposing effects on AGI. Understanding social dynamics in communities could improve approaches to intervention implementation.

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TEMPERATURE AND ALL-CAUSE DIARRHEA IN NORTHERN ECUADOR: CHANGING SEASONALITY AFTER ROTAVIRUS VACCINE INTRODUCTION

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In general, diarrhea is more prevalent in warmer seasons. However, this effect varies by pathogen—warm temperatures are generally associated with bacterial diarrhea whereas rotavirus diarrhea is more common in cooler seasons. In this analysis, we assess the relationship between temperature and all-cause diarrhea in rural northwestern coastal Ecuadorian communities in two different surveillance periods: 2003-2007 and 2011-2013. We examine whether the introduction of rotavirus vaccination late in 2008, between the two periods, modifies the overall seasonality of diarrhea. We use generalized Poisson regression models with an offset for population size to estimate the effect of temperature on all-cause diarrhea separately for the two surveillance periods. We account for community clustering using an auto-regressive correlation structure and ran these analyses stratified by age group (<5 and >5 years) and for both age groups combined. While the two surveillance periods had similar overall rates of diarrhea, higher vaccine coverage was associated with lower rates of diarrhea (IRR=0.304, 95% CI: 0.118, 0.785). This association was present for all age groups, including among older, unvaccinated children and adults. In the pre-vaccine period, diarrhea outbreaks were more common during the cooler weather months, with incidence peaking in September-November. Transient drops in temperature one week prior were associated with increased diarrhea among young children (<5 years) (IRR=0.770, 95% CI: 0.612, 0.968), but not adults, consistent with viral diarrhea. Seasonality was similar in the post-vaccine compared to the pre-vaccine period, but the overall peak was earlier (July-September) and the transient drops in temperature were attenuated (IRR=0.866, 95% CI: 0.621, 1.21). The effect of temperature was not modified by vaccine coverage, community hygiene score, coverage of improved sanitation, or remoteness. These analyses are suggestive that diarrhea has been associated with cooler temperatures in Ecuador but that this tendency may be shifting due to the introduction of rotavirus vaccination.

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EFFECT OF DRINKING WATER CHLORINATION AT THE POINT-OF-COLLECTION ON CHILD DIARRHEA IN DHAKA, BANGLADESH: A DOUBLE-BLIND CLUSTER-RANDOMIZED CONTROLLED TRIAL

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In cities of low-income countries, few utilities are able to maintain fully pressurized piped systems that deliver water 24 hours a day, resulting in contaminated water at the point-of-collection. We evaluated the effect of a decentralized, point-of-collection water chlorination intervention on child health through a double-blind cluster-randomized controlled trial among 100 shared water points in Dhaka, Bangladesh. The treatment group received drinking water automatically chlorinated by a solid tablet chlorine doser, while the control group received water treated by a doser identical in appearance that supplied vitamin C. Drinking water at treatment taps had detectable chlorine residual 81% of the time (compared to 0% at control taps). The mean total chlorine residual at taps in the treatment group was 0.4 ppm, which is above international minimum guidelines but below the average dose of typical point-of-use chlorine treatment products. When asked to guess their treatment status, 30% of respondents in the treatment group and 20% in the control group thought the device was dosing chlorine. Children in the treatment group had 23% less caregiver reported diarrhea than children in the control group (Treatment: 10%, Control: 7.5%; PR: 0.77, 95% CI 0.65-0.91; N=4227). Reported illness-related expenditures and antibiotic consumption in the past two months per child were significantly lower in the treatment group than the control group. There were no significant differences between groups in weight-for-age z-scores or negative control outcomes (e.g. bruising). Whereas many previous water intervention trials have focused on household-level water treatment, our findings demonstrated effectiveness of an automatic point-of-collection water

treatment intervention. While previous water treatment blinded trials have failed to detect health impacts, we estimated a significant reduction in child diarrhea in a low-income urban setting. Our results also suggest targeting a low chlorine residual dose (0.3-0.5ppm) in an effort to increase acceptability of chlorinated water can still improve water quality and reduce the risk of diarrhea.

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PERCEPTIONS, EXPERIENCES AND ACCEPTABILITY OF A WATER INTERVENTION USING RIVERBANK FILTRATION TECHNOLOGY IN RURAL INDIA

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Universal and equitable access to safe and affordable drinking water is one of the United Nations' Sustainable Development Goals. While global gains towards this goal have been made, many communities are unable to afford technical water treatment solutions. Riverbank filtration technology (RBF) is an inexpensive water treatment technique that can be delivered at a community level and sustained using local resources. In the setting of a stepped-wedge cluster randomised controlled trial of a water intervention in rural Karnataka, India, we performed a mixed methods study to evaluate the perceptions, experiences and acceptability of RBF-treated water delivered to community village tanks. Quantitative data was collected through household surveys and analysed using Stata version 14. Qualitative data were collected through semi-structured household-level interviews and focus groups with data collectors. All discussions were audio-recorded, transcribed and translated verbatim prior to thematic analysis using NVivo version 11. Uptake of RBF water supply was not universal; at the conclusion of the study, less than 20% of households described it as their primary source of drinking water. Qualitative analysis revealed reluctance among many villagers to adopt a new primary drinking water source due to the belief that changes in water source would result in diarrhoea and other symptoms. Other barriers to uptake included salinity and hardness of the water, which affected taste, cooking time and its ability to form a lather with soap when washing clothes. The storage of RBF water in plastic tanks was perceived to affect the quality and taste of the water, and many householders expressed that their preference would be to have water piped directly to their homes. While RBF has potential as a sustainable drinking water solution for some rural communities in India, we identified significant barriers to its uptake and use, including its mode of delivery. The design and delivery of future RBF interventions should ensure that community consultation and education are built into the intervention delivery.

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ADAPTIVE SITES ALLOCATION FOR TYPHOID ENVIRONMENTAL SURVEILLANCE IN TWO INDIAN CITIES

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Environmental surveillance (ES) detects circulation of pathogens in the environment and has been used to complement clinical surveillance to monitor disease (e.g. poliovirus) transmission in communities. Although WHO published guidelines for ES for poliovirus in 2003, a systematic method to determine the locations of ES sites does not exist. Our goal is to choose ES sites for typhoid to achieve high sensitivity in two Indian cities. We developed an adaptive ES sites allocation method that dynamically updates sites based on their performance. In the pilot phase, 30 sewage samples from different areas of Kolkata were analyzed for *E. coli*, human-specific phage, *Salmonella* Typhi and *Salmonella* Paratyphi A to determine an initial range of detection frequency and concentration for each target microorganisms. Presences of *S*. Typhi and *S*. Paratyphi A in specific geospatial areas and the sensitivities of different potential ES sites were simulated. A certain number of ES sites were initialized (selected from potential ES sites) based on prior information/randomization and periodically updated based on their performance. The sensitivity of a site (how often it can detect fecal contamination, human fecal contamination, and *S*. Typhi or *S*. Paratyphi A) and "information loss" by removing a site will be periodically evaluated to determine which site(s) should be relocated. Parameters and settings (the number of sites initialized, the number of sites relocated per update, the time between two updates etc.) for the site initialization and update process can be optimized to rapidly achieve an ES system with high sensitivity. 72 scenarios have been tested. With appropriate parameters and settings, a high average sensitivity of sites (80%) were reached within 10 updates (12 weeks per update) from randomized initial sites (average sensitivity around 50%). This study also examined how the adaptive site allocation method performs for spatially-correlated sites, seasonality of *S*. Typhi and *S*. Paratyphi A circulation, and temporal changes in high-risk areas. In those scenarios, the adaptive sites allocation method consistently outperformed the fixed sites.

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INVESTIGATING IMMUNE SIGNATURES IN MALARIA-EXPOSED CHILDREN

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While naturally acquired immunity to Malaria provides promise of developing an effective vaccine, decades of study have yielded no definitive correlates of protection and the most advanced vaccine candidate provides only partial and short-lived protection. Given the complicated host-pathogen interactions involved it is unlikely that approaches focused on few parameters will unravel the underlying immune mechanisms responsible for immunity. We used a systems approach comprising whole blood transcriptomic, cellular and plasma analyses on a cohort of children who had been under active surveillance for malaria for several years. We developed and verified systems immunology tools capable of identifying molecular and cellular signatures of children of similar age who have experienced a "high" or "low" number of clinical malaria episodes. Transcriptomic, cellular, cytokine and active malaria surveillance were integrated to define signatures associated with malaria experience. High-episode children are distinguishable from low-episode children by enhanced expression of genes involved in immune activation and regulation, with modular analysis revealing enrichment in interferon-inducible genes. For a subset of high-episode children we also note a distinct signature related to hemoglobin biosynthesis, which appears to correlate with clinical anaemia, and may reflect enhanced erythropoiesis in response to malaria-induced anaemia. The transcriptomic signature of enhanced immune activation in high-episode children is supported by elevated levels of pro-inflammatory cytokines (including IL-6 and TNF-α), while high IL-10 and a subset of $\gamma\delta$ T cells distinguished high- from low- episode children. Through cellular deconvolution of the transcriptomic data, high-episode children appear to be associated with functionally altered B cells, $CD8^+$ T cells and neutrophils compared to low-episode children. Whole blood analysis reveals a distinct immune signature

associated with repeated episodes of clinical malaria. The implications of this signature for anti-malarial immunity are the focus of an on-going project.

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B CELL MEDIATED AUTOIMMUNE ANEMIA IN MALARIA PATIENTS

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Malaria is still one of the leading infectious diseases in the world and a global health burden. Severe anemia is one of the most common malaria-associated complications, which accounts to both increased morbidity and mortality. Our lab has recently identified autoantibodies that target the membrane lipid phosphatidylserine (PS) on uninfected erythrocytes as a major promoter of malarial anemia in a rodent model. High levels of anti-PS antibodies also correlate with *P. falciparum* malarial anemia in patients. Additionally, we have recently characterized an atypical population of B-cells expressing the transcription factor T-bet and surface marker CD11c as being major producers of anti-PS IgG antibodies during malarial anemia in a mouse model and in *ex-vivo* cultures of naïve human PBMCs exposed to *P. falciparum* infected erythrocyte lysates. The aim of this work was to identify the presence of T-bet$^+$ B-cells and its possible correlation with anti-PS IgG antibodies and anemia in malaria patients. To test this hypothesis, we assessed PBMCs from German *P. falciparum* malaria patients for different B cell populations such as Plasma cells, Naïve, Immature, Activated memory, Classical memory, Atypical memory, CD11c$^+$ and T-bet$^+$ B-cells. Furthermore, we assessed the plasma of these patients for anti-PS and anti-red blood cell (RBC) lysate IgG antibodies, hemoglobin as a measurement of anemia, along with other clinical parameters such RBC and thrombocyte counts. Our results showed an inverse correlation between percentage of T-bet$^+$ B-cells and hemoglobin hence associating these B-cells with anemia development in this cohort of malaria patients. Moreover, we also observed that Atypical Memory B-cells (CD21$^-$, CD27$^-$, CD20$^+$ CD10$^-$), along with Fcrl5$^+$ B-cells, that are characteristic of malaria patients and also express T-bet, correlate with anemia development. Lastly, we observed CD11c$^+$ B-cells correlating positively with plasma anti-RBC antibody levels and a positive trend with anti-PS antibody levels. These results suggest that T-bet B cells may contribute to anemia in malaria patients through the generation of autoimmune antibodies.

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EX VIVO ANALYSIS OF *PLASMODIUM FALCIPARUM*-SPECIFIC B CELL RESPONSES TO NATURAL MALARIA INFECTION IN CHILDREN AND ADULTS

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In naturally acquired immunity to malaria, antibodies that reliably protect are only acquired after years of repeated *Plasmodium falciparum* (*Pf*) infections. We have shown in Mali that this inefficiency in humoral immunity to malaria is associated with a large expansion of CD21^{lo}CD27$^-$ 'atypical' MBCs. The function of atypical MBCs remains elusive, as they exhibit altered B cell receptor (BCR) signaling and when activated fail to secrete cytokines and antibodies. We set out to study whether atypical or classical B cells are recruited into the immune response to a febrile malaria infection: using *Pf* antigen probes, we characterized *Pf*AMA1/*Pf*MSP1-specific B cells in Malian children and adults (ages 2 to 36 years), at well-defined time points before, during and after a febrile malaria episode. Our preliminary analysis shows that *Pf*-specific atypical MBCs display signs of activation after a febrile malaria infection. Using antigen probes to track influenza hemagglutinin-specific B cells in the same individuals, we have gained insight into the relative role of *Pf* in driving atypical MBC expansion and have also compared the magnitude and kinetics of *Pf*- and influenza-specific atypical and classical MBC responses. We further show that classical *Pf*-specific IgG$^+$ MBCs are activated and expanded, but interestingly, that the majority of activated *Pf*-specific MBCs are unswitched, expressing surface IgM. Additionally, we have single cell sorted *Pf*- and influenza-specific MBCs to compare their BCR features (VH gene diversity and somatic hypermutation rates). This analysis provides important new insights into the mechanisms underlying the inefficient acquisition of naturally acquired immunity to malaria in children.

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PREDOMINANT LOSS OF LOW AVIDITY IGG ANTIMALARIAL ANTIBODIES ASSOCIATED WITH REDUCTION IN INFECTION AFTER INDOOR RESIDUAL SPRAYING IN NAGONGERA, UGANDA

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Immunity to *Plasmodium falciparum* (*Pf*) malaria is acquired slowly after repeated exposure and poorly maintained, with antibodies playing a critical role. We evaluated total and high avidity IgG antibodies to *Pf* blood stage antigens in 160 participants from Nagongera, Uganda, where childhood malaria incidence declined from 4 episodes per person per year to near zero following indoor residual spraying of insecticide (IRS). Samples were obtained at 4 time points, from a longitudinal cohort with all episodes of *Pf* infection and malaria recorded: 12 and 6 months before IRS and 6 and 12 months post IRS. IgG antibodies against 19 antigens were measured using a multiplex bead array (MagPix platform). Levels of high avidity (strongly binding) IgG antibodies were measured by adding guanidine hydrochloride (GuHCl) to dissociate weakly binding antibodies. The avidity index (AI) was calculated as median fluorescence intensity (MFI) of GuHCl treated samples (avid) divided by MFI of untreated samples (total). We observed preferential net loss of of total vs. avid antibody for all antigens (e.g. Rh4.2; median 1.04 vs 0.32 log MFI, SBP1; 0.91 vs 0.21, HSP40; 0.74 vs 0.15, Hpy2; 0.74 vs 0.08, EBA140; 1.02 vs 0.47) between 6 months pre (highest incidence) and 12 months post IRS (lowest incidence). Including all 4 time points in a generalized estimation equation model, net loss of both total and avid antibodies was significantly associated with days since last infection in all antigens, independent of age. Preferential persistence of avid antibodies post infection manifested into net increased median AI for 17/19 antigens (e.g. SBP1; 0.25, HSP40; 0.17, Hpy2; 0.16, EBA140; 0.12). Our results suggest that frequent infection with *Pf* results in the acquisition of IgG antibodies with predominantly low avidity, but that low avidity antibodies wane more rapidly than high avidity antibodies following a reduction in exposure. We postulate that high avidity antibody pool is from affinity matured long lived plasma cells. This pool may be responsible for maintenance of circulating antibody levels in absence of infection and may play a role in maintenance of acquired immunity.

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FUNCTIONAL ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* SPOROZOITES ARE ASSOCIATED WITH A LONGER TIME TO NATURALLY ACQUIRED PCR-DETECTED INFECTIONS AMONG SCHOOLCHILDREN IN BURKINA FASO

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Individuals living in malaria-endemic regions can develop naturally acquired immunity against malaria severe disease and death, but it is unclear if sterilizing immunity does develop even after years of exposure. This was addressed in a longitudinal study of schoolchildren in Burkina Faso. Healthy schoolchildren (parasite-free by microscopy) received a curative dose of antimalarial to clear possible sub-patent infections before the start of the transmission season. Subsequently, *P. falciparum* infections were detected by PCR during weekly visits. Upon parasite detection, children were followed daily basis until day 7 then weekly basis up to 35 days. Participants were closely monitored for the development of malaria symptoms. Anti-malarial was given upon the detection of symptoms or 35 days after parasite detection, whichever came first. Venous blood sample were collected at enrolment for immunological assessments. Antibodies to circumsporozoite protein (CSP) were detected by ELISA. Sporozoite gliding inhibition of purified IgG was assessed using NF54 sporozoites; hepatocyte invasion using primary hepatocytes. 51 infections were monitored; the median time to first detection of infection by PCR was 27 days. Although antibodies responses to pre-erythrocytic antigens were low, IgG of the majority of schoolchildren reduced *in vitro* sporozoite gliding motility with a median gliding inhibition of 59.6%. Gliding inhibition and antisporozoites antibodies levels were strongly correlated ($P<0.0001$). Also, there was a positive correlation between gliding inhibition and reductions in hepatocyte invasion ($P=0.02$). Survival analysis indicated a longer time to infection detection in individuals displaying strong gliding inhibition. Naturally exposed children with functional anti-sporozoite antibody responses have an increased time to developed *P. falciparum* infection compared to children with lower responses. This response does not prevent them from becoming infected during the season but is important for the development and evaluation of interventions that aim to prevent malaria infections by active or passive immunisation.

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IGG3 SUBCLASS ANTIBODIES MEDIATE THE MOST EFFECTIVE FUNCTIONAL RESPONSES AGAINST *PLASMODIUM FALCIPARUM* MEROZOITE INVASION LIGANDS

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Naturally-acquired antibodies are important for protection from symptomatic malaria. Some antibodies target merozoites and inhibit erythrocyte invasion. Field studies suggest that these antibody responses are typically skewed to IgG1 or IgG3 subclasses, and that IgG3 responses often have a stronger association with protection. However, it is unclear how their different properties impact protective immunity, and therefore which IgG subclass is required for the most effective vaccine response. We hypothesised that the different effector responses of these IgG subclasses would impact functional immunity. To assess this, we made IgG subclass-switch antibodies; these are mAbs engineered with the same variable region, i.e. same epitope target, but different human IgG subclass backbones, and then compared them using *in vitro* functional assays. We focused on merozoite antigens EBA-175, Rh5 and AMA-1, as they are important invasion ligands and leading vaccine candidates. For each antigen, existing monoclonal antibodies (mAbs) that target known neutralising epitopes were selected, and used to express IgG subclass-switch mAbs. Characterisation confirmed their expected size, subclass, similar N-glycosylation patterns and ability to bind their respective recombinant and native proteins. Unexpectedly, IgG3 variants showed significantly greater inhibition of merozoite invasion compared to other IgG subclasses. IgG3 variants were most effective at C1q fixation and subsequent deposition of the membrane attack complex. These results indicate the key role of IgG3 in mediating functional immunity and support findings from field studies that show strong associations between IgG3 responses and protection. It is likely that the superior Fab flexibility of IgG3 enables more effective antigen binding leading to enhanced neutralisation and formation of IgG complexes for complement fixation. These findings provide a strong rationale to explore the development and efficacy of vaccines that induce strong IgG3 responses.

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IL-15 COMPLEX TREATMENT PROTECTS MICE FROM CEREBRAL MALARIA BY INDUCING IL-10-PRODUCING NK CELLS

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Cerebral malaria (CM) is one of the most lethal complications of *Plasmodium falciparum* infection, responsible for a large fraction of the nearly 500,000 malaria-related deaths annually. In the experimental mouse model of CM (ECM), mice are inoculated with red blood cells infected with *Plasmodium berghei* ANKA (PbA) parasites and develop a CM-like disease within 6-10 days post-infection (dpi). We found that treatment of C57BL/6 mice with interleukin (IL)-15 complexes (IL-15C; IL-15 bound to an IL-15Rα-Fc fusion protein) prevented the development of PbA-induced ECM. IL-15C-expanded natural killer (NK) cells were necessary and sufficient for protection against ECM. IL-15C treatment also decreased CD8+ T cell activation in the brain and prevented blood brain barrier breakdown without influencing parasite load. Moreover, we found that IL-15C treatment of mice induces IL-10 expression in NK cells, and that IL-10 is required for IL-15C-mediated protection from ECM. Additionally, primary human NK cells produce IL-10 following *in vitro* culture with IL-15 or with ALT-803, a modified human IL-15C. Finally, we show that ALT-803 mediates similar induction of IL-10 in mouse NK cells and protection against ECM. Using conditional genetic deletion strategies, we are investigating the cellular target(s) of NK cell-derived IL-10, including CD8+ T cells, endothelial cells, and myeloid cells. We are also investigating the signaling pathway required for IL-15C-mediated induction of IL-10 in NK cells. In summary, these data indicate that NK cells – which are typically involved in promoting inflammatory responses – can also restrain damaging immune responses, suggesting that IL-15C holds promise as an adjunctive therapy for immune-mediated diseases, potentially including CM.

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EVALUATION OF THE PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF PRAZIQUANTEL IN THE *SCHISTOSOMA MANSONI* MOUSE MODEL

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After more than 40 years of use, Praziquantel (PZQ) still remains the drug of choice for the treatment of intestinal and urogenital schistosomiasis. Its anti-parasitic activity resides primarily in the (*R*)-enantiomer. Hitherto neither the molecular target nor the pharmacokinetic-pharmacodynamic relationship have been fully elucidated. Here we investigated the efficacy and pharmacokinetics of PZQ in the *Schistosoma mansoni* mouse model to determine the key factors that drive its efficacy. Dose-response studies with racemic PZQ with or without addition of an irreversible pan-cytochrome P450 (CYP) inhibitor, 1-aminobenzotriazole (ABT), were performed. In addition, efficacy of PZQ in the presence of the CYP inducer, dexamethasone (DEX), was determined. Plasma samples were obtained by tail vein bleeding at 4 time points. The (*R*)-PZQ levels were determined using a LC-MS/MS method. Non-compartmental pharmacokinetic analysis was performed using PKsolver. In addition, experiments using an enhanced *in vitro* assay were conducted. We found that the use of ABT increased (*R*)-PZQ plasma exposures in the systemic circulation by ~10 to 20 fold but the latter were not predictive of efficacy. The use of DEX decreased plasma exposures of (*R*)-PZQ in the systemic circulation by ~10 fold without reducing efficacy. We extrapolated the (*R*)-PZQ concentrations in mouse portal vein / mesenteric veins from the systemic exposures and found that a free exposure of (*R*)-PZQ of ~ 20 µM*h in the portal vein was needed to obtain a worm burden reduction >60%. It is suggested that the high (*R*)-PZQ concentrations available before the hepatic first pass metabolism drive the efficacy against *S. mansoni* adult worms residing in the mesenteric veins. It is then possible that the current dosing regimen of 40 mg/kg in preventive chemotherapy programs may provide suboptimal concentrations in low-weight patients such as children, due to smaller total amounts of drug administered, and may consequently result in lower cure rates.

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SAFETY AND EFFICACY OF NEW ORAL DISPERSIBLE TABLET (ODT) FORMULATIONS OF RACEMATE- AND L-PRAZIQUANTEL IN 2 TO 6 YEARS OLD CHILDREN INFECTED WITH SCHISTOSOMIASIS

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The Pediatric Praziquantel Consortium is developing a praziquantel (PZQ) orally dispersible tablet (ODT) 150 mg that is suitable and palatable for children ≤6 years infected with schistosomiasis. Two candidate ODTs are currently being tested: one containing the L-PZQ enantiopure active pharmaceutical ingredient and an ODT containing the racemate PZQ molecule. Results of an open label, dose-finding phase II trial in children 2 to 6 years infected with *Schistosoma mansoni* in Côte d'Ivoire (ClinicalTrials.gov Identifier: NCT02806232) will be presented. A total of 420 children, 60 in each of 7 cohorts, were randomized to receive 3x20 mg/kg or a single dose of 40 mg/kg of Biltricide®, 40 mg/kg or 60 mg/kg of racemate-PZQ ODT, or 30 mg/kg, 45 mg/kg or 60 mg/kg of L-PZQ ODT. Cure rate (CR) 14 to 21 days post-treatment was the primary efficacy endpoint; egg reduction rate (ERR) and rate of related treatment-emergent adverse events (TEAEs) were secondary endpoints. CRs and ERRs were assessed using triplicate Kato-Katz on 2 faecal samples collected within 5 consecutive days. CRs for participants with follow-up Kato-Katz results and no clinically important protocol deviations (n=372) were >80% for all cohorts. L-PZQ 60 mg/kg and 45 mg/kg showed the highest efficacy among ODTs: CR= 89.3% (95% confidence interval [CI]: 78.1%, 96.0%) and CR= 88.0% (95% CI: 75.7%, 95.5%), respectively. ERRs were ≥90% for all cohorts. Somewhat lower CRs were observed in moderate/heavy *versus* light infected patients for all cohorts. No differences in CR were observed for males versus females or for 4- to 6-year-old children versus younger participants. CRs diagnosed using the Point-of-Care Circulating Cathodic Antigen test were consistent with the Kato Katz results but lower for all cohorts. Only 64 subjects (15.2% of 420 treated) had 68 related TEAEs, of which most were mild and transient, and resolved within 24 hours. Both ODT formulations were well tolerated at all doses tested.

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DEVELOPMENT OF NOVEL DRUGS AGAINST SCHISTOSOMIASIS

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Human schistosomiasis is a debilitating, life-threatening disease affecting more than 250 million people in as many as 78 countries. Schistosomiasis is a neglected tropical disease and is second only to malaria as "the most devastating parasitic disease". There is only one drug of choice effective against all three major species of *Schistosoma*, Praziquantel (PZQ). However, as with many monotherapies, resistance is emerging in the field and can be selected for in the laboratory. Previously used therapies include Oxamniquine (OXA), however shortcomings such as toxicity and affordability resulted in discontinuation. Our collaborations with medicinal chemists and structural biologists have enabled us to develop and test novel drug derivatives of OXA to treat this disease. Using an iterative process for drug development, we have successfully identified one derivative that is effective against all three species of the parasite at varying levels and shows promising preliminary *in vivo* efficacy, CIDD-0066790. As CIDD-0066790 is a racemic mixture, we have isolated the R and S enantiomers of this derivative and identified the R form as most effective. Furthermore, we have identified three more efficacious derivatives, one of which CIDD-0072398 kills 100% of *S. mansoni* worms. We will present data on the *in vitro* and *in vivo* efficacy of these 3 novel drugs. Our goal is to generate a secondary therapeutic that can be used in conjunction with Praziquantel to overcome the ever-growing threat of resistance. In this regard we are testing our best derivatives in conjunction with PZQ to determine efficacy of combination therapy. The ability and need to design, screen, and develop future, affordable therapeutics to treat human schistosomiasis is critical for successful control program outcomes.

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SCHISTOSOMA MANSONI EGGS, ANTIGENS AND DNA CLEARANCE CURVES AFTER PRAZIQUANTEL TREATMENT AND REINFECTION RATES OVER SIX MONTHS: DIFFERENTIATING BETWEEN RAPID REINFECTION AND DRUG RESISTANCE

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Schistosoma mansoni causes the debilitating disease schistosomiasis. The World Health Organization recommends using Kato-Katz to detect eggs in stool and infection prevalences guide mass treatment decisions. However, Kato-Katz has low sensitivity, particularly with low intensities and/or post treatment. Rapid circulating cathodic antigen tests (POC-CCA) are more sensitive and WHO interim guidelines have been published for their use in disease mapping. While there are several methods for schistosomiasis diagnosis, including DNA analyses in blood and stool, it remains unclear which are best for monitoring drug efficacy in different endemic settings. Guidelines recommend using Kato-Katz 2 to 4 weeks post treatment. However, previous data have shown large discrepancies between cure rates measured by Kato-Katz and POC-CCA. Low egg counts, but high POC-CCA results post treatment could be due to juvenile worms tolerant to praziquantel and rapid reinfection, or due to worm surviving treatment (resistance) but with reduced egg production (embryostasis). Embryostasis would be undetected by Kato-Katz, and POC-CCAs cannot differentiate it from reinfection. There is a need to optimize drug-efficacy protocols and timeframes to capture true cure rates (adult worms dying) and differentiate reinfection from resistant worm embryostasis to inform control interventions. We present data from 55 children over six months following praziquantel treatment: characterizing parasite egg, antigen and DNA levels twice a week in stool, urine, and blood to elucidate parasite clearance and reinfection in a high endemic setting. Using sibship techniques, we identified full- and half-siblings through time, inferring if antigens are caused by worms surviving treatment or maturing juveniles. These data provide novel information on worm clearance, effective breeding populations, density-dependent egg production, fecundity compensation and embryostasis following treatment within individual children. Findings will inform optimal protocols and timelines for monitoring clearance and reinfection in high endemic settings.

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OVERCOMING CHALLENGES IN THE DIAGNOSIS OF *SCHISTOSOMA MANSONI* INFECTIONS USING POINT OF CARE METHODS, RECOMBINANT PROTEIN AND MONOCLONAL ANTIBODY TECHNOLOGIES

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Control constraints of Schistosomiasis include the lack of diagnostic methods with high sensitivity. We develop a prospective study in southeast Brazil to standardize new sensitive and rapid diagnostic methods for *Schistosoma mansoni* infection. Currently, we are investigating 6 endemic areas (>1000 individuals with chronic infection) and 84 travelers infected in a freshwater pool (with acute infection). Sera, urine, feces and saliva samples were used for the standardization/validation of innovative methods, including acute, chronic and post-treatment patients. Comparisons are performed with eggs in feces by 24 Kato-Katz slides and 2 analyses of Saline Gradient and clinical symptoms. Within our new point-of-care methods, we use selected recombinant proteins (MEAr, CCAr) and their respective monoclonal antibodies. We were able to detect the disease early as 10 days post-infection and more than 95% of positive cases from chronic low endemicity areas with our Dot Blot, IMS and Fluo-IMS rapid and cheap methods. Plus, POC FLT immunochromatography method uses concentrated urine to increase POC-CCA® sensitivity from 6% to 56% with a accuracy of 0.9, being equivalent to 21 Kato-Katz slides. Monoclonal antibody and recombinant protein technologies allowed a superior detection method when comparing to the conventional methods. In conclusion, data showed 100% of sensitivity of chronic patients and 98% of acute patients with new improvements and innovative POC methods.

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URINE POC-CCA STRIP ASSAY: A QUANTITATIVE METHOD FOR DIAGNOSING INTESTINAL SCHISTOSOMIASIS?

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In intestinal schistosomiasis light intensity infections are difficult to detect in the field, especially in endemic regions when using non-sensitive methods like the Kato-Katz technique. The point-of-care strip assay for the detection of the schistosome Circulating Cathodic Antigen (POC-CCA) in urine has shown to be a user-friendly and more sensitive alternative for the diagnosis of *Schistosoma mansoni* infections, but the reading is observer dependent and is often interpreted in a qualitative way. Therefore, in particular when the infection burden is low and the test lines become less strong, misinterpretation of the trace test lines might lead to a different result interpretation. The current study evaluates different approaches for quantification of the POC-CCA readings in the field and a standardized set-up for the strip readings with a focus on the low intensity signals. The innovative approach uses 3 different techniques to read the cassettes: (1) using a portable Qiagen ESEQuant Lateral Flow Reader; (2) performing visual readings compared against a series of artificial cassettes (inkjet-printed strips of different intensities to allow scoring on a G1-G10 scale); (3) generating a digital image of the test and control lines with quantitative interpretation using appropriate software. All techniques can be calibrated with a set of reference standards to allow appropriate quantification resulting in CCA concentration per ml urine. These are then compared with the visual scoring system of negative, trace, 1+, 2+ and 3+ signals. Endemic urine samples (n=100) were tested with POC-CCA and the results read by image processing using various computer software programmes. Results showed excellent correlations with the visual reading. In addition, 100 urine samples from different *S. mansoni* endemic areas were tested with POC-CCA using the above techniques. Overall, associations were highly significant, also in the lower intensity signals, indicating the usefulness and applicability of the different techniques in quantitation as well as for the resolve of the trace readings of the POC-CCA in different (endemic) settings.

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GLYCAN MICROARRAY-ASSISTED ANALYSIS OF DIAGNOSTIC ANTI-GLYCAN ANTIBODIES IN SCHISTOSOMIASIS

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During *Schistosoma* infections, antibodies (Abs) are raised against numerous antigens expressed by parasite larvae, adult worms and eggs. A large proportion of these Abs is directed against antigenic glycans that are part of the parasite's glycoprotein and glycolipid repertoire. If and how anti-glycan antibodies might play a role in immunity to schistosomiasis

remains poorly understood. However, it is clear that serum antibodies to defined schistosome glycans may constitute valuable parameters in detecting schistosome exposure or infection, and monitoring treatment and schistosomiasis elimination programs. Through a detailed glycomics analysis of *Schistosoma mansoni* cercariae, schistosomula, adult male and female worms and eggs, we have isolated and identified hundreds of glycans from the parasite. These native parasite glycans in combination with a number of synthetic glycans related to schistosomes and other helminths were used to construct a glycan microarray covering a large portion of the potential glycan antigen repertoire of schistosomes. The microarray was used to analyse longitudinally sera from experimentally infected animals, as well as cross-sectional human cohorts from *S. mansoni* and *S. haematobium* endemic areas, with respect to IgM, IgG and IgE to each glycan motif, and in relation to infection duration, intensity and treatment. Overall, the most intense IgM and IgG responses are against a range of highly fucosylated glycans associated with cercarial and egg glycoproteins and glycolipids, but IgE responses were restricted to glycoprotein N-glycan core modifications only. We observed age-dependent differences in anti-glycan responses, especially when considering changes induced by treatment with PZQ. In general anti-glycan IgG is more sustained than IgM after treatment. Currently we are investigating the development of glycan-specific Ab responses in a controlled human *S. mansoni* infection model that has been developed at the LUMC. Detailed data on the diagnostic potential of specific anti-glycan Abs will be presented.

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ANNUAL VERSUS BIANNUAL MASS AZITHROMYCIN DISTRIBUTION AND SEROLOGIC MARKERS OF TRACHOMA AMONG CHILDREN IN NIGER: A COMMUNITY RANDOMIZED TRIAL

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Annual mass drug administration (MDA) with azithromycin to affected districts to treat the causative agent, *Chlamydia trachomatis (Ct)*, is a core component of the World Health Organization's trachoma elimination strategy. In some areas, repeated rounds of annual MDA have not reached the elimination target of <5% prevalence of the clinical sign trachomatous inflammation-follicular (TF). We conducted a cluster randomized trial comparing annual azithromycin MDA to the entire community to biannual MDA to children ≤12 years of age ("targeted treatment") in a mesoendemic region of Niger (N=24 communities). After 36 months (3 treatment rounds versus 6 targeted treatment rounds), we collected dried blood spots from a random sample of 50 children aged 1 to 5 years per community. A multiplex bead assay on a Luminex 200 platform was used to measure antibody (Ab) responses against the *Ct*-derived antigens Pgp3 and CT694. We compared log-transformed quantitative Ab levels to Pgp3 and CT694 in children in communities randomized to annual versus biannual azithromycin distribution using generalized linear models with standard errors clustered by community. Of 1,005 children with serologic data, median age was 3 years and 51.4% were female. At 36 months, TF prevalence was 7.7% and ocular *Ct* prevalence was 5.2%. The prevalence of Ab positivity to Pgp3 was 27.3% and to CT694 was 23.6%. Seropositivity to Pgp3 and/or CT694 was significantly associated with TF at the individual (prevalence ratio [PR] 1.90, P<0.001) and community level (correlation coefficient 0.67, P=0.0003) and with ocular *Ct* infection at the community level (correlation coefficient 0.62, P=0.001). The probability of seropositivity increased with increasing age (PR 1.25 per one-year increase in age, P<0.001). Children in communities receiving targeted treatment had non-significantly reduced relative risk of Pgp3 (RR=0.71) and CT694 seropositivity (RR=0.81) compared to annual treatment. There was no difference in the shape of the age-seroprevalence curve between the two arms. While Ab responses were correlated with signs of trachoma, biannual MDA had no observable effect on TF prevalence.

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PROGRESS TOWARDS ACHIEVING TRACHOMA ELIMINATION: LONGITUDINAL TRENDS OVER SIX YEARS UNDER THE SAFE STRATEGY IN AL RAHAD LOCALITY, SUDAN

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In 2009, the Sudan Federal Ministry of Health conducted a trachoma baseline survey in Al Rahad locality (district), Gedarif state, to determine if trachoma programmatic interventions were needed. The baseline prevalence of trachomatous inflammation-follicular (TF), a key programmatic indicator, was 7.1% (95% Confidence Interval (CI): 5.4-8.4%) in children ages 1 - 9 years and the prevalence of trachomatous trichiasis (TT) in those ≥15 years was 4.8% (CI: 3.3-6.8). Health education and TT surgical camps were conducted in 2012. In 2013, as part of operational research on survey methodology, a survey was conducted in 3 administrative units (sub-districts) of Al Rahad. Aggregating the data to a locality level produced a TF prevalence of 29.4% among children ages 1 - 9 years and a TT prevalence of 1.8% in those ≥15 years, demonstrating Al Rahad as the most endemic locality in Sudan. 3 rounds of mass drug administration (MDA) with azithromycin were conducted from 2015 to 2017 and a coverage survey was implemented after the last round of MDA from which the overall self-reported treatment coverage was estimated to be 76.0% (CI: 68.8-82.0%). During this period, TT surgical camps, health education and the trachoma school curriculum were also implemented in the locality. In December 2017, a multi-stage cluster random impact survey was conducted in Al Rahad, sampling 25 clusters of 25 households. The TF prevalence was 6.3% (CI: 4.0-10.0%) in children 1 - 9 years and therefore 1 additional round of MDA was required. The prevalence of TT among those ≥15 years was 0.59% (CI: 0.24-1.47) demonstrating that further surgical campaigns were required. Among children 1 - 9 years, 72.3% (CI: 60.2-81.8%) were found to be free from ocular and nasal discharge and 59.8% (CI: 44.8-73.1) of households surveyed were found to own a latrine. Conjunctival swabs collected from children ages 1 - 9 years are still being processed to estimate the prevalence of *Chlamydia trachomatis* infection. It is anticipated that the upcoming single round of MDA and surgical camps to be conducted in 2018 will enable Al Rahad to achieve the trachoma elimination target and move into surveillance mode in 2019.

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UNDERSTANDING AND QUANTIFYING THE COMPLEX RELATIONSHIP BETWEEN DIFFERENT DIAGNOSTIC INDICATORS FOR TRACHOMA SURVEILLANCE

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By 2020 the World Health Organization aims to eliminate trachoma as a public health problem in all endemic countries. The first of two steps towards achieving elimination is to reduce TF prevalence in all endemic districts to <5%. Serology has been suggested as an additional diagnostic to help confirm elimination. However, to understand observations from serological data it must be understood how estimates of transmission

intensity inferred from serological data relate to those from standard surveillance by TF or those from. In this study we used serological and epidemiological data from 6000 participants (aged 1-90 years) across 6 different regions (Nepal, Solomon Islands, Kiribati, Gambia, Ethiopia and Malawi) to understand the relationship between different diagnostic test results. We fitted sero-catalytic and antibody-acquisition models to serological data from each study-site. We quantified the relationship between: PCR and estimated sero-conversion rates and TF and sero-conversion rates (SCR) for each study-site, using a regression framework. We estimated the sample sizes required to determine the true prevalence using all 3 diagnostics. Through our estimated relationship between the 3 diagnostic indicators we suggest that for TF <5% we would expect 0.5% (Confidence interval (CI) 95%: 0-2%) of individuals in the community to test PCR positive and 7% (CI 95%: 4-9.3) of individuals to test sero-positive, with an SCR of 0.014 (Credible Interval (CrI): 0.007-0.021) year^{-1} corresponding to TF <5% in 1-9 year olds. We demonstrated that at least, if not a smaller sample size would be required to estimate the SCR (400 samples)in comparison to that needed for TF <5% (700 samples), but at least 3000 samples would be required to determine the true PCR prevalence. We provide the first estimate of an operational threshold for elimination with PCR. We quantified the relationship between all 3 diagnostics currently being considered for elimination surveillance to understand how findings from different diagnostics relate to one another, a vital step in understanding how different surveillance diagnostics can be used to complement one another.

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FIELD TESTING AND OPTIMIZATION OF A RAPID TEST FOR ANTIBODIES TO THE CHLAMYDIA TRACHOMATIS ANTIGEN PGP3

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Trachoma, caused by repeated ocular infection with *Chlamydia trachomatis* (Ct), is targeted for elimination by 2020. We have been investigating antibody responses against the Ct antigen Pgp3 as a surveillance tool using the multiplex bead assay (MBA) and a recently developed lateral flow assay (LFA). We evaluated the LFA in two villages in Kongwa district, Tanzania. Blood was collected into microtubes for field testing and onto filter paper as dried blood spots (DBS) for lab testing. All LFAs tested in the field were manufactured at CDC encased in cassettes ("standard" method); field testing was done using blood (N=506) and plasma (N=505) from the same individuals. DBS were tested at CDC by standard LFA (N=458) as well as a recently modified "dipstick" LFA (N=506). DBS were also tested by MBA (N=506). LFAs were read as positive, negative or invalid by an expert rater (rater 1) and at least one of three other raters. Interrater agreement as measured by Cohen's Kappa (κ). In field-based testing, the percent positive for blood was 42.5% (95% CI 38.2%-46.8%) and for plasma was 48.4 % (95% CI 44.0%-52.9%) by standard LFA. In lab-based testing of DBS, the percent positive for standard LFA was 44.1% (95% CI 39.4-48.8), for dipstick LFA was 44.3% (95% CI 39.9-48.8), and for MBA was 45.5% (95% CI 41.1-49.9). The percent agreement between the MBA and each type of LFA ranged from 92.3%-94.7%. In the field, agreement between raters 1 and 2 was κ=0.916 (95% CI 0.873-0.959) for plasma and κ=0.921 (95% CI 0.886-0.956) for blood and between raters 1 and 3 was κ=0.884 (95% CI 0.831-0.937) for plasma and κ=0.803 (0.740-0.865) for blood. In the lab, the agreement between rater 1 and 4 was κ=0.885 (0.836-0.934) for standard LFA and κ=0.952 (0.925-0.979) for dipstick LFA. The lower kappa (<0.9) seen with two raters suggest an automated reader may benefit the assay. Interrater agreement was best with dipstick LFA on DBS, suggesting this iteration is easiest to read. The Pgp3 LFA is a robust assay, with all iterations providing comparable community-level seropositivity data, and good performance in a dipstick format optimized for ease of manufacture and use.

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PLANNING FOR TRANSITIONING SERVICES WHEN ELIMINATION THRESHOLDS ARE MET: LESSONS LEARNED FROM TRACHOMA TRANSITION PLANNING

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The work to eliminate trachoma as a public health problem has involved a large scale up of dedicated outreach services, actively searching for patients and taking specific trachomatous trichiasis (TT) management to the patients, along with various rounds of mass drug administration (MDA) with azithromycin and dedicated interventions to support behavioural change related to facial cleanliness and environmental improvement (F+E). By the time elimination thresholds for active trachoma (TF) and TT are met specific services should be integrated back into ongoing eye care, general health and WASH systems. Transition planning also serves as the link between dedicated interventions and preparation of the national verification of elimination and dossier preparation. Guidelines for planning for the effective transition of dedicated services (for TT, MDA, and F&E) back into existing government systems have been developed and deployed in a number of countries. Two large scale trachoma elimination programmes in 9 countries, have been gathering experiences on the successes (and factors contributing) and challenges of transition planning. Experience indicates that for successful post-elimination transition planning there is a need for engagement of the relevant stakeholders from the start of the programme, on-going communication at the national and district level, improved human resource management, a strong referral system, effective supply chain management procedures, and knowledge and awareness about the disease, intervention, and elimination at all levels. Challenges to transition planning have included resistance by stakeholders to the 'cessation' of dedicated services, lack of budget allocation by the ministries, lack of adequate information regarding elimination by district health authorities, and poor integration of F+E work into ongoing WASH programmes. Based upon the experiences in trachoma as well as the other NTD elimination programmes transition planning should enable countries to ensure that through the programmes themselves, the health system is strengthened and elimination is maintained.

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THE POPULATION-BASED PREVALENCE OF TRACHOMATOUS SCARRING IN A TRACHOMA HYPERENDEMIC SETTING: RESULTS FROM 152 IMPACT SURVEYS IN AMHARA, ETHIOPIA

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Trachomatous scarring (TS) is thought to result from repeated infection with *Chlamydia trachomatis* throughout childhood, it commonly develops in late childhood in endemic settings, and it progresses throughout the lifetime. Pronounced scarring is an underlying cause of trichiasis, which can lead to blindness. Since the condition is irreversible, scarring in adults has been considered a marker of past exposure to Chlamydial infection within a population. Because early surveys demonstrated that Amhara, Ethiopia was endemic for trachoma, interventions including mass drug administration (MDA) with azithromycin were scaled up to the estimated 22 million people in all districts within the 10 administrative zones. District-level multi-stage cluster surveys were conducted in all districts between 2010-2015 after an average of 5 rounds of MDA to monitor the impact

of trachoma interventions. Before each survey round, trachoma graders participated in a 7 to 10-day training and a field reliability exam which included cases of TS. From all 152 districts in Amhara, 208,265 individuals ages ≥1 years were examined for the signs of trachoma including TS. Region-wide, the population-based prevalence of TS was 8.2%, (95% Confidence Interval [CI]: 7.8, 8.7%). Among children ages 1-9 years (n=73,406) the prevalence of TS was 1.1% (95%CI: 1.0, 1.2%), while among individuals ≥15 years (n=110,137) the prevalence was 12.6% (95%CI: 11.9, 13.3%). The zonal prevalence of TS among those ≥15 years ranged from 7.0% in South Wollo zone to 23.2% in South Gondar zone, and the district-level prevalence ranged from 0.9% to 36.9%. The prevalence of TS increased with age, reaching 22.4% among those 56-60 years and 24.1% among those ages 61-65 years, while the highest prevalence observed was among women ages 61-65 years, 26.1%. These results suggest that Amhara has had a long history of trachoma exposure, and that a large population remains at risk for developing trichiasis. It is promising, however, that children ages 1-9 years, many born after the MDA program began, have low levels of TS.

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DOES INFECTION AND ANTIBODY DATA ADD EVIDENCE TO THE UNDERSTANDING OF TRACHOMA PREVALENCE IN LOW ENDEMIC AREAS?

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Trachoma is a public health problem in Mpwapwa district, Tanzania. A recent pre-validation survey done three years after stopping mass drug administration (MDA) with azithromycin suggested there was resurgence of active disease. In settings where prevalence of trachomatous inflammation-follicular (TF) is <5% and chlamydial infection is <2% evidence suggests that resurgence of active disease is unlikely to occur. Recent studies suggest that tests for chlamydial antigen can inform interruption of transmission of *Chlamydial trachomatis*. Mathematical models suggest that TF prevalence of <5% approximates to 7% chlamydial antibody sero-positivity. We aimed to investigate novel strategies for evaluating the resurgence of trachoma by testing markers for *C. trachomatis* infection and pgp3 antibodies. The study participants were children aged 1-9 years who were sampled during a routine trachoma survey. Children were examined for trachoma signs using the World Health Organization simplified grading system. Ocular swab samples were tested for chlamydial infection using Cepheid GeneXpert PCR platform. Antibodies against immunodominant antigen (*pgp3*) were detected using lateral flow-based assay (LFA). A total of 1,000 children were included in the study. The mean (standard deviation) age was 4.5(2.4) and the majority (52.7%) were male. The prevalence of TF was 10.2% (95% confidence interval [CI] 9.6-12.2). Based of TF prevalence, restarting MDA is required. The results of *C. trachomatis* infection and pgp3 are being processed and data will be presented at the conference. Analysis will include correlation of TF, infection and *pgp3*. The findings will provide evidence to support the identification of the resurgence of trachoma and monitor MDA. Further longitudinal monitoring of TF, *C. trachomatis* infection, and pgp3 will be needed to assess effect of MDA and track progress to elimination.

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GLOBAL BURDEN OF MALARIA AND DENGUE CO-INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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The epidemiology and the geography of malaria-dengue co-infection are poorly understood. We conducted a systematic review and meta-analysis to determine the geographic limits of malaria and dengue co-infection and to estimate the prevalence of the co-infection. Electronic databases regardless of language were searched from inception until May 2017 for studies that reported the concurrent detection of *Plasmodium* and dengue. Each study site location was geo-coded, and Google fusion were used to generate individual maps. For the meta-analysis, we excluded case report/case series and pooled the study-specific estimates of prevalence rate using a random-effects model to obtain an overall summary estimate of the crude prevalence across studies. Data were analyzed using the statistical software Comprehensive Meta-Analysis. We identified 1718 unique papers and included 73. Malaria-dengue co-infection has been found in South and South-East Asia, Africa, and Americas regions, with 53 (72%), 10 (13.6%), and 10 (13.6%) articles, respectively. India is the country with most articles 33 (45%). Thirty-four studies included adult patients (≥18 years), while 27 studies included patients of all ages. Forty papers were eligible for meta-analysis corresponding to 19645 individuals. The proportion of malaria/dengue co-infection varies from 0.31-82%. The crude co-infection prevalence in the pooled analysis was 0.5% (95% CI 0.4-0.85) (I^2=95.3%, *p*-heterogeneity<0.0001). The most frequently reported *Plasmodium spp*. were *P. vivax* (*n*=331), followed by *P. falciparum* (*n*=141), mixed infection (*n*=12), *P. knowlesi* (*n*=4), and *P. ovale* (*n*=1). Fatal outcome was rare (*n*=4). Of the 73 studies, nine (12.3%) compared clinical and laboratory features between co-infected and either mono-infection. Evidence of the negative impact of malaria/dengue comorbidity was lacking. In conclusion, although malaria and dengue vectors are widely distributed in nature, the prevalence of co-infection is rare, heterogeneous distributed, mainly confined to South and South-East Asia; and with a predominance of *P. vivax*.

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ECONOMIC IMPACT OF DENGUE ON PATIENTS HOSPITALIZED IN SOUTHERN SRI LANKA - AN ANALYSIS OF DIRECT AND INDIRECT COSTS

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Dengue is an important cause of hospitalization in the tropics. The economic impact of dengue on patients has not been clearly defined in Sri Lanka. We determined costs associated with illness for patients hospitalized with dengue in southern Sri Lanka. A prospective cohort study was conducted at a tertiary and a secondary-care public hospital in Southern Province. Consecutive patients hospitalized with risk for severe dengue (platelet <100x10^9/L) were enrolled from Jun-Oct 2017. Data regarding direct patient expenditures (travel, medical visits, medications, and investigations) and indirect costs (loss of productivity) were gathered using interviews and a telephone call 2-4 weeks after discharge. Among 323 patients with dengue, 200 (61.9%) were male and median age was

33 years (IQR 25-46). Median duration of hospitalization was 4 days. Overall, 249 (77.1%) patients reported 1 to 4 prior outpatient visits for same illness: 70.3%- general practitioner, 4.0%- Outpatient Department, and 4.6%- consultant physician. Among 300 adults, 217 (72.3%) missed work (median 3 days, IQR 2-5) and among 23 children, 9 (39.1%) missed school (median 4 days, IQR 2-4) before admission. Of 77.1% who received a post-discharge call, 14 (16.5%) reported another outpatient visit and 8 (3.2%) reported another admission. Most adults (184, 80.0%) reported missing work following discharge (median 14 days, IQR 7-21) and 8 (42.1%) children reported missing school following discharge (median 14 days, IQR 5-17.5). Patients reported a total median expenditure of 2050 Sri Lankan Rupees (IQR 1000-3500) for the illness: 1350LKR (IQR 450- 2050) for medical care before admission, 0 (IQR 0-0) LKR during admission, and 800 (500- 1500) after discharge. Most expenditures were for medical visits. Given a national daily average income of 1147 LKR/ person and median values for days of work lost, 24,087 LKR in wages were lost by an adult for the illness. Dengue resulted in a significant economic burden to hospitalized patients. These data highlight the need for preventative public health actions for dengue and may help inform decisions such as vaccination or vector control activities.

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GAPS IN SERVICE UTILIZATION AND SERVICE PROVISION: AN ANALYSIS OF DHS AND SPA MALARIA DATA FROM MALAWI, SENEGAL, AND TANZANIA

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Ensuring universal access to malaria diagnosis and treatment is a key component to Pillar 1 of the WHO Global Technical Strategy for Malaria 2016-2030. To achieve this goal, it is essential to know the types of facilities where the population seeks care as well as the malaria service readiness of these facilities in endemic countries. To investigate the gaps between the seeking and provision of malaria services, we examined malaria service utilization data in children under five with fever from the household-based Demographic and Health Survey (DHS) and provision of care data from the facility-based Service Provision Assessment (SPA) from Malawi, Senegal, and Tanzania. Facility types were harmonized (considering managing authority and facility level) between DHS and SPA surveys to examine the types of facilities where people seek care and the malaria service readiness of the facilities. Facilities categorized as "malaria service ready" included those with 1) malaria diagnostic capacity through either rapid diagnostic tests or laboratory microscopy testing, 2) malaria treatment guidelines available, 3) first-line medicines available on the day of the survey, and 4) personnel recently trained in malaria diagnosis and/ or treatment. In Malawi and Tanzania, the largest proportion of children under five with fever were taken to a government health centers (39% and 25%, respectively). However, among government health center offering child curative services and/or malaria diagnosis/ treatment, 32% of those in Malawi and 20% of those in Tanzania were classified as malaria service ready. Similarly, in Senegal, 36% of children under five with fever were taken to a government health post, mobile clinic, or health hut, however, only 51% of these facilities were classified as malaria service ready. Across all three countries, the facilities with the highest level of malaria service readiness included government hospitals, although less than 10% of children in each country received care at those facilities. More focus should be given to improving the malaria service readiness in secondary health care facilities where the population is seeking care.

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SUBNATIONAL PROFILING USING CHI-SQUARE AUTOMATIC INTERACTION DETECTOR (CHAID) ANALYSIS TO INFORM TARGETING OF MALARIA INTERVENTIONS: A CASE STUDY FROM NIGERIA

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The epidemiology of malaria in sub-Saharan Africa has changed substantially over the past two decades, owing to large investments in malaria prevention and case management. Many countries have seen significant declines in malaria transmission, with several shifting to more moderate and heterogeneous transmission environments. Malaria prevention and treatment interventions have expanded, though progress has been uneven, with large variation in coverage in many countries. Given these complex and evolving settings, countries require finer-scale data to target interventions. We present results from a subnational profiling study conducted in Nigeria using 2015 Nigeria Malaria Indicator Survey data. We ran Chi-square automatic interaction detector (CHAID) models to identify the most significant predictors of intervention coverage and specific subgroups that had lower coverage of interventions. We ran models for household ownership of at least one insecticide-treated net (ITN); ITN use in children under five, pregnant women, and the general population; intermittent preventive treatment in pregnancy (IPTp) uptake; and use of artemisinin-based combination therapy (ACT) for treatment of children with fever. The CHAID models identified "region of the country" as the most significant predictor of ITN ownership (range: 55%-89%, $p<0.001$) and use across the different populations (range: general population: 21%-54%, children under five: 25%-62%, pregnant women: 28%-69%; $p<0.001$ for all models), and place of residence as the best predictor of IPTp uptake (range: 17%-27%, $p<0.001$) and use of ACT treatment (range: 13%-21%, $p<0.001$). The CHAID gain index results for each model identified key subgroups for targeted interventions. For example, the household ITN ownership model identified small households (1-4 members) in the South West, North Central, and South Central regions of the country as subgroups to target (total 40% gain percent). The use of CHAID analysis is new to the global health field and can provide valuable information for national malaria control programs on how to target interventions more efficiently to maximize impact.

1396

MAKING THE CASE FOR INVESTMENT IN SOCIAL AND BEHAVIOR CHANGE INTERVENTIONS FOR MALARIA: RESULTS FROM A SECONDARY ANALYSIS IN NIGERIA

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Social and behavior change (SBC) interventions have been an integral component of malaria intervention packages in Nigeria, but evidence of their effectiveness in improving uptake of malaria interventions is limited. In this study, we used 2015 Malaria Indicator Survey data to examine the relationship between maternal recall of malaria messages in the past six months and care-seeking for children under five with fever, insecticide-treated net (ITN) use in children under five, and ITN use and uptake of intermittent preventive treatment (IPTp) in pregnant women. Multiple logistic regression was conducted to assess the relationship between maternal recall of at least one specific topic-related message (e.g., "seek

treatment for fever," "sleeping inside a mosquito net is important") and the care-seeking and ITN use outcomes. A case-control study approach explored the relationship between recall of at least one malaria SBC message and IPTp uptake, with cases defined as "women who received ≥2 doses of IPTp" and controls defined as "women who had a live birth in the past two years but did not receive ≥2 doses of IPTp." Results indicate positive associations between maternal SBC recall and all measured outcomes. The odds of ITN use among children whose mother recalled an ITN message were 2.1 times greater than among children whose mother did not recall a message (OR: 2.12, 95% CI: 1.65–2.72); and the odds of ITN use among pregnant women who recalled an ITN message were 2.4 times greater than pregnant women who did not recall a message (OR: 2.42, 95% CI: 1.46–4.00). A borderline significant association was observed for care-seeking. The odds of care-seeking for children with fever were 1.7 times greater for children whose mother recalled a care-seeking message than for children whose mother did not recall a message (OR: 1.69, 95% CI: 0.94–3.04). The odds of recall of a malaria message among women who received IPTp were 1.7 times greater than among women who did not receive IPTp (OR: 1.70, 95% CI: 1.43–2.03). The results suggest an important role for SBC in Nigeria. Given low maternal recall of SBC messages (36% in 2015), efforts should be made to improve the reach of SBC.

1397

LARVAL SOURCE REDUCTION WITH A PURPOSE: DESIGNING AND EVALUATING A SCHOOL AND COMMUNITY INTERVENTION IN COASTAL KENYA

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Since *Aedes aegypti* mosquitoes preferentially breed in domestic containers, control efforts focus on larval source reduction. Our objectives were to design and test the effectiveness of a school and community source reduction intervention to improve knowledge and behaviors and reduce the abundance of immature mosquitoes and household breeding sites in coastal Kenya. During a 10-month formative phase, we interviewed 80 adults and children to understand current knowledge and practices and surveyed 200 households to assess mosquito breeding. We enrolled 50 children (ages 10-16) and their parents from 5 control and 5 intervention schools. Baseline and 3-month follow-up assessments measured knowledge, behaviors, and household mosquito breeding before and after the intervention. Our formative phase indicated that source reduction awareness and behaviors were minimal, female heads of household primarily manage water containers, and more than 80% of mosquito breeding occurred in containers with no purpose. Using this information, we designed the resulting intervention to involve female heads of households during 1-hour home sessions, children during 5-hour interactive after-school lessons using games and poetry, and everyone during school recycling events to collect and reuse "no purpose" containers. By the 3-month follow-up, the intervention arm had significantly more knowledge and self-reported behavior than the control arm ($p<0.05$): 69% vs. 30% of intervention vs. control adults and 83% vs. 17% of children knew stages of the mosquito life cycle; 68% vs. 29% of adults and 41% vs. 20% of children knew how to reduce mosquito breeding; and 56% vs. 19% of adults practiced source reduction. During recycling events, participants collected 17,200 containers (1 ton of plastics) and planted 4,000 native trees. At follow-up, intervention households had fewer no purpose containers and immature mosquitoes than control households. We will assess the sustainability of outcomes after one year. Our study demonstrates that source reduction interventions can be effective if designed with an understanding of the social and entomological context.

1398

PARTNERING WITH MIDWIVES AND HEALTH CENTER PERSONNEL TO DETECT AND TREAT CONGENITAL CHAGAS DISEASE IN RURAL GUATEMALA

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Chagas disease is a significant challenge for public health in Latin America. The incidence of new congenital infections is estimated to be 5-6.6%. In 2015, a prevalence survey of Chagas disease in Comapa, a region in Guatemala with previously high levels of triatomine infestation, revealed that 1.4% ±1% (95% CI) of children 7-14 years old and 10%±3% (95% CI) of women of childbearing age (15-44 years) are seropositive to *Trypanosoma cruzi*. We implemented a community-based program for prenatal and neonatal *T. cruzi* screening for early congenital Chagas treatment. Between 2014 and 2015, a participatory strategy was developed with midwives from high risk communities, health center personnel and other stakeholders. In 2015, 135 pregnant women attending prenatal care at the health center in Comapa were tested with a Chagas rapid antibody test, compared with 454 in 2017. Positives are confirmed by ELISA. Neonates (most within one month of birth) are now brought to the health center and screened for parasitemia through microscopic observation of heel prick blood in capillary tubes. A real-time PCR (qPCR) that targets a variable region of the minicircle is performed on blood samples preserved in nucleic acid preservation cards. In 2017, 17 neonates born to seropositive mothers were negative by microscopy. By qPCR, 3 of 9 (33%) samples are positive for *T. cruzi* DNA, with unique melting curves. Infants born to seropositive mothers will be followed at 9 months for antibody to provide treatment. Current guidelines indicate treatment only after proven parasitemia or antibody detection at nine months. Enhancing a partnership, based on the synergy of an existing social structure and local resources and processes, facilitates access to congenital Chagas detection in rural areas with low access to health services. Early detection of congenital infection and expeditious treatment of infected newborns are essential for Chagas elimination. Future efforts should promote access to treatment for seropositive women to reduce risk of congenital transmission and use of pediatric formulations to facilitate treatment of congenital disease.

1399

A NOVEL, FLUORESCENCE-BASED ASSAY TO ASSESS THE REPLICATIVE ABILITY OF *TRYPANOSOMA CRUZI* PARASITES BY DETECTION OF THYMIDINE INCORPORATION INTO PARASITE DNA

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is considered one of the world's 20 most neglected tropical diseases by the World Health Organisation (WHO). *T. cruzi* is endemic to 21 countries within the Americas, infects approximately 28 000 people and causes some 12 000 deaths, per year. The drugs currently used to treat *T. cruzi* infection have questionable efficacy and associated toxicity. Inevitably, the result is ineffective or incomplete treatment, therefore new compounds are needed for the drug discovery pipeline. Attrition of compounds is an ongoing issue for Chagas discovery and improvement of *in vitro* assays to further understand the efficacy and mode of action of new compounds would support new discoveries. We have developed a high-throughput, high-content assay to assess compound activity against intracellular *T. cruzi* amastigotes utilising the fluorescent markers Hoechst and HCS

CellMask Green. To understand the replicative capability of *T. cruzi*, we have employed a fluorescent flou-488 marker to identify incorporation of a collection of 6 thymidine analogues into *T. cruzi* DNA, visualised using click chemistry. Analogues were co-incubated with intracellular *T. cruzi* parasites over time to identify 5-ethynyl-2′-deoxyuridine (EdU) as the most effectively incorporated into parasite DNA. Identification of DNA synthesis by EdU has previously been used to label replicating mammalian cells. EdU was employed to develop a novel image-based assay to assess the activity of compounds against parasite replication. The effects of the drugs used to treat Chagas disease on *T. cruzi* replication were investigated, in addition to posaconazole, which causes a sub-efficacious effect against the parasite *in vitro* and has failed clinical trials to treat Chagas disease. Active compounds against *T. cruzi* from the Medicines for Malaria Venture (MMV) Pathogen Box were also assessed for their effect upon parasite replication. This methodology provides new insights into the MOA and static/cidal nature of the action of these compounds and drugs and is a promising tool to aid the prioritisation of compounds in drug discovery.

1400

MOLECULAR CHARACTERIZATION OF *LEISHMANIA* DNA FROM ARCHIVED GIEMSA-STAINED SLIDES OF PATIENTS FROM SALTA, ARGENTINA

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Cutaneous leishmaniasis is endemic in the northwestern province of Salta in Argentina. *Leishmania* DNA from Giemsa-stained slides of up to 12 years in storage of patients from a University based reference center in Oran, Salta was characterized through PCR-Restriction Fragment Length Polymorphism (RFLP). One hundred smears from individuals with suspicious lesions and epidemiologic exposure plus microbiologic confirmation through positive microscopy were analyzed. The samples had been originally taken 2, 3, 5, 6 and 12 years prior to performing PCR-RFLP (20 samples from each year) and were maintained at room temperature in a tropical environment. All the cases were classified in a semiquantitative scale for amastigote density, and Leishmanin skin test (LST) results were included. DNA extraction was done applying lysis buffer with proteinase K, and then DNA was amplified with ribosomal internal transcribed spacer 1(ITS1) primers. PCR products were digested with HaeIII enzyme. All PCR positive smears (74/100) belonged to *Viannia* subgenus. A statistically significant directly proportional relationship between semiquantitative microscopy and PCR results was detected ($p < 0.001$). All patients had LST positive results (induration ≥ 5 mm), and the smears of those with positive but smaller induration (LST < 19 mm) had a higher proportion of positive PCR results. This study determined that smear age did not affect PCR positivity, which allows retrospective analyzes and suggests smears might be useful for molecular complementary diagnosis. Since *Leishmania* (*Viannia*) *braziliensis* is the main circulating species in the study area, determining *Viannia* subgenus in all analyzed samples confirms previous findings. PCR positivity showed statistically significant differences according to semiquantitative microscopy, highlighting the importance of parasite burden in the diagnostic sensitivity of the method. Considering that smears of patients with smaller LST induration were more positive in PCR, a negative smear from patients with positive LST response, but < 19 mm, could actually represent a false negative smear result.

1401

PREVALENCE AND ASSOCIATION OF *LEISHMANIA* RNA VIRUS-1 (LRV-1) IN SEVERE AND NON-SEVERE PHENOTYPES OF AMERICAN TEGUMENTARY LEISHMANIASIS FROM PERU

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American tegumentary leishmaniasis (ATL) comprises a discrete set of clinical presentations of leishmaniasis endemic to Latin America. *Leishmania* RNA virus-1 (LRV-1) is a double stranded RNA virus identified in 20-25% of the *Leishmania Viannia* complex, and is believed to be a predictive biomarker of severe ATL. Our objective was to determine the baseline prevalence of LRV-1 and associations with clinical phenotypes in ATL patients from Peru. Banked clinical isolates of patients residing in or traveling to Peru between 2006 and 2016 were species identified by PCR, RFLP analysis, and Sanger sequencing, and screened for LRV-1 by real-time PCR. Patient isolates were stratified according to clinical phenotype: localized cutaneous leishmaniasis (LCL) was defined as "non-severe" ATL, whereas "severe ATL" was defined as mucosal or mucocutaneous leishmaniasis (ML/MCL); erythematous, purulent, or painful ulcers and/or lymphatic involvement (inflammatory ulcers); or multifocal/disseminated ulcers (greater than 4 in greater than 2 anatomic sites). Of 132 patients enrolled, 64 (48%) and 68 (52%) of ATL cases had the severe and non-severe phenotypes, respectively. Twenty-seven (42%) of 64 severe ATL cases and 29 (43%) of 68 non-severe ATL cases were positive for LRV-1, respectively ($p=1.00$). The severe phenotype was over-represented among older patients (median age 41.5 years vs. 29 years in non-severe ATL, $p=0.0002$), while the median age did not differ by LRV-1 status ($p=0.27$). A trend in disease severity was observed by sex, whereby 62 (59%) males had a severe phenotype compared to 10 (37%) females ($p=0.05$). No difference in LRV-1 status was observed by sex ($p=0.50$). Twenty-three (41%) of 56 quantified LRV-1 positive patients revealed greater LRV-1 copy numbers in those with ML/MCL compared to those with all forms of CL ($p=0.04$). Our findings suggest age as a contributing factor to disease severity. Although an association between LRV-1 status was clinical phenotype was not demonstrated, LRV-1 copy number was higher in patients with ML/MCL. Therefore, the role of LRV-1 viral burden in severe disease requires further exploration.

1402

TRANSCRIPTOME ANALYSIS OF SPLENIC ASPIRATES IN HUMAN VISCERAL LEISHMANIASIS REVEALS IMPAIRED TISSUE ORGANIZATION/REPAIR AND INHIBITED PARASITE KILLING

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Changes in the spleen environment that contribute to the pathogenesis of human visceral leishmaniais (VL) are under explored. We performed transcriptional profiling (RNAseq) on the remnants of diagnostic splenic aspirates from 8 Kenyan patients with visceral leishmaniasis. Differentially expressed genes were identified by comparison to splenic RNA from normal controls obtained through commercial sources. A total of 2,749 differentially expressed genes were identified with a cutoff of as 2-fold change and false discovery rate (FDR) ≤0.05. Functional analysis was done with the Ingenuity Pathways Analysis (IPA) software. We found that half of the top 10 down-regulated canonical pathways were related to cell assembly and organization with decreased expression of actin, integrins

and cadherins. Decreased expression of extracellular matrix proteins (ECM), vitronectin, fibronectin, collagen and endothelins indicated defects in ECM and decreased tensile strength of the tissue. IL8 signaling was a highly down-regulated pathway, which together with the ECM changes, would lead to impaired neutrophil recruitment, transmigration, and activation. Pathways involved in Leishmania killing, including production of nitric oxide and NFkB signaling were also represented in 2 of the top 10 canonical pathways that were inhibited. Transcript expression was compatible (Z<-2.0) with impaired tissue repair and healing (decreased angiogenesis, lymphangiogenesis, endothelial cell development and connective tissue differentiation). In contrast, functions predicted to be activated (Z>2.0) indicated tissue damage (mediated by upstream gene regulators IFNγ, TNFα and CxCL10), bleeding (by coagulation system defects), dysgenesis, and edema. Collectively these data indicate that impaired splenic tissue organization and repair is likely to be part of the pathogenesis of human VL.

1403

CHAGAS DISEASE-INDUCED FUNCTIONAL PERTURBATIONS OF THE GASTROINTESTINAL MICROBIOME

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Chagas disease is a tropical disease endemic to Latin America, caused by infection with *Trypanosoma cruzi* parasites. Six to seven million people are *T. cruzi*-positive, thirty to forty percent of which will develop cardiac symptoms, including arrhythmias, aneurysms and fatal heart failure. A minority of patients will develop gastrointestinal manifestations (megaoesophagus and megacolon), alone or in combination with cardiac symptoms. Gastrointestinal tissues are also a major site of chronic parasite persistence, in mouse models of disease, and may serve as reservoir for cardiac re-seeding following unsuccessful treatment. However, the pathogenesis of gastrointestinal Chagas disease is still poorly understood. To address this, we performed joint microbiome and metabolome characterization of fecal samples over the course of experimental acute and chronic Chagas disease, using 16S rRNA sequencing and liquid chromatography-tandem mass spectrometry. These results showed functional changes in the fecal microbiome, beginning in the acute stage prior to peak parasite burden. Importantly, these changes persisted in the chronic stage of disease. Alterations include perturbations in conjugated linoleic acid (CLA) derivatives and in specific members of families *Ruminococcaceae* and *Lachnospiraceae*, as well as changes in secondary bile acid levels and members of order Clostridiales, all of which could have local as well as systemic effects on *T. cruzi* persistence and inflammation. In addition, we assessed microbial and metabolic changes systematically throughout the gastrointestinal tract. This enabled us to build a three-dimensional model of functional microbiome disturbances, in correlation with localized parasite burden. The greatest microbial disruptions were observed in the colon, where parasite burden was the highest, and in the esophagus, in association with changes in small molecule profile. Overall, these results are expanding our understanding of Chagasic megacolon and megaoesophagus pathogenesis, with great potential for the development of novel intervention strategies against this neglected tropical disease.

1404

MODELLING THE EFFECTS OF ATTRACTIVE TOXIC SUGAR BAITS (ATSBS) ON MALARIA PREVALENCE IN HUMANS; A TOOL FOR EPIDEMIOLOGICAL STUDY DESIGN

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Attractive toxic sugar baits (ATSBs) are a promising new tool for malaria control as they can potentially target outdoor-feeding mosquito populations, in contrast to the current vector control tools which predominantly focus on indoor-feeding mosquitoes. A Phase II efficacy trial with entomological endpoints is currently underway in Mali. To inform the design of subsequent Phase III epidemiological trials, we developed mathematical models to explore the potential impact on human endpoints of ATSBs in different transmission settings. A critical parameter determining efficacy is the sugar-feeding rate, which can vary between locations (due to availability of other food sources) and seasonally (due to variation in abundance of vegetation with rainfall). In a moderate-to-high-transmission setting such as Mali, with a 3-4 month peak transmission season and an annual average human prevalence in 2-10 year olds (PrP_{2-10}) of 50%, a sugar-feeding rate of 0.4/day (similar to that estimated from past field trials) is predicted to reduce the year-round average mosquito density in the second year of ATSB implementation by ~90% compared to the pre-intervention average. Because the increase in death rate caused by ATSBs induces a greater reduction in the population of older mosquitoes, we predict a higher reduction in EIR (>99.9%) and hence a ~92% reduction in PrP_{2-10}. Smaller but still significant effects on PrP_{2-10} are predicted with a lower sugar feeding rate (~90% for a rate of 0.2/day, ~68% for a rate of 0.1/day, ~36% for a rate of 0.05/day). Higher sugar feeding rates during the dry season and lower rates during the rainy season are predicted to modify the impact whilst movement of mosquitoes from untreated into treated areas is also expected to reduce the overall efficacy. Further outputs for a range of locations and additional human endpoints such as clinical incidence will be presented, allowing a full exploration of the potential impact of this new vector control tool across malaria-endemic settings.

1405

SIMULATION STUDIES TO QUANTIFY THE IMPACT OF COMPETING RISK EVENTS IN A HYPOTHETICAL SCENARIO OF FALLING ANTIMALARIAL DRUG EFFICACY IN SINGLE-ARMED AND COMPARATIVE DRUG TRIALS

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A new infection (NI) can potentially preclude the occurrence of a subsequent recrudescence, thus constituting a competing risk event (CRE). In the presence of CRE, the complement of the Kaplan Meier (K-M) estimate is known to result in an overestimate of failure. The overall aim of this simulation work was to quantify the bias in derived failure due to the use of the K-M method (which censors NI) compared to the Cumulative Incidence Function (CIF) approach (which considers NI as a CRE), and to assess the impact of CRE on comparative efficacy. A clinical trial (n=500 patients) was simulated with 5, 10 and 15% documented recrudescences. For each scenario, different proportions of NI were simulated to represent areas of progressively increasing transmission (<10%, 10-20%, 20-40% and >40%). Time to recrudescence and NI were simulated using biologically plausible hazard functions. For comparative studies, nine different scenarios which could be observed in a field trial when comparing two drugs (drug A and drug B) were simulated. For each of these scenarios, log-rank test for comparing the equality of K-M curves and Gray's test for comparing the equality of the CIFs were used. In single-armed trial, the overestimation of failure by the K-M method increased with increasing proportion of NI. In high transmission areas, the maximum overestimation in efficacy was 0.75% at 5% recrudescence and this rose to 3.1% and 4.3% when the drug efficacy fell to 90% and 85% respectively. In comparative trial, where drug B was associated with 2-fold increase in both recrudescence and NI (compared to drug A), the log-rank test appeared to be the more powerful test with a rejection probability of 99% compared to 90% with Gray's test. However, when drug B exerted differential effect on recrudescence and NI (i.e. reduced

recrudescence but increased NI), the Gray's test was the more powerful of the two tests. In high transmission areas, where the risk of new infection is high, the competing risk analysis approach should be used for deriving failure estimates. For comparative trials, the choice of the test to establish difference should be guided by the research question of interest.

1406

MULTIPLE FIRST LINE THERAPIES VERSUS REDUCING OVERPRESCRIPTION OF ANTIMALARIALS TO SLOW ANTIMALARIAL RESISTANCE

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The emergence of artemisinin and partner drug resistance prompts examination of which strategies can best delay or prevent the spread of resistance. It is unlikely that countries can implement all strategies simultaneously given resource constraints. Some countries are adopting a policy of multiple first-line therapies (MFT), where different patients are allocated to different ACTs, to increase the diversity of selective pressures acting on the parasite. However, there are barriers to implementing MFT, in particular the logistics of distribution as well as patient acceptance. Another measure, which many countries are also implementing, is to reduce overprescription of antimalarials, which can occur in the absence of diagnostic tests. We use model-based analysis to investigate whether reducing overprescription of antimalarials could be as effective as MFT in delaying drug resistance. We developed an individual based model of malaria transmission incorporating treatment with combination therapies, malaria parasites with different genotypes (wild type, resistant to each possible combination of antimalarials), heterogeneity in exposure to infectious bites, immunity to clinical manifestations of malaria, and fitness costs of resistance. We varied key parameters such as treatment coverage, the proportion of infections which develop symptoms, and the degree of resistance to each strain. We quantified the effectiveness of each strategy as the cumulative number of treatment failures over time. Initial results show that reducing overprescription of antimalarials is predicted to have more relative effect in delaying resistance in high transmission areas, where selection occurs during reinfection. The effect of reducing overprescription is small in low to moderate transmission areas. There is a non-linear relationship between treatment coverage with any one drug among clinical cases, and time to reach 10% resistance, such that if it were possible to use 5 or more different drugs for MFT, resistance development may be substantially delayed. Ongoing analysis will compare the benefits of the two strategies under different scenarios.

1407

PHARMACOKINETIC/PHARMACODYNAMIC MODELING TO OPTIMIZE DIHYDROARTEMISININ-PIPERAQUINE EXPOSURE AND PREVENT SELECTION FOR MARKERS OF DECREASED ANTIMALARIAL DRUG SENSITIVITY DURING INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY

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Dihydroartemisinin-piperaquine (DP) is under study for intermittent preventive treatment during pregnancy (IPTp), but its use may accelerate selection for drug resistance. We aimed first, to define the relationship between piperaquine (PQ) exposure and genetic markers associated with decreased drug susceptibility, and second, to predict optimized DP dosing regimens to minimize risk of resistance. Clinical data, PQ concentrations, and *P. falciparum* genotypes associated with decreased PQ sensitivity in Africa (*pfmdr1* 86Y, *pfcrt* 76T) were obtained from pregnant Ugandan women who received IPTp with sulfadoxine-pyrimethamine (SP) every 8 weeks (n=106), DP every 4 weeks (n=100), or DP every 8 weeks (n=94). Joint pharmacokinetic/pharmacodynamic models were developed to describe the relationship between PQ concentration and the probability of detecting genotypes of interest using nonlinear mixed effects modeling; monthly, weekly, and daily DP regimens were simulated. Increasing PQ concentration was associated with a log-linear decrease in detection of parasitemia. Higher median PQ concentrations were needed to provide 99% protection against mutant infections as compared to wild type (N86: 7.2 ng/mL; 86Y: 10.8 ng/mL; K76: 4.9 ng/mL; 76T: 11.0 ng/mL). The median percentage of time at risk (PQ levels below those offering 99% protection) with monthly DP was 4.2% (interquartile range (IQR): 0-21%) for *pfmdr1* N86, 34% (IQR: 9.3-52%) for *pfmdr1* 86Y ($p<.01$), 0% (IQR: 0-2.6%) for *pfcrt* K76, and 31% (IQR: 10-54%) for *pfcrt* 76T ($p<.01$). Daily low dose DP was predicted to reduce the median percentage of time at risk for any parasitemia to <1% and to result in the fewest mutant infections (episodes of *pfmdr1* 86Y parasitemia per 1,000 pregnancies: SP: 332, monthly DP: 190, weekly DP: 52, daily DP: 0). Our models predict that 1) higher PQ concentrations are needed to prevent infections with mutant compared to wild type parasites, 2) the overall burden of infections with mutant parasites is lower for DP than SP, and 3) daily low dose DP best reduced the estimated number of mutant infections. Consideration of dose optimization of DP for IPTp in Africa is warranted.

1408

PHARMACOKINETIC-PHARMACODYNAMIC MODELLING OF ARTESUNATE IN PATIENTS WITH DRUG RESISTANT AND SENSITIVE MALARIA (TRAC)

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The emergence of artemisinin resistance in Southeast Asia is a substantial threat to currently used antimalarial therapies and it is important to optimize current treatments to maximize therapeutic efficacy. This project aimed to investigate the pharmacokinetic and pharmacodynamic properties of artesunate, and to quantify the impact of resistance on therapeutic outcome. Pharmacokinetic and pharmacodynamic data presented here were generated from the Tracking Resistance to Artemisinin Collaboration (TRAC). Hitherto, this is the largest study ever conducted, investigating the pharmacokinetic and pharmacodynamic properties of artesunate. A total of 1,180 patients in Asia and Africa with uncomplicated *falciparum* malaria, receiving daily monotherapy of artesunate for three days, contributed data to this analysis. Densely collected plasma concentrations of artesunate and dihydroartemisinin, microscopy parasite counts and molecular markers for drug resistance were collected and analysed using nonlinear mixed-effect modelling. Artesunate and dihydroartemisinin pharmacokinetics were well-described by a parent-metabolite model, assuming full *in-vivo* conversion of artesunate into dihydroartemisinin. Parasite density measurements were modelled using a fixed 10-fold multiplication growth rate per parasite life-cycle. The elimination of parasites was dependent on the concentration of dihydroartemisinin and was described by an E_{MAX} model. Mutation of the K13-propeller, associated with reduced drug susceptibility, had a significant impact on the maximum effect, resulting in a slower killing of parasites in patients with drug resistant infections. The developed model was used to simulate different treatment alternatives for patients with artemisinin resistant infections, demonstrating that an increased treatment duration of current combination therapy is necessary in these patients. Alternatively, triple combination of antimalarial drugs are under evaluation to treat resistant infections.

1409

SCHOOL-BASED ANTIMALARIA INTERVENTIONS EFFICIENTLY REDUCE COMMUNITY-LEVEL *PLASMODIUM FALCIPARUM* PREVALENCE IN A HIGH-TRANSMISSION SETTING: A MATHEMATICAL MODELING STUDY

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Global antimalaria scale-up has significantly decreased malaria morbidity and mortality in many regions of the world. However, countries with the highest *Plasmodium falciparum* transmission have seen the slowest declines in malaria disease incidence and infection prevalence. In Malawi, uptake of antimalaria interventions is high among groups with the highest burden of disease (pregnant women and under-five children). Most *P. falciparum* infections, however, occur in school-aged children (5-15 years old) and prevention efforts have not been effective at reducing infection prevalence among this group. School-aged children are also less likely to receive anti-*P. falciparum* treatment, as many school-aged children's infections are asymptomatic. By targeting this highly-infected group, school-based interventions may be a cost-effective approach to reduce population-level transmission. We used a differential equation-based, deterministic, compartmental simulation model to evaluate how school-based interventions might affect community-level *P. falciparum* prevalence compared to current strategies (targeting pregnant women and under-five children) or community-based application of mass drug administrations (MDA) and mass screen-and-treat (MST) programs. The model, which accounted for asymptomatic infections, was fit to data from cross-sectional surveillance and longitudinal cohort studies in southern Malawi. We aimed to achieve a 50% decrease in community malaria prevalence while minimizing the number, frequency, and coverage of treatment campaigns as well as minimizing the number of individuals treated. The decrease in community infection prevalence was greatest when interventions were distributed randomly throughout the community. However, school-based interventions led to greater decreases in prevalence than MDA or MST targeting under-five children and pregnant women. While school-based interventions may not produce the greatest decrease in community infection prevalence, their comparative efficiency and potential cost-effectiveness may make them a better strategy for future malaria control programs.

1410

PROBABILISTIC MODEL OF *PLASMODIUM VIVAX* RELAPSE FOR IMPROVED ESTIMATION OF TREATMENT EFFICACY USING TIME-TO-RECURRENCE DYNAMICS

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Plasmodium vivax causes most of the malaria outside Sub-Saharan Africa. Vivax malaria in South East Asia exhibits the frequent relapse phenotype, with infections recurring at short intervals (3-4 weeks). As relapses from liver stage hypnozoites cannot be distinguished genetically from reinfections the radical curative efficacy of primaquine can only be characterized properly with placebo controlled trials (i.e. randomization to no radical cure) which is not possible in many countries where the standard of care includes radical cure. Data from a three way randomized controlled trial (n=600) comparing chloroquine (a slowly eliminated blood stage drug), chloroquine and primaquine together, and artesunate monotherapy (which is rapidly eliminated) were used to fit a Bayesian hierarchical model to the times to recurrent episodes (>1300 episodes recorded) conditioned on the treatments given. The relapse hazard rate is non-constant after acute vivax malaria. Tropical frequent relapse *P. vivax* on the Thai-Myanmar border area was well described by a triple mixture model with a relapse component (exponential waiting time), a `rapid' relapse component (normally distributed), and a `slow' relapse component (exponential waiting time). Time to recurrent episode provides discriminatory information between relapse and reinfection, thus providing a probabilistic framework for adjusting radical cure efficacy.

1411

NEUTROPHILS INFLUENCE ANTIGEN PRESENTATION DURING IMMUNE RESPONSE TO LIVE ATTENUATED LEISHMANIAL VACCINE

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No vaccine exists against Visceral Leishmaniasis. We have earlier reported the protective role of live attenuated centrin gene deleted *L. donovani* (*LdCen*$^{-/-}$) parasite vaccine in animal models. *LdCen*$^{-/-}$ induces strong innate immunity leading towards protective Th1 response. Neutrophils are indispensable for first line of defense against pathogens. Additionally, role of neutrophils as antigen presenting cells (APCs) has been demonstrated in enhancing virus based vaccine induced responses and tuberculosis vaccination. Emerging evidence suggests that neutrophils should be considered as important modulators of leishmaniasis. Although studies have shown the importance of neutrophils during *Leishmania* infection, none have shown its role in development of specific response to a Leishmania vaccine. Hence, we studied the role of neutrophils as APCs in the induction of specific response to *LdCen*$^{-/-}$ intradermal vaccination. Increased neutrophil migration with heightened microbicidal attributes was observed after infection with *LdCen*$^{-/-}$ compared to *LdWT in vitro*. *LdCen*$^{-/-}$ infection induced TLR activation and *NF-KB* pathway induction in macrophages was found to be essential for higher neutrophil recruitment in response to infection . Likewise, *in vivo* study showed that intradermal injection with *LdCen*$^{-/-}$ induces higher neutrophil recruitment at the site of injection (ear) and lymph nodes compared to *LdWT* parasites. Phenotypic characterization of recruited dermal neutrophils revealed the presence of heterogeneous neutrophil population. Low density neutrophils sort selected from *LdCen*$^{-/-}$ infected mice exhibited attenuated expression of pro-parasitic molecules compared to *LdWT*. Adoptive transfer of *LdCen*$^{-/-}$ parasite bearing neutrophils were able to induce heightened Th1 differentiation in visceral organs compared to *LdWT* thereby highlighting the robust APC feature of neutrophils in *LdCen*$^{-/-}$ induced immunity. Also, the engulfment of *LdCen*$^{-/-}$ parasitized neutrophil by dendritic cells (DCs) enhanced Ag presentation capability of DCs compared to *LdWT*. Thus neutrophils play novel role in shaping early vaccine immunity.

1412

PHARMACOKINETIC-PHARMACODYNAMIC ASSESSMENT OF THE SAFETY OF FEXINIDAZOLE FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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Fexinidazole is a new oral treatment for *Trypanosoma brucei* (*T.b. gambiense*) human African trypanosomiasis (g-HAT). Fexinidazole *in vitro* is also active against other human kinetoplastid parasites, *T. cruzi* and *Leishmania donovani,* the causative agents of Chagas disease and visceral leishmaniasis, respectively. During a dose-ranging study conducted

in asymptomatic Bolivian Chagas patients, delayed neutropenia and significant increases in hepatic transaminases were observed and the clinical study was suspended. We analysed retrospectively all available pharmacokinetic (PK) and pharmacodynamic (PD) data on fexinidazole in healthy volunteers, Chagas patients and HAT patients to characterise the PK-PD relationships for oral fexinidazole in different regimens. A population PK model was fitted to data from three Phase I studies, two g-HAT field trials and one Chagas field trial. Bayesian exposure-response models were fitted to haematological indices and liver related PD outcomes in asymptomatic Chagas patients. These models were compared with observed data from the g-HAT trials and used to predict likely adverse events from the recommended g-HAT regimen. Asymptomatic delayed neutropenia and thrombocytopenia, and elevations in liver transaminases were found to be exposure related and thus dose-dependent in asymptomatic Chagas patients. Mild, asymptomatic haematological outcomes consistent with the exposure-response curves were observed in the g-HAT trials, confirming a haematological effect of fexinidazole. However, liver toxicity and biochemistry abnormalities were not observed in healthy volunteer studies nor in any g-HAT trials. Fexinidazole treatment results in a dose dependent delayed and reversible bone marrow suppression. The currently recommended g-HAT regimen (adult dose 1800mg for 4 days, then 1200mg for 6 days) provides exposures within a satisfactory safety margin for both neutropenia and hepatotoxicity. Liver toxicity appears to be specific to the Chagas population.

1413

INTERROGATING CHAGAS ANTIBODY RESPONSES WITH HIGH-THROUGHPUT PRECISION TOOLS: TOWARDS A COMPREHENSIVE CATALOG OF *TRYPANOSOMA CRUZI* ANTIGENS AND EPITOPES ACROSS AMERICA.

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Using state of the art high-density peptide microarrays, we developed a highly multiplexed screening array design containing ~2.8 million unique peptides derived from the complete proteomes of Trypanosoma cruzi strains CL-Brener (hybrid DTU TcVI; 19,668 proteins) and Sylvio X10 (DTU TcI, 10,832 proteins). The full length of all 30,500 proteins encoded in these genomes were scanned using 16mer peptides with an overlap of 12 residues between each peptide. Using this platform, we screened sera pools from Chagas-positive and healthy volunteers from several regions across America: Argentina, Brazil, Colombia, Mexico and the United States. Screening of negative sera pools from healthy subjects allowed us to define a background baseline of antibody-binding signal against peptides, as well as to identify cross-reactive epitopes. Screening of Chagas-positive sera pools allowed us to identify specific antibody-binding against *Trypanosoma cruzi* peptides. Using a conservative signal threshold to define antigenic peaks in proteins, our analysis led to the discovery of more than 4,500 antigens (mostly novel), and to the identification of 22,747 antibody-binding peaks (epitopes) across all samples. Also, the comparative analysis of antibody-binding in different samples allowed us to define a core set of pan-Chagas antigens and epitopes with reactivity in all human samples, as well as sets of unique/differential antigens (only reactive in one population) or antigens with shared reactivity in a few samples (populations). In the presentation we will highlight interesting cases of shared/differential antibody responses in different samples, and will discuss next steps to use this high-throughput platform to decompose the sera pools and gain insights into the seroprevalence of these epitopes in each population. To the best of our knowledge this is the first proteome-wide catalog of antigenic determinants for a human infectious disease.

1414

EVALUATION OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) KIT AS A MOLECULAR DIAGNOSTIC TEST FOR CONGENITAL CHAGAS DISEASE

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Molecular tests are used to diagnose congenital Chagas disease in some countries, mainly in Europe. Their use in endemic countries is still anecdotal. Technical and economic factors have been put forward to explain the poor penetration of molecular tests to diagnose Chagas disease in low and middle-income countries (LMIC). The use of loop-mediated isothermal amplification (LAMP) methods may overcome some of these limitations. A newly developed LAMP kit designed for detection of *Trypanosoma cruzi* DNA in human blood showed a good analytical performance and was easy to use. We evaluated the performance of a *T. cruzi* LAMP kit in stored clinical samples of congenital Chagas disease cases in Argentina and Spain. Clinical samples from 49 congenital Chagas disease cases (10 from Argentina and 39 from Spain) and 74 controls (26 from Argentina and 48 from Spain) were tested by the *T. cruzi* LAMP kit and qPCR. The *T. cruzi* LAMP results were read by naked eye and fluorimeter. The sensitivity and specificity of the *T. cruzi* LAMP was estimated using the standard case definition as reference e.g. baby with a positive microscopy, or culture between 0 and 1 months, or a positive serology for congenital Chagas disease at 9-10 months. We evaluated the agreement between *T. cruzi* LAMP read by naked eye and fluorimeter and between *T. cruzi* LAMP and qPCR using Cohen's kappa statistics (K). The sensitivity of *T. cruzi* LAMP was 98.0% (95% CI: 89.3 – 99.6%) both by naked eye and fluorimeter. Specificity was 93.2% (95% CI: 85.1 – 97.1%) by naked eye and 94.6% (95% CI: 86.9 – 97.9%) by fluorimeter. The sensitivity and specificity of *T. cruzi* LAMP improved when only the samples taken 0 to 1 months after birth were considered. There was very good agreement between *T. cruzi* LAMP read by naked eye and fluorimeter (K=0.98) and between *T. cruzi* LAMP and qPCR (K=0.87). The *T. cruzi* LAMP kit could be used as a diagnostic tool for congenital Chagas disease cases in endemic and non-endemic countries.

1415

DEVELOPMENT OF A NOVEL BENZTHIAZOLE SCAFFOLD FOR CHAGAS DISEASE

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An estimated 7 million people, mostly in Latin America, are infected with *Trypanosoma cruzi,* the etiologic agent of Chagas disease. This chronic infection leads to cardiomyopathy or mega-syndromes of the intestines in ~30% of infected individuals. The existing anti-parasitic drugs (two nitroaromatic compounds) are associated with high rates of side-effects that greatly limit their use. A better tolerated and safe drug with potent anti-*Trypanosoma cruzi* activity is desperately needed to treat scores of infected people before they develop irreversible end-organ disease. Towards this end we are developing a novel compound series built around a benzthiazole core. The initial hit compound was

discovered in a high-throughput screen against the closely related parasite, *Trypanosoma brucei.* Subsequent testing against *T. cruzi* demonstrated an EC_{50} of 0.83 μM. Through iterative rounds of medicinal chemistry a lead compound (45DAP076) has been developed with an EC_{50} against *T. cruzi* of 0.12 μM. It has no cytotoxicity on mammalian cells at the highest concentration tested of 50 μM. When dosed to mice at 50 mg/kg by oral gavage, 45DAP076 achieved a peak plasma concentration of 23.2 μM with a robust area under the curve (AUC) of 13,217 min*μM. The compound was tested in an acute murine model of *Trypanosoma cruzi* infection with a 5 day treatment course beginning 7 days after the initial infection. 45DAP076 resulted in complete suppression of parasites but recrudescence occurred later (a result that was comparable to the clinical drug, benznidazole). The compound is currently undergoing testing in the more predictive chronic murine infection model. Analogs of 45DAP076 with further improved pharmacokinetic properties are also being tested. The near-term goal is to identify an optimized lead compound to advance for pre-clinical safety and toxicology testing.

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DISCOVERY AND FINE EPITOPE MAPPING OF NOVEL SEROLOGY-BASED MARKERS FOR DIAGNOSIS OF CONGENITAL CHAGAS DISEASE USING HIGH-DENSITY PEPTIDE CHIPS

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Chagas Disease affects 6-8 million people in the Americas and at least 2 million women of fertile age are estimated to be chronically infected with *Trypanosoma cruzi*. Congenital infections represent a global problem, occurring both in endemic and non-endemic areas. Current diagnostic techniques for Congenital Chagas Disease (CCD) are cumbersome, have poor performance or require re-testing of newborns during the first year of age. New and improved diagnostics that can be deployed in primary health centers are urgently needed. Serology-based assays are preferred due to their low cost and simplicity, but currently suffer of poor performance in newborns due to the presence of confounding maternal antibodies. Furthermore, there is a very limited set of antigens characterized from congenital infections. Here we present a set of defined antigens and epitopes with an excellent potential for diagnosis of CCD. These were identified using a parallelized screening platform based on high-density peptide-arrays displaying >175,000 short peptides derived from 457 T. cruzi proteins. These arrays were screened with 2 pools of sera from 10 infected newborns, and with 2 paired pools of sera from their mothers. Each array was assayed with anti-human IgG (Cy3 labeled), and anti-human IgM (Cy5 labeled) secondary antibodies, and imaged in a fluorescence scanner. After data normalization, we reconstructed antibody-binding profiles for each protein and validated the assay by profiling known antigens. Comparative analysis of IgG reactivity between paired sera pools of newborns and their mothers allowed the identification of antigens with high IgG reactivity in infected newborns, and low or null reactivity in the paired samples from their mothers. We also identified candidate antigens with strong IgM reactivity in infected newborns. We prioritized 125 highly reactive peptides from the top 5% of IgG and IgM sets, and selected 48 short peptides for further validation and assessment of seroprevalence in standard ELISA format (currently underway). This study represents the largest screening so far for discovery of congenital markers of infection with *T. cruzi*.

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MODELING AND SIMULATIONS OF FEXINIDAZOLE TREATMENT REGIMEN FOR HUMAN AFRICAN *TRYPANOSOMIA BRUCEI GAMBIENSE* TRYPANOSOMIASIS (G-HAT) AND THEIR SUCCESSFUL CLINICAL APPLICATION IN ADULT AND CHILD PATIENTS

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Fexinidazole is an oral formulation developed for the treatment of g-HAT. Fexinidazole is rapidly metabolized to sulfoxide (M1) and sulfone (M2). A population pharmacokinetic (POPPK) model which simultaneously models fexinidazole and its two metabolites in healthy volunteers (HV) was developed and then used to: (i) simulate dosing regimens in patients to obtain M2 plasma concentrations reaching the efficacious target, (ii) determine an optimized sparse PK dried blood sampling for patient studies, (iii) characterize the PK of fexinidazole, M1 and M2 in patients. POPPK modeling and simulations in HV were used to test several multiple dosing regimens. The identified dosing regimen was verified in HV and tested in three phase II/III clinical studies of stage 2 and early stage g-HAT (adults and children). A POPPK analysis of fexinidazole and metabolites in patients was performed based on sparse PK sampling. Based on the POPPK model simulations, the best tolerated regimen was once a day administration with food of 1800 mg for 4 days + 1200 mg for 6 days. The PK of fexinidazole and the two metabolites were characterized in adult and child patients. The expected level of efficacy in plasma (10μg/mL) was reached over the duration of treatment. The POPPK modeling and simulations based on fexinidazole and the two metabolites in HV predicted the PK parameters obtained in child and adult g-HAT patients. The resulting treatment regimen was well tolerated in children and adults, and led to the intended exposure.

1418

COMPREHENSIVE INNATE IMMUNE PROFILING OF ZIKA CASES REVEALS A KEY ROLE OF MONOCYTES IN THE ACUTE PHASE OF INFECTION AND NO EFFECT OF PRIOR DENGUE VIRUS INFECTION ON THE INNATE IMMUNE RESPONSE

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Zika virus (ZIKV) is an emerging, mosquito-borne flavivirus responsible for the recent pandemic in the Americas. Much remains to be defined regarding the human immune response to ZIKV infection. To generate a comprehensive innate immune profile after ZIKV infection, we collected blood in PAXgene solution and plasma at acute (days 1-3 and days 4-6 post-onset of symptoms) and convalescent (days 14-16) time-points and peripheral blood mononuclear cells (PBMCs) at days 4-6 and convalescence from symptomatic RT-PCR-confirmed ZIKV-infected children in our long-term pediatric cohort study in Managua, Nicaragua. Patients were documented to be either previously exposed to the closely related dengue flavivirus (DENV) (n=42) or DENV-naïve (n=45). Innate immune responses were comprehensively profiled by CyTOF, Luminex cytokine/chemokine

assays, and RNA-seq. Interestingly, our results reveal that prior exposure to DENV does not impact the innate immune response and clinical symptoms. CyTOF results of all Zika cases showed that the frequency of CD14+ monocytes and CD14+CD16+ monocytes are expanded during the acute phase of infection. Luminex results revealed that levels of monocyte-attracting and pro-inflammatory cytokines, including CCL2, CCL3, CCL4, TNF-α, IFN-γ and IL-1α were elevated during acute ZIKV infection. Additionally, 4 distinct patterns of cytokine levels in serum were detected over time. Strikingly distinct transcriptomic and immunophenotyping signatures were associated with time-point, even between early and late acute phases. Within the significant transcriptomic signatures, we found that interferon signaling pathways and pattern recognition pathways were differentially regulated in acute and convalescent stages of infection. All data are being integrated for network modeling to generate global, unbiased maps of regulatory relationships and to uncover novel host-virus pathways and driver genes. To our knowledge, this study is the most comprehensive immune profiling and network analysis of the response to ZIKV infection in children to date and reveals no effect of prior DENV infection on innate immune responses.

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RECENT PRIOR DENGUE VIRUS INFECTION PROTECTS AGAINST ZIKA IN A PEDIATRIC COHORT IN NICARAGUA

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Zika virus (ZIKV) emerged in northeast Brazil in 2015 and spread rapidly across the Americas, including Nicaragua, where it caused an explosive epidemic in 2016. We analyzed the epidemiology of Zika in an ongoing, community-based cohort study of dengue that follows ~3,700 children aged 2-14 in Managua, Nicaragua, with well-characterized DENV immune histories. To identify Zika cases, RT-PCR was performed on acute blood and urine samples and serological assays were performed on paired acute and convalescent samples. Annual blood samples, collected every March/April, were used to serologically identify inapparent ZIKV infections. Multivariable Poisson regression was used to examine the relation between documented prior DENV exposure in the cohort and incidence of symptomatic and inapparent ZIKV infection. From January 2016 to February 2017, 545 Zika cases and 1226 ZIKV infections were identified in the cohort, for an overall incidence of 13.6 cases (95% confidence interval [CI]: 12.5, 14.8) and 37.2 infections (95% CI: 35.2, 39.3) per 100 person-years. Incidence of cases and infections was higher in females and in older children. Prior documented DENV infection was protective against developing Zika disease among those infected (Incidence Rate Ratio [IRR]: 0.60; 95% CI: 0.43-0.84) and in the total cohort population (IRR: 0.61; 95% CI: 0.47, 0.80) when adjusted for age, sex, and recent DENV infection (1-2 years before ZIKV infection). Recent DENV infection was protective against symptomatic ZIKV infection when adjusted for age and sex, but not when prior DENV immunity was included in the model. Prior or recent DENV infection did not affect the rate of ZIKV infection. These findings support that previous DENV infection protects individuals from symptomatic disease when they are infected with ZIKV.

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EPITOPE TARGETS OF THE HUMAN ANTIBODY RESPONSE TO PRIMARY ZIKA VIRUS INFECTION

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Zika virus (ZIKV) transmission became a global public health emergency after the recent epidemic in Latin America and beyond revealed rare but dire manifestations of infection such as severe birth defects and Guillain-Barré syndrome. ZIKV's emergence in areas where other related flaviviruses such as dengue are endemic creates challenges in terms of accurately diagnosing infections, conducting reliable surveillance, but also in understanding the distinguishing aspects of the host immune response to ZIKV. Because vaccines represent a key strategy for prevention of infectious diseases and typically rely on a robust antibody responses, we sought to analyze the durable antibody responses in individuals infected by Zika as a first flavivirus infection. We observed complex populations of antibodies that bind to epitopes on intact virions, simpler epitopes on envelope protein monomers as well as envelope subdomains. Moreover, strong neutralizing antibody responses that minimally cross-react with dengue viruses were consistently detected. To better understand the molecular determinants of the neutralizing antibody response to ZIKV and to develop tools that could aid vaccine development, we isolated two potently neutralizing monoclonal antibodies (mAbs) from one primary ZIKV case and mapped key amino acid residues involved in mAb binding and neutralization by multiple complimentary methods including generation of neutralization escape mutants and alanine scanning mutagenesis. The mAbs recognize different epitopes on domain I and domain II of the viral envelope protein. Functionally, both mAbs were protective in a lethal mouse model of ZIKV infection. The antigenic sites on the virus defined by these mAbs appear to be common targets of the human response to ZIKV as their binding is consistently blocked by ZIKV- but not dengue-immune sera. This work provides new knowledge and tools that may be useful as diagnostic reagents or as therapeutics and will advance vaccine development.

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HOW CLOSE IS CLOSE? CHARACTERIZATION OF ZIKA AND DENGUE VIRUS CROSS-REACTIVE MEMORY B CELLS AND SERUM RESPONSES

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Zika virus (ZIKV) recently spread rapidly throughout the Americas, co-circulating with dengue virus (DENV). The antigenic similarity of ZIKV and DENV raises important concerns regarding the effect of immunological cross-reactivity on protection and potential enhancement of ZIKV and DENV infections and vaccines. Here, we analyzed memory B cells (MBCs) from peripheral blood mononuclear cells (PBMCs) collected 2 weeks or ~7 months after RT-PCR-confirmed Zika cases from children who were DENV-naïve or had experienced 1 or >1 previous DENV infections (n=8-12/group) in a cohort study in Nicaragua. Analysis of activated MBCs using a

Multi-color FluoroSpot assay that enables measurement of B cell reactivity to DENV1-4 and ZIKV at a single-cell level showed that all ZIKV-infected patients mounted a highly type-specific response to ZIKV regardless of prior DENV infection, with 64% type-specific (TS) response in DENV-naïve and 45% TS response in previously DENV-infected groups ~7 months post-ZIKV infection. Previously DENV-infected groups displayed substantial DENV-ZIKV cross-reactivity 14 days post-ZIKV infection that decreased by ~7 months, while the TS proportion increased. The magnitude of DENV-ZIKV cross-reactive MBC responses decreased over time to a greater extent than TS responses. At ~7 months post-ZIKV infection, we characterized serum antibodies derived from plasma cells. In the great majority of both DENV-naive and previously DENV-infected individuals, depletion of cross-reactive antibodies did not substantially affect ZIKV neutralizing antibody titers, though these titers in a few patients were reduced, possibly due to shared neutralizing antibody epitopes between specific DENV serotypes and ZIKV. Prior infection by 1 versus >1 DENV before ZIKV infection did not differentially affect DENV-ZIKV cross-reactive responses, and DENV responses were lower than DENV-ZIKV responses at both 2 weeks and ~7 months post-ZIKV infection. This study improves our knowledge of B cell and serum antibody responses to closely related flaviviruses with implications for protection and pathogenesis of subsequent infections and vaccines.

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PROSPECTIVE INVESTIGATION OF A COMMUNITY-BASED COHORT OF PREGNANT WOMEN BEFORE AND DURING THE ZIKA EPIDEMIC IN NORTHEAST BRAZIL: SILENT TRANSMISSION AND INFANT OUTCOMES

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Little prospective information exists on Zika virus (ZIKV) transmission among pregnant women and clinical outcomes of their infants. We conducted a community-based prospective study among urban slum residents in Salvador, Brazil. We identified pregnant women from January 2015 to June 2016 and identified incident Zika seroconversions by analyzing biannually collected samples in the ZIKV35 monoclonal antibody Blockade of Binding (BoB) assay. We evaluated adverse outcomes by interviewing mothers and performing anthropometric measurements, neurologic (Hammersmith Exam, HINE), audiometric and ophthalmological evaluations and examinations with Bayley Scales of Infant Development III among infants. Among a cohort of 655 participants, 66 (10%) had at least one pregnancy during the study period. We completed follow-up for 46 (70%) of the 66 women, of whom Zika seroconversion occurred prior to, during and after the pregnancy in 25 (54%), 13 (28%), and 1 (2%), respectively. The earliest seroconversion event occurred between February and September 2013. Among the 48 pregnancies, 46 resulted in live births and two in spontaneous abortions. The 46 infants did not have evidence of microcephaly or congenital defects at birth. However, the 13 infants of mothers who seroconverted during pregnancy had increased risk of having low/borderline cognitive development scores (RR 5.08; 95% CI 1.05-24.43) than the 33 infants of mothers who did not seroconvert during pregnancy (4 [31%] of 13 vs. 2 [6%] of 33, respectively). Similarly infants of mothers who seroconverted during pregnancy had increased risk (RR 6.82: 1.54-30.17) of having altered behavior-observed audiometry findings than infants of mothers who did not seroconvert during pregnancy (5 [45%] of 11 vs. 2 [7%] of 30, respectively [missing data for 5 individuals]). These finding indicate that silent transmission of ZIKV occurred prior to the initial epidemic in early 2015. Furthermore, these findings suggest that a significant proportion of infants exposed *in utero* to ZIKV develop developmental deficiencies despite the absence of clinically evident neurological sequelae.

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EVIDENCE OF SHORT-TERM CROSS-IMMUNITY BETWEEN DENGUE AND ZIKA VIRUSES INFERRED USING LONGITUDINAL SEROLOGICAL AND SURVEILLANCE DATA FROM FIJI, 2013-2017

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The Zika (ZIKV) and dengue (DENV) viruses are two closely related flaviviruses that share the same primary vector, the *Aedes* genus of mosquitos. Given the antigenic similarity between these viruses it has been hypothesised that DENV antibodies may cross-react with ZIKV. To investigate the role of DENV cross-protection on ZIKV dynamics during overlapping outbreaks, we combined a transmission dynamic model with serological and surveillance data from Central Division, Fiji. Longitudinal population representative seroepidemiological data were available from participants sampled in 2013, 2015 and 2017. In addition, surveillance data were available from a major DENV-3 outbreak in 2013/14, and ZIKV case reports from 2015 and 2016. Using a Bayesian approach, we fitted a transmission dynamic model with a seasonally varying transmission to these data to estimate the probability that DENV would confer transient immunity against ZIKV infection, as well as the length of this immunity. We found evidence that DENV infections generated strong, short-term, immunity against ZIKV infection and that this relationship could explain the multi-wave transmission dynamics of ZIKV in Fiji. We found evidence that the virus was introduced in early 2013 and persisted for several years with minor outbreaks in consecutive years, with dynamics determined by seasonality of transmission, pre-existing immunity and cross-immunity following DENV infection. Our analysis provides ecological evidence of interaction between ZIKV and DENV and shows the value of linking multiple data sources to reconstruct ZIKV dynamics. The availability of detailed case and serology data in an island outbreak setting – combined with mathematical models – presented a unique opportunity to gain crucial insights into these infections. Seasonal variation in transmission, combined with other co-circulating flaviviruses, means the timing of ZIKV introduction can have a major impact on outbreak transmission dynamics.

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MAPPING REACTIVITY TO ZIKA VIRUS IN MEMORY B CELLS FOLLOWING DISTINCT ANTECEDENT FLAVIVIRUS EXPOSURES

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Flaviviruses such as dengue (DENV) and Zika viruses (ZIKV) encompass increasingly overlapping circulation areas. Serologic cross-reactivity amongst flaviviruses is a potential result of new flaviviruses emerging into an area endemic for other flaviviruses. This phenomenon can lead to diagnostic difficulties, enhancement of disease, or potentially broader protective, natural immunity. However, there is only a limited understanding of the evolution of the human antibody response at the clonal level to sequential flavivirus infections, particularly those involving different serogroups. To address this problem, we recruited travelers exposed to ZIKV virus and characterized neutralizing antibody titers to

DENV and ZIKV in their plasma to assess flavivirus exposure history. Based on serology and self-reported travel and medical histories, we classified subjects as having either ZIKV as a primary flavivirus infection or ZIKV as a post-primary flavivirus infection (typically meaning ZIKV infection in a DENV-immune subject). To assess the extent to which primary or post-primary ZIKV infection led to distinct memory B cell (MBC) repertoires, we immortalized these cells from peripheral blood and assessed reactivity to ZIKV and different DENV serotypes. The frequency of ZIKV-binding MBCs was higher in primary ZIKV subjects (0.9-1.2% of immortalized MBCs) compared to post-primary ZIKV subjects (0.1-0.17%). Strikingly, we also found that regardless of exposure history, ZIKV infection induced a dominant ZIKV-specific response (81-96%) with limited cross-reactivity to DENV (4-19%). Further studies are aimed at understanding the clonality of primary or post-primary ZIKV responses with regard to VH/VL gene usage, CDR3 length, and level of somatic hypermutation, and whether patterns change over time.

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HIGH RESOLUTION MAPPING OF GLOBAL CHILD MORTALITY

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We present findings from an effort to produce high resolution maps of mortality rates and death counts for neonates, infants, and children under-5 across 120 low and middle income countries. Some 4.8 million, or 97% of all global child deaths, occurred within these countries in 2016. We compiled and geo-referenced data on nearly 28 million child deaths across nearly two decades, from 1998-2016. Sources include complete and summary birth histories from sample surveys and censuses, in combination with vital registration data. This is a methodological first. We developed new methods to account for biases in these various data sources across space and time, including systematic under-reporting in vital registration data. We also developed a new geostatistical model which accounts for residual spatiotemporal correlation across age groups. Our findings highlight great improvements since 2000, yet entrenched subnational inequalities. We focus specifically on subnational heterogeneity in the proportion of child deaths in different age groups under 5. In general, progress in reducing neonatal mortality has been slower than in older children, but to greatly varying degrees even within countries. Reducing neonatal mortality will require a different set of interventions than those which have successfully reduced mortality in older children. Finally, in addition maps of mortality rates, we also provide estimates of numbers of child deaths by 5x5 pixel and by subnational units (districts and provinces). Such maps will arm policymakers with information critical in targeting public health interventions to children at highest risk.

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CAUSES OF DEATH AMONG CHILDREN UNDER 5 YEARS AND STILLBIRTHS, CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK, DECEMBER 2016 - JUNE 2018

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Children die disproportionately in Africa and South Asia. The CHAMPS Network aims to improve understanding about causes of child mortality through longitudinal surveillance and etiologic investigation of child deaths and stillbirths by combining post-mortem minimally invasive tissue sampling (MITS), molecular, microbiologic, and histopathologic testing, clinical records, and verbal autopsy. From 635 cases with MITS as of 6/6/2018, we analyzed 270 (43%) cases with causes of death (COD) assigned by CHAMPS expert panels using standardized approaches. Cases were from South Africa (162, 60%), Mozambique (46, 17%), Kenya (46, 17%), Mali (11, 4%), and Bangladesh (5, 2%); 243 (90%) died in a health facility. Among 138 neonates, 40 died on day zero, and 60 within 6 days. Most common neonatal underlying COD were preterm birth complications (35), neonatal encephalopathy (13), maternal hypertension (12), congenital birth defects (13), and sepsis (8). Main factors in stillbirths (n=31) were maternal hypertension (7), placental hemorrhage (4), and labor and delivery complications (4). Main underlying causes in children 1-59 mos old (n=101) were lower respiratory infections (16), malnutrition (12), congenital birth defects (12), neonatal preterm birth complications (11), and diarrheal diseases (9). Pathogens contributed to death in 58% of cases; 31% had multiple causal pathogens. Leading pathogens were *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *E. coli*, cytomegalovirus, *Staphylococcus aureus*, respiratory syncytial virus; 42% had no causal pathogen. Among 49 preterm cases, 36 (73%) had >1 pathogen in the causal chain. Fatal injuries (burns, transport, poisoning) and noncommunicable conditions (sickle cell, cancer) were also observed. Panelists deemed 67% of all cases to be preventable. These early data highlight the importance of understanding multicausal patterns, maternal factors, and interplay between infectious and chronic conditions in child deaths. As data accrue with more community deaths represented, CHAMPS will produce new actionable knowledge to reduce child and perinatal mortality.

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REAL-TIME UNDER-5 MORTALITY SURVEILLANCE, CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE SYSTEM (CHAMPS), DECEMBER 2016-FEBRUARY 2018

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Approximately 5 million children per year die before their fifth birthday, with disproportionate mortality in sub-Saharan Africa and South Asia.

Vital registration systems capture only a fraction of these deaths, often with low specificity for causes of death. The Child Health and Mortality Prevention Surveillance (CHAMPS) Network was established to define the causes of under-5 mortality and stillbirth using population-based surveillance and novel diagnostic approaches, including minimally invasive tissue sampling (MITS). We describe initial results from rapid notification systems and consent for the MITS procedure. Surveillance for under-5 deaths and stillbirths began in one site in December 2016, with other sites beginning over the course of 2017. As of February, 2018, 5 sites (Manhiça, Mozambique; Soweto, South Africa; Bamako, Mali; Kisumu, Kenya; Baliakandi, Bangladesh) were actively collecting mortality surveillance and laboratory data. Cumulatively through 15 Feb 2018, CHAMPS had received 2634 unique notifications (68% from health facilities; 16% community reporter or health worker; 5% demographic surveillance staff; 11% other source). Screening determined 1074 cases (42% of unique notifications) to be eligible for inclusion. Most eligible deaths (80%) occurred in health facilities, with 50% from a large tertiary hospital in Soweto. Of all eligible deaths, 707 (66%) were notified within 24 hours of death (23% over 24 hours; 11% missing), with this proportion increasing from 47% in Dec 2016 to 75% in January, 2018. A total of 687 families were approached for consent for the MITS procedure. Of these, 75% provided consent (range by site:58% to 96%). A total of 500 MITS procedures had been conducted by February 15, 2018. These data suggest that it is feasible to obtain notifications of deaths in <24 hours in diverse high-under-5 mortality settings and obtain post-mortem tissue samples in a substantial proportion which over time will provide opportunities to provide more specificity for mortality burden estimates.

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MATERNAL FACTORS ASSOCIATED WITH PERINATAL MORTALITY SHOULD BE EASILY IDENTIFIED DURING ANTENATAL VISITS. THE 1000 *DAYS* COHORT STUDY IN RURAL NIGER

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Epidemiologic data on perinatal mortality, defined as stillbirth or neonatal death within 7 days of birth, is scarce in African rural areas. From April 2015-June 2016, a cohort study was implemented in 5 health facilities in rural Niger (Zinder region, Mirriah department), enrolling all pregnant women presenting for a first antenatal care (ANC) visit. They received a standard ANC package defined by the Ministry of Health. We used a multivariate generalized linear mixed model to examine factors associated with perinatal mortality, including study site as a random effect. A complication score was created based on the following six danger signs identified during ANC: age ≤18 years or ≥35 years, primiparity, multiple gestation, severe anemia (<7g/dL), positive malaria rapid diagnostic test (RDT), pre-eclampsia (high blood pressure and proteinuria). Of 1,745 pregnant women included in the cohort study, 1,679 women gave birth (4 maternal deaths, 12 abortions, and 50 unknown pregnancy outcomes). In total, 84 (5.0%) births were considered as perinatal deaths and 1,595 children were alive at 7 days of life. Perinatal mortality was associated with fewer than 4 ANC visits (aOR 1.71; 95%CI 1.01–2.88; p=0.05), primiparity (aOR 2.18; 95%CI 1.32–3.61; p<0.01), positive malaria RDT during pregnancy (aOR 2.55; 95%CI 1.29–5.06; p<0.01), severe anaemia during pregnancy (aOR 3.01; 95%CI 1.06–8.52; p=0.04). Overall, 19%, 10% and 1% of women presented with one, two and three danger signs, respectively. Perinatal mortality in babies born to women without danger signs was 3.5% (41/1,171) compared to 8.5% (43/508) among those born to women with at least one danger sign (aOR 2.45, 95%CI 1.58-3.81). We found no association between the occurrence of danger signs and place of delivery: only 37.5% (449/1,171) of women without danger signs and 37.4% (190/508) of those with at least one danger sign gave birth in health facilities (p=0.97). Perinatal mortality was associated with maternal risk factors easily identified during ANC, however reducing perinatal deaths in this at-risk group would require delivery by more highly skilled medical personnel.

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PREDICTING IN-HOSPITAL MORTALITY USING ROUTINE DATA TO GUIDE DECISION MAKING FOR ESSENTIAL NEONATAL CARE

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More than 98% of neonatal deaths occur in low and middle-income countries (LMICs) and hospitals in these regions are expected to play a key role toward achievement of Sustainable Development Goal 3.2 "reduce neonatal mortality to 12 per 1000 live births and below". To deliver quality care in LMICs, rationally designed hospital services are required. The study aimed to generate better neonatal prognostic data and use it to develop locally relevant prognostic models and explore their potential to support decision making for essential neonatal care. Using the largest neonatal unit routine data set in Kenya (n=11,850), logistic regression was applied to (1) develop a model to predict in-hospital mortality using five treatments derived from essential neonatal care prescribed at admission, (2) develop an alternative model using symptoms and signs of severe illness documented at admission. Calibration plots were used to measure model discrimination and calibration. Decision curve analysis was used to compare the potential clinical utility of the models using the net benefit. The Neonatal Essential Treatment Score (NETS) and the Score for Essential Symptoms and Signs (SENSS) have good discrimination (each with c-statistic of 0.89) but the SENSS model is better calibrated (calibration slope 0.90 compared to 0.76 for NETS). Net benefit is observed over a wider range of predicted probabilities for SENSS (0 to greater than 80%) in comparison to NETS (0 to 80%) due to the superior calibration of SENSS. The NETS model would result in a higher number of patients classified at high risk of inpatient death across the range of predicted probabilities. Both models can be presented as simple score charts for use by clinicians at the point of care. NETS and SENSS are based on predictors available in basic routine data and may support clinical decisions such as referrals and aid in the organisation of different levels of neonatal hospital care within a region. The performance of these models may be evaluated further by geographical external validation using data from similar neonatal units followed by impact assessment in a cluster randomised controlled trial.

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ENVIRONMENTAL ENTERIC DYSFUNCTION AND OTHER FACTORS DURING EARLY CHILDHOOD ASSOCIATED WITH ATTAINED SIZE AT FIVE YEARS: FINDINGS FROM THE MAL-ED BIRTH COHORT STUDY

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Poor growth in early childhood is considered to have both short- and long-term consequences on health and development in low-income settings. The goal of this analysis was to identify factors (illness, pathogen burden, dietary intake, gut function, micronutrient status, and socioeconomic factors) in the first two years of life that are associated with growth outcomes at five years of age. The data are from seven sites in the MAL-ED cohort study, including 1,004 children enrolled from 0-17 days after birth and followed until five years of age. Gut function biomarkers were related to size at five years. Mean L:M Z-scores during the first 2 years of life were negatively associated with all of the growth outcome measures in this study (HAZ: -0.12 (95% CI -0.20, -0.04); WAZ: -0.17 (95% CI -0.27, -0.07); BMIZ: (-0.12 (95% CI -0.24, 0.0)). Myeloperoxidase was negatively associated with the weight measures (WAZ: -0.54 (95% CI -0.82, -0.26) and BMIZ: -0.59 (95% CI -0.91, -0.27)), but not with HAZ; whereas alpha-1-antitrypsin had a negative association with HAZ (-0.26 (95% CI -0.50, -0.02), but not with the weight measures. Indicators of iron status were also related to size at five years. Transferrin receptor was positively related to HAZ (0.21 (95% CI 0.09, 0.33)) and WAZ (0.23 (95% CI 0.09, 0.37)) at five years. Haemoglobin was positively related to HAZ (0.07 (95% CI 0.01, 0.13)), and ferritin was negatively related to HAZ (-0.08 (95% CI -0.12, -0.04)). Among the infection/illness factors that we tested, bacterial density in stool was negatively associated with HAZ (-0.04 HAZ per 10% increase in bacteria positive samples (95% CI -0.08, 0.00)), but illness symptoms did not have an effect on size at five years. Size at enrolment and socioeconomic status were both positively related to all of the growth outcomes. Longitudinal markers of environmental enteric dysfunction (EED) and bacterial density are associated with poor growth outcomes at five years of age. Iron markers are also related to growth outcomes. Environmental interventions to reduce bacterial burdens and EED, including vaccines where appropriate, may improve long-term growth in low-income settings.

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HOUSEHOLD-LEVEL FACTORS ASSOCIATED WITH CHILDHOOD GROWTH IN BANGLADESH: AN ANALYSIS OF THE MULTIPLE INDICATOR CLUSTER SURVEY, 2012-2013

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Growth impairment in children continues to be a leading global health concern, despite improvement in recent decades. An understanding of factors associated with growth could contribute to public health efforts. The study examined the household-level factors associated with children growth using data from the Multiple Indicator Cluster Survey (MICS) in Bangladesh. A total of 7851 children 0-24 months of age were included in the analysis. A linear mixed model analysis with household as a random effect was used to test for the effect of different household factors independently associated with height-for-age z-score (HAZ). Interactions were also tested to understand the relationships between factors. Significant factors were included in a stepwise regression to identify the final combination of factors influencing the outcome. Children in our sample were on average 1.45 standard deviations shorter than the WHO reference standard. Age of the child was also negatively associated with the outcome, with HAZ scores decreasing with increasing child age. Maternal education and presence of a refrigerator in the household were positively associated with HAZ, while male sex, unimproved toilet facilities, and shared toilet facilities had a negative effect on HAZ. In addition, maternal education was found to reduce the negative association of unimproved toilet facilities and shared toilet facility with HAZ. The results indicate that growth impairment continues to be a public health concern in Bangladesh, and that child growth, as measured by HAZ, is associated with several household-level factors. Maternal factors were also important factors in modifying the effect of some of these factors. Interventions should therefore not only target these factors but also consider the relationships that exist between them.

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VECTOR BIONOMICS AND TRANSMISSION INTENSITIES OF MALARIA VECTORS ON BIOKO ISLAND OVER 14 YEARS OF INTEGRATED VECTOR CONTROL

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Entomological surveillance has been an integral part of the Bioko Island Malaria Control Project (BIMCP) since the implementation of the project in 2004. Systematic vector surveillance over the years continued to inform and guide the vector control interventions in attaining remarkable outcomes. This study analyses the trend in the vector bionomics and transmission intensities of the local vectors since the inception of the BIMCP. The feeding and resting behaviors, as well as the compositions of the local vectors were monitored using window traps, CDC light traps, and human landing catches. Trends in vector densities, sporozoite rates, and the entomological inoculation rates (EIR) were determined. Phenotypic resistance profile of the malaria vectors as well as target-site resistance and metabolic resistance patterns were also monitored. *An gambiae s.s*, (S and M forms) constituted 45% of the local vectors at baseline with *An funestus* 45% and *An melas* 10%. However after two years of IRS *An. funestus s.l.* was eliminated. In 2009, *An gambiae s.s. S*. was also eliminated and as of 2017, *An gambiae s.s. M* (*An. coluzzii)* (70%) and *An. melas* (30%) remained the main vectors on the Island. Biting rates have reduced from an average of 35 bites per person per night in 2009 to an average of 8 bites per person per night in 2017. Vectors biting behavior shifted to more of outdoor biting between 2004 and 2014. The EIR has dropped from 1,214 infective bites person per annum at baseline to 13 infective bites per person per annum in 2017. The frequency of *kdr-w* has increased to over 85% in the vector population in addition to the presence of P450s pyrethroid metabolizers. However, AChE mutations have not been detected. The planning, implementation, monitoring and evaluation of vector control interventions rely on the knowledge of the local vectors for effective programs. Changes in vector behaviors and transmission intensities are essential in directing vector control interventions and measuring the impacts of such interventions.

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ANNUAL INDOOR AND OUTDOOR ENTOMOLOGICAL INOCULATION RATES IN *ANOPHELES GAMBIAE, AN. COLUZZII*, AND *AN. ARABIENSIS* ACROSS FIVE ECOZONES OF NIGERIA

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Vector control is the primary transmission reduction strategy against malaria in Nigeria. The entomological inoculation rate (EIR) measures exposure to infective bites received by an individual during a specific time interval. Investigations were carried out in all six sentinel sites where *Plasmodium falciparum* is transmitted mainly by *Anopheles gambiae, An. coluzzii* and *An. arabiensis*. Entomological surveillance occurred monthly over one year (Jan-Dec 2017) across five ecozones in Nigeria. Malaria vectors were collected using human-baited CDC light traps (4 houses/site) both indoor and outdoor, and pyrethrum spray catches indoors (32 houses/site). The vectors were identified morphologically and the sibling species were identified using PCR assays. *P. falciparum s*porozoite rates were determined by ELISA. EIRs were calculated using standard methods. Indoor EIR values were recorded for *An. gambiae* with a range from 1.2 infective bites per person per year(ib/p/yr) in the Sahel-savannah (Sokoto) to 23.9 ib/p/yr recorded in the mangrove swamp/rainforest (Akwa Ibom). Indoor EIR values for *An. coluzzii* ranged from 0.9 ib/p/yr in the forest (Oyo) to 11.4 Ib/p/yr in the rainforest (Ebonyi). However, higher indoor EIR were recorded among *An. coluzzii* only in the rainforest, though this did not vary significantly from *An. gambiae* (p=0.059). For *An. arabiensis,* the highest indoor EIR of 4.7 Ib/p/yr was recorded in the Sahel-savannah. For outdoor EIR, *An. gambiae* remained the dominant malaria vector with the highest EIR of 19.2 ib/p/yr recorded in the Sahel-savannah and the lowest EIR of 0.8 Ib/p/yr recorded in the forest. For *An. coluzzii,* outdoor EIR ranged from 0.1 Ib/p/yr in the mangrove to 0.7 ib/p/yr in the Guinea-savannah. Outdoor EIR *for An. arabiensis* ranged from 2.9 ib/p/yr in the Guinea-savannah and 0.2 ib/p/yr in the forest and mangrove swamp/rainforest areas. The high outdoor EIR for *An. gambiae* in the Sahel-savannah could be a factor in persistently high parasite prevalence in the Sahel region despite LLIN coverage of >80%. New tools targeting outdoor transmission may be required to achieve national malaria control targets.

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REPLACEMENT OF INDOOR RESIDUAL SPRAYING ''IRS" BY LONG LASTING INSECTICIDAL NETS ''LLINS" AND SEASONAL MALARIA CHEMOPREVENTION ''SMC" ASSOCIATED WITH CHANGES OF KEY ENTOMOLOGICAL INDICATORS OF MALARIA TRANSMISSION IN SOUTHERN MALI

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Malaria vector control by IRS was implemented in Southern Mali in 2013-2014 with bendiocarb and in 2015-2016 with Actellic 300CS with support from PMI/USAID. IRS was relocated to Mopti Region in 2017 given particularly high malaria incidence at health facilities. LLINs and SMC were provided to populations in former IRS sites, as part of national policy. Entomological monitoring was conducted from June to December in 3 districts (2 sprayed: Koulikoro & Baroueli and 1 adjacent unsprayed: Kati) in 2016 and 2017, covering the last year of IRS and 1st year of IRS relocation. Monthly collections of mosquitoes were performed by pyrethrum spray catch in 20 houses/site to determine indoor resting densities and by human landing catches in 8 houses/site to determine human biting rates (HBR), species composition and entomological inoculation rate (EIR). Molecular analysis was conducted to determine sporozoite rates (SR) and species identification. In sites where IRS was withdrawn, the vector density doubled after IRS cessation (mean of 6.0 *An. gambiae*/house in 2017 versus 3.0 in 2016), while in the control site there was a decrease (5.4 *An. gambiae*/house in 2017 versus 12.1 in 2016). Similarly, in sites where IRS was withdrawn, HBR was extremely high at 28.7 bites/human/night (b/h/n) in 2017 compared to 2016 (6.1 b/h/n); a 4.7 fold increase after IRS cessation. In the control site, there was a lower magnitude increase in HBR (36.5 b/h/n in 2017 versus 22.1 in 2016, 0.6 fold increase). *An. coluzzii* was the major vector (95%). In IRS sites, the SR was similar for both years at 0.8% (95%CI: 0.2-1.5) in 2017 versus 1.3% (95%CI: 0.2-2.5) in 2016; p=0.42. The trend was the same for the control site at 0.8% (95%CI: 0-1.7) in 2017 versus 0.4% (95% CI: 0-1.2) in 2016, p=0.5. Following IRS relocation, EIR in IRS areas increased two fold to 15.37 infectious bites/human/5months in 2017 versus 7.05 ib/h/5months in 2016. In the control site, there was a threefold decrease (6.21 ib/h/5months in 2017 versus 18.6 ib/h/5months in 2016). Overall, following IRS relocation there was no significant increase in SR, but the biting rate increased substantially, resulting in a doubling of EIR.

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THE BEHAVIORAL AND PHYSIOLOGICAL ADAPTATIONS OF MALARIAL MOSQUITOES SINCE 2000 AND CONSEQUENCES FOR PUBLIC HEALTH IMPACT IN AFRICA

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The anti-malarial efficacy of key vector control interventions - long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) - are reliant on technologies that interrupt human biting by exploiting the night time, indoor feeding patterns of primary African vectors. There is a potential public health impact due to increasing physiological resistance, enabling mosquitoes to overcome toxic insecticidal effects, or behavioural adaptations that facilitate avoidance of toxic exposures. The impact of physiological resistance on the efficacy of nets and IRS has been quantified using WHO discriminatory dose bioassay tests and experimental hut studies. A systematic review of mosquito and human behaviour and an established transmission model for malaria are then used to estimate i) a public health impact of changes in exposure risk; ii) the consequential impact of both resistance mechanisms together, and; iii) the residual transmission across Africa and consequences for the efficacy of indoor vector control. There are few studies on human indoor or sleeping behaviours which is crucial for evaluating indoor vector interventions. However, temporal data suggest a transition to increased outdoor biting for key malaria-transmitting mosquitoes. Physiological and behavioural resistance were not found to be associated suggesting these may be independent mechanisms assisting mosquito survival in the presence of insecticides. As mosquito communities transition to become increasingly able to avoid deleterious impacts of indoor interventions, the burden of residual transmission increases. We find an association between places with more outdoor biting and a reduced efficacy of indoor vector control. This is the first study to quantify both physiological and behavioural resistance mechanisms of mosquitoes and extrapolate the public health impact. Novel interventions targeting the control of vectors outdoors will be critical for malaria control going forward.

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SCREENING AND FIELD PERFORMANCE OF POWDER-FORMULATED INSECTICIDES ON EAVE TUBE INSERTS AGAINST PYRETHROID RESISTANT *ANOPHELES GAMBIAE*: AN INVESTIGATION INTO ACTIVES PRIOR TO A RANDOMIZED CONTROLLED TRIAL IN CÔTE D'IVOIRE

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Electrostatic coating is a new way of delivering insecticides to mosquitoes visiting homes for blood source. Although previous tests demonstrated the resistance breaking potential of this chemical application method, studies screening and investigating the residual efficacy of broader range insecticides are necessary. Eleven insecticide powder formulations belonging to seven insecticide classes were initially screened for residual activity over 4 weeks against pyrethroid resistant *Anopheles gambiae s.l.*. Tests were performed using the eave tube assay. With the best performing insecticide we monitored the persistence over 12 months and explored the actual contact time lethal to mosquitoes, using a range of transient exposure time (5s, 30s, 1min up to 2 min) in the tube assays in laboratory. The mortality data were calibrated against overnight release recapture data from enclosure around experimental huts incorporating treated inserts. Natural recruitment rate of mosquitoes to the tube without insecticide treatment was assessed using fluorescent dust particles. Although most insecticides assayed during the initial screening induced significant mortality (45-100%) of pyrethroid resistant *An. gambiae s.l.* during the first two weeks, only 10% beta-cyfluthrin retained high residual efficacy, killing 100% of An. gambiae during the first month and >80% over 8 subsequent months. Transient exposure for 5 seconds of mosquitoes to 10% beta-cyfluthrin produced 56% mortality, with an increase to 98% when contact time was extended to 2min (P = 0.001). In the experimental huts with enclosure, mortality of *An. gambiae* with 10% beta-cyfluthrin treated inserts was 55% compared to similar rate (44%) of mosquitoes that contacted the inserts treated with fluorescent dusts. This indicates that all host-seeking female mosquitoes that contacted beta-cyfluthrin treated inserts during host-seeking were killed.Beta-cyfluthrin showed great promise for providing prolonged control of pyrethroid resistant *An. gambiae s.l.* and has potential to be deployed year-round for the eave tube technology.

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IMPACT OF THE DUPLICATED P450 GENE, CYP6P9A AND CYP6P9B ON THE EFFECTIVENESS OF VARIOUS BED NETS AGAINST *ANOPHELES FUNES*TUS, A MAJOR MALARIA VECTOR IN AFRICA

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Insecticide-based interventions are reducing malaria burden. Unfortunately, growing insecticide resistance in malaria vectors is threatening these successes. However, the extent of the impact of this resistance notably, metabolic resistance on the effectiveness of insecticide-based interventions such as long lasting Insecticidal Nets (LLINs) remains unclear. Taking advantage of the recent detection of the first DNA-based molecular marker for metabolic resistance in the Cytochrome P450 genes (CYP6P9a and CYP6P9b), we used an experimental hut study in Cameroon to assess the impact of metabolic resistance driven by the duplicated P450 genes, CYP6P9a and CYP6P9b on the performance of five LLINs (Olyset, Olyset Plus, PermaNet 2.0 and PermaNet 3.0). After an experimental hut trial on the field and cone assay in the laboratory, mosquitoes were genotyped for the CYP6P9a and CYP6P9b to assess its impact. A reduced efficacy of LLINs was observed against the pyrethroid resistant fumoz-fang lab strain (*An. funestus*) with significantly lower mortality rates (<31.4%; P<0.0001 for PermaNet 2.0 and <56.7%; P<0.0001 for Olyset) although PBO-based nets had the highest mortality. However, the blood feeding rate is significantly reduced in the LLINs (10.2% to 6.8%, p<0.0001) vs control (28.7%). A strong association was observed between the presence of the CYP6P9a and the ability to survive in the presence of the bed nets: PermaNet 2.0 (OR= 15.5; p<0.0001), PermaNet 3.0 (OR= 11.6; p<0.0001), Olyset (OR= 7.7; p<0.0001), Olyset Plus (OR= 2.5; p<0.0001). The same association was observed between the CYP6P9a and the ability to blood feed in the presence of bed nets: Olyset (OR= 2.3; p<0.0001), PermaNet 3.0 (OR= 4.5; p<0.0001). The loss of efficacy of LLINs against pyrethroid resistant *An. funestus* and the increased ability to survive and blood feed of CYP6P9a resistant mosquitoes highlight the impact of resistance on effectiveness of insecticide-based interventions.

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MALARIA VECTOR SURVEILLANCE - CAN VECTOR DATA GUIDE COUNTRY POLICY AND PROGRAMMATIC MALARIA CONTROL DECISIONS?

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National malaria program policies for vector control interventions recommend the universal use of either insecticide treated nets (ITNs/ LLINs) or indoor residual spraying (IRS) with larval source management as a supplemental intervention in certain circumstances. ITNs and IRS have been successful, being responsible for >80% of the reductions in *P. falciparum* malaria in Africa. In this environment of limited recommended interventions choices, vector surveillance has had a limited programmatic role in decision-making. However, the threat posed by increasing insecticide resistance and residual transmission has led to an anticipated rollout of new interventions incorporating new/multiple active ingredients in established and novel interventions. The soon-to-be increasingly complex vector control landscape argues for increased vector surveillance to select the most appropriate vector control measures based on vector ecology and malaria epidemiology. The increasing cost (time and money) of randomized control trials (RCTs) also raises the question of whether vector-monitoring data can replace the RCT as the basis for policy recommendations on new interventions. Questions that vector data must address include "What is the status of vector surveillance in malaria national control programs?", "Do vector monitoring tools have the precision to guide programmatic decisions on deployment of vector control interventions?' Are vector data used today to guide programmatic decisions?", and "Are countries' vector control programs able to collect vector data and use it for decision-making?" Vector surveillance activities of >30 malaria endemic countries in Africa, Asia, the Pacific and the Americans were analysed against best practice recommendations. How vector data is being collected and used today and the limitations of the data argue for an invigoration and investment in the public health entomologist discipline. The need to utilize vector data requires us to elevate the role of the public health entomologist in the decision-making process, especially in countries seeking to eliminate malaria.

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PLASMODIUM VIVAX ERYTHROCYTE BINDING PROTEIN GENE COPY NUMBER, BUT NOT DUFFY BINDING PROTEIN, IS SIGNIFICANTLY HIGHER IN MALAGASY ISOLATES THAN IN CAMBODIAN ONES

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Among the five species of *Plasmodium* that infect humans, *P. vivax* is the most widespread, with billions of people at risk in nearly 100 different endemic countries in South America, Africa and Asia. *P. vivax* being responsible for the majority of malaria cases outside Africa, a blood stage vaccine would considerably alleviate the burden inflicted by this parasite. Invasion mechanisms of *P. vivax* are still unclear and the prevailing paradigm that makes the interaction between *Plasmodium vivax* Duffy binding protein (PvDBP) and Duffy antigen receptor for chemokines (DARC) essential for *P. vivax* invasion is questioned since this parasite has been observed in individuals lacking the Duffy antigen. The pathways by which the parasite is able to enter the Duffy negative reticulocytes are unknown but two possible mechanisms have been suggested in the literature: the amplification of the gene coding for PvDBP as well as the involvement of alternate proteins, in particular a newly described Erythrocyte Binding Protein, PvEBP, which is able to bind Duffy negative red blood cells. In this context, to further study these two hypothesis, we analyzed the genomic polymorphisms (copy number variation and sequence polymorphism) in the second exon of PvDBP and PvEBP in isolates from two different locations: Cambodia where the vast majority of people are Duffy positive and Madagascar, where an admixture of Duffy positive and Duffy negative individuals coexists. We also studied the location of polymorphic residues for these two genes by 3-D modeling. Our molecular epidemiology approach demonstrated that PvEBP was far more conserved than PvDBP and that the copy number variation of PvEBP was significantly different between isolates from Cambodia and Madagascar. Interestingly we observed no significant difference for the copy number variation of PvDBP. Those results further strengthen the possible role of PvEBP in the invasion of Duffy negative individuals.

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ADULT SEVERE MALARIA PATIENTS WITH ACUTE KIDNEY INJURY PRESENT A DISTINCT VAR PROFILE

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Malaria symptoms and mortality rates vary depending on patient age. In areas with lower malaria transmission intensity, such as India, the risk of renal failure, severe jaundice, pulmonary edema, and multi-organ failure increases with age. Despite the different disease spectrum and higher mortality rates in adults, the pathological mechanisms that lead to adult organ complications remain poorly understood. Acute kidney injury (AKI) is one of the most frequent complications in adult severe malaria and it is an independent predictor of mortality. Parasite sequestration in kidney peritubular endothelial cells has been documented in autopsy studies in both children and adults. However, the role that specific parasite binding phenotypes play in the development of AKI has never been studied. To address this question, we analyzed the parasite *var* profile in blood of Indian adults that presented severe malaria with kidney involvement. Patient serum creatinine levels directly correlated with a biomarker of parasite biomass. In addition, machine learning models revealed a divergence of parasite adhesion types in disease complications, with higher DC8 *var* transcripts in AKI patients and higher Group A *var* transcripts in patients with multi-organ failure. Both DC8 and Group A *var* genes encode EPCR binding activity, but Group A binding to endothelium is additionally mediated by ICAM-1. Our findings suggest that AKI is associated with the expansion of DC8 EPCR-binding parasites, and that an expansion of Group A-expressing parasites is present in patients with multi-organ complications. Thus, different parasites might contribute to the variable disease presentations observed in adult patients with severe malaria. This finding has important implications in the future development of malaria adjunctive therapies.

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IDENTIFICATION OF HOST IMMUNOLOGICAL AND *PLASMODIUM* INTRINSIC FACTORS INFLUENCING GAMETOCYTES INFECTIVITY TO *ANOPHELES* MOSQUITOES IN THE FIELD

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Sustainable malaria prevention and control measures have permitted a significant decrease in the disease burden in many endemic countries. However, the global agenda for malaria elimination and eradication may not be achieved without the development of transmission-blocking interventions. Malaria transmission to the mosquito relies on gametocytes infectivity for the continuation of the ife cycle and the spread of resistant parasites. Identification of biomarkers associated with gametocyte infectivity can help in the development of new therapeutic strategies. This study aimed to identify host and parasite biomarkers related to gametocyte infectivity in the field. From October to December 2017, a cross-sectional study was conducted in Faladje to enroll asymptomatic *Plasmodium ssp*. gametocyte carriers. Gametocyte carriage was assessed by light microscopy. Venous blood was collected for gametocytes purification, plasma collection and direct membrane feeding assay (DMFA) with both whole blood and RBC supplemented with AB serum. Mosquito's oocyte positivity was determined day-8 post feeding. Protein microarrays covering 250 *P. falciparum* antigens were probed with plasma from volunteers. Gametocyte's RNA and DNA were extracted for RNA and DNA sequencing. 70 volunteers were enrolled. For the 68 malaria positive samples used for DMFA, mosquito's infectivity rate was 28% (n=19). The number of oocyst positive mosquito and oocyst load per mosquito were significantly higher in RBC+AB serum DMFA for 73.7% (n= 14) of the infectious sample. Ab levels to 7 antigens were significantly higher in plasma from non-infectious gametocyte blood meals compared to those who were infectious. Candidate antigens included a *Plasmodium* Deoxyhypusine hydroxylase, four putative proteins highly expressed in gametocytes, ookinetes and/or sporozoites, and an ATG autophagic pathway associated protein. Dissecting factors influencing gametocytes infectivity will highlight biomarkers associated with malaria transmission and provide potential targets for transmission blocking strategies and new tools for malaria transmission screening.

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FERROPTOSIS-LIKE SIGNALING FACILITATES A POTENT INNATE DEFENSE AGAINST *PLASMODIUM* INFECTION

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The facets of host control during *Plasmodium* infection, and the mechanisms by which they alter susceptibility in the field, remains largely unknown. Surprisingly, conventional innate regulatory pathways are only minimally effective at eliminating parasites during the Liver Stage of infection. Ferroptosis, a recently described form of iron-dependent cell death that drives accumulation of reactive oxygen species and lipid peroxides, has not yet been shown to function as an innate immune response. We demonstrate that inducing ferroptosis with small molecules or by genetic perturbation of its negative regulators, GPX4 and SLC7a11, dramatically reduces survival of the Plasmodium Liver Stage in mice. Knockdown or knockout of NOX1 or knockdown of TFR1, which are required for ferroptosis, increases the number of Liver Stage parasites. Moreover, we demonstrate that blocking ferroptosis renders parasite-infected hepatocytes resistant to P53-mediated hepatocyte death. Interestingly, preliminary analysis indicates that individuals with differential susceptibility to malaria in Mali exhibit altered levels of ferroptosis-related genes. These findings suggest that the ferroptosis pathway might not only regulate malaria in a mouse model, but may also extend to regulating susceptibility to disease in humans. Our work also raises the possibility that ferroptosis operates as an axis of the innate immune system to defend against intracellular pathogens.

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IMPACT OF NATURALLY-ACQUIRED HEMOZOIN ON SEVERE MALARIAL ANEMIA AND ERYTHROPOIESIS AMONG CHILDREN RESIDENT IN *PLASMODIUM FALCIPARUM* HOLOENDEMIC REGION OF KENYA

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In holoendemic *Plasmodium falciparum* transmission areas, malaria manifests primarily as severe malarial anemia [SMA; hemoglobin (Hb)<5.0 g/dL] among children aged <48 months. Although our *in vitro* studies demonstrate that enhanced uptake of malarial pigment (hemozoin, *Pf*Hz) suppresses immune response genes and gene pathways, the impact of naturally-acquired P*f*Hz on SMA and erythropoietic responses are largely unexplored. Intraleukocytic P*f*Hz and inefficient erythropoiesis (reticulocyte production index; RPI<2.0) were determined among parasitized children (<36 mos.) stratified into SMA (n=280) and non-SMA (Hb≥5.0 g/dL; n=1,032). Pigmented monocyte (PCM) levels (%) were classified as follows: PCM [(-), no PCM], Low PCM (≤10%), Moderate PCM (>10<26.7%), and High PCM (≥26.7%). Demographic (age, admission temperature), hematological parameters, and parasitological indices (parasite density, geomean parasitemia) varied across PCM groups (P<0.001). Proportions of children presenting with SMA and insufficient erythropoiesis also varied across PCM groups (P<0.001). Binary logistic regression analyses, controlling for covariates, demonstrated that presence of PCMs increased susceptibility to SMA [High PCM, odds ratio (OR)=4.957 (95% CI=2.820-8.712), P<0.001; Moderate PCM, OR=3.906 (95% CI=2.299-6.635), P<0.001, and low PCM, OR=1.732 (95% CI=1.154-2.599), P=0.008]. Additional analysis showed that high PCM levels increased the odds of children presenting with RPI≥2.0 [OR=2.827 (95% CI=1.462-5.469), P=0.002]. No associations were observed between RPI≥2.0 and either moderate PCM [OR=1.484 (95% CI=0.721-3.053), P=0.283] or low PCM [OR=1.098 (95% CI=0.643-1.876), P=0.731]. Taken together, these results suggest increased that intramonocytic P*f*Hz is associated with enhanced susceptibility to SMA, an event that appears independent of its effect on erythropoiesis.

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NEUREGULIN-1 ATTENUATES EXPERIMENTAL CEREBRAL MALARIA PATHOGENESIS BY REGULATING ERBB4/AKT/STAT3 SIGNALING

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Human Cerebral Malaria (HCM) is a severe form of malaria characterized by sequestration of infected erythrocytes (IRBC) in brain microvessels, increased levels of circulating free heme and pro-inflammatory cytokines and chemokines, brain swelling, vascular dysfunction, coma and increased mortality. Utilizing an experimental cerebral malaria (ECM) model, we reported that NRG-1 played a cytoprotective role in ECM and that circulating levels were inversely correlated with ECM severity. Intravenous infusion of NRG-1 reduced ECM mortality in mice by promoting a robust anti-inflammatory response coupled with reduction in accumulation of IRBCs in microvessels and reduced tissue damage. In the current study, we explored a potential molecular mechanism by which NRG-1 regulates downstream pathways to improve/restore the integrity of the blood brain barrier (BBB) and attenuate ECM pathogenesis. We examined whether NRG-1 protects against CXCL10- and heme-induced apoptosis using human brain microvascular endothelial (hCMEC/D3) cells and the M059K neuroglial cells. hCMEC/D3 cells grown in a monolayer and a co-culture system with 30μM heme and NRG-1 (100ng/ml) were used to examine the role of NRG-1 on BBB integrity. Using the *in vivo* ECM model, we examined whether the reduction of mortality was associated with the activation of ErbB4 and AKT and inactivation of STAT3 signaling pathways. We found that NRG-1 protects against cell death/apoptosis of human brain microvascular endothelial cells and neroglial cells, the two major components of BBB. NRG-1 treatment improved heme-induced disruption of the *in vitro* BBB model consisting of hCMEC/D3 and human M059K cells. In the ECM murine model, NRG-1 treatment stimulated ErbB4 phosphorylation followed by activation of AKT and inactivation of STAT3, which attenuated ECM mortality. Our results indicate a potential pathway by which NRG-1 treatment maintains BBB integrity *in vitro,* attenuates ECM-induced tissue injury and reduces mortality. Furthermore, we postulate that augmenting NRG-1 during ECM therapy may be an effective adjunctive therapy to reduce CNS tissue injury of HCM.

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ANTIBODY TO *PLASMODIUM FALCIPARUM* VARIANT SURFACE ANTIGENS AND TRANSCRIPTION OF VAR GENES IN PAPUA NEW GUINEAN CHILDREN WITH SEVERE OR UNCOMPLICATED MALARIA

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The *Plasmodium falciparum* Erythrocyte Membrane Protein 1 family of proteins mediate sequestration of infected erythrocytes (IEs) in the deep vasculature, contributing to pathogenesis of falciparum malaria. Antibody responses to PfEMP1 proteins develop in convalescence from malaria, particularly against homologous isolates. 71 parasite isolates from children with severe (N=41) or uncomplicated malaria (N= 30) from Papua New Guinea were cultured in first *in vitro* cycle to trophozoite stage and magnet purified. Isolates were divided according to donor ABO

blood group, and incubated with matched homologous and heterologous acute and convalescent plasma. IgG antibodies to variant surface antigens (predominantly PfEMP1) were measured by flow cytometry. We extracted RNA from ring stage IEs and used quantitative real time PCR to assess transcription of 46 *var* genes in 40 isolates which grew in culture. Homologous boosting of antibody in convalescence was common. Among children with non-O blood groups, cluster analysis of antibody profiles showed that in comparison to children on presentation with severe malaria, there were broadly higher levels of antibody to IE surface antigens in convalescence from severe malaria and in acute or convalescent samples from uncomplicated malaria. By contrast, in children with blood group O, only convalescent plasma from severe malaria showed higher recognition of tested isolates. These differences may relate to the differential susceptibility of children with blood group O to severe malaria. Several *var* genes were upregulated in severe malaria including mostly *var* type A, but also some *var* types B/A, B and B/C, while none were upregulated in mild relative to severe malaria. Increased transcription, predominantly of *var* genes associated with severe malaria in Africa, was common in severe malaria in PNG. The relationship between antibody response and *var* gene profiles is currently being investigated and will be discussed.

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COORDINATED THAILAND MINISTRY OF PUBLIC HEALTH AND ROYAL THAI ARMY-US ARMY CIVILIAN-MILITARY RESPONSE TO A MALARIA OUTBREAK IN NORTHEAST THAILAND

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In April 2017, the Thailand Ministry of Public Health (MoPH) reported a malaria outbreak in Sisaket Province, along the Thai-Cambodian border. Between April and November 2017, 713 cases were reported: a five-fold increase from the same time period in 2016. A similar increase in cases (n=119 versus n=16) was found in a Royal Thai Army (RTA) cohort stationed in the Sisaket. The majority of cases were reported from three malaria-endemic districts: Khun Han (47%), Phu Sing (25%), and Kantharalak (24%). Cases were primarily *P. vivax* infections (76%), among males (88%) working in rubber plantations (63%) or as military and/or border police (15%). To stem the outbreak, the Sisaket Provincial Governor launched an Emergency Operations Center (EOC) including representatives of MoPH, RTA and US components of the Armed Forces Research Institute of Medical Sciences (AFRIMS), the University of California, San Francisco, forest service staff, local military personnel, border police, and local administrative organizations. The response focused on three main efforts derived from the MoPH's National Malaria Elimination Strategy: 1) proactive case detection; 2) a "1-3-7" approach, in which both civilian and military malaria cases were reported within 1 day, confirmed and investigated within 3 days and responded to within 7 days; and 3) analysis of genetic markers among *P. falciparum* cases to assess for drug resistance. A total of 6,299 people were screened by microscopy and PCR in 24 high-risk villages and military bases. All cases were promptly treated using supervised treatment. Confirmed cases were followed for up to 90 days, with microscopy smears and dried blood samples collected at each follow-up visit. In addition, 61 key informant interviews were conducted. To date, a total of 24 case investigations among RTA have been conducted. Analysis of the root causes of the outbreak, findings from outbreak response activities, and lessons learned in integrating civilian and military efforts will be presented. The Sisaket outbreak response demonstrates the necessity of civilian-military cooperation to support Thailand malaria elimination goals.

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ASSESSMENT OF RESIDUAL MALARIA TRANSMISSION IN LETPAN VILLAGE, SOUTHERN RAKHINE STATE, MYANMAR

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The PMI-supported Defeat Malaria Project continuously monitors malaria data in its target areas. A general pattern of declining malaria has been reported in recent years in project areas, with marked heterogeneity. In Letpan village (South Rakhine State), an increasing Annual Parasite Incidence (API) has been registered despite continuous community-based malaria control activities implemented since 2013. An assessment was performed in 2017 to identify underlying causes of this pattern and suggest appropriate responses. Staff reviewed epidemiological data, conducted a cross-sectional household survey, key informant interviews, and a parasitological survey. The village has 790 residents of Rakhine (85%) and Chin (15%) ethnicities. Approximately 30% Rakhine and most Chin are forest workers. Epidemiological data from 2014 to 2017 revealed an increasing API of 18.3, 15.5, 30.2 and 40.4, respectively. Receptivity analysis showed intrusion of river water creating small ponds, some water reservoirs during the rainy season, and nearby paddy fields. A village malaria worker was recruited in July 2013 and Annual Blood Examination Rates were over 10% from 2014 through 2017. Nearly 62% of villagers reported sleeping under an insecticide-treated net the previous night. From 5 to 9 PM, 97% of respondents reported staying outdoors, increasing exposure to mosquitoes bites. A parasitological survey of 232 people (29% of total population) conveniently sampled, conducted in August 2017, did not detect any malaria positive case by conventional RDT. A detailed review of malaria cases reported from October 2016 to September 2017 revealed that 30 out of 32 cases (94%) were forest workers and 4 were children under-5 who accompanied family members into the forest. Letpan's residual malaria transmission, despite good coverage of malaria services, is most likely due to the risk group of forest-goers infected outside the village, and Letpan has been classified as a residual non-active focus. Active surveillance of fever cases among returning forest goers, and intensified measures to prevent vector-human contacts among forest goers should be strengthened.

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CASE BASED MALARIA SURVEILLANCE SYSTEMS IN ELIMINATION SETTINGS OF ZANZIBAR

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Since 2012, ZAMEP has operated both a passive, facility-based and active case-based surveillance and response system for malaria. In 2017, ZAMEP enhanced the case-based system to capture additional information to permit the classification of individual malaria cases and their associated *Foci* according to WHO definitions. Our objectives were to describe the classification of malaria cases and the *Foci* investigated in Zanzibar. All health facilities report individual malaria cases to the central server in real time using mobile phone. Messages transmitted from the server alert the respective District Malaria Surveillance Officer, who obtains detailed clinical and epidemiological information about individual cases through the health facility that reported the case and during case follow-up and *Foci* investigations at household level. All household members receive testing and treatment (if positive). Malaria cases and *Foci* are classified by WHO definitions. From January 2017 to February 2018, a total of 774 cases were reported from 78 facilities, with 76 *Foci* investigated and classified. During case investigation, 68.9% cases were classified as imported, 27.4% cases as indigenous, 2.7% cases as introduced, 0.8% cases as relapsed, and 0.3% cases as induced. During *Foci* investigation, 55.2% *Foci* were classified as residual active and 44.8% *Foci* were classified as residual non-active. Geographically, 13 *Foci* were identified in Pemba, of which 46.2% residual active *Foci* were in Micheweni district and 53.8% in Chake chake district. In Unguja, 29 residual active *Foci* were identified; 62.1% *Foci* in North B, 20.7% *Foci* in West, and 17.2% *Foci* in Urban. The enhanced case-based surveillance system demonstrates that case and *Foci* investigations and classification are feasible in Zanzibar. Interventions focusing on imported cases, alongside other interventions for residual active *Foci* might reduce malaria transmission and morbidity, and accelerate malaria elimination efforts in Zanzibar.

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ACCESSING HARD-TO-REACH POPULATIONS: FOREST INTERVENTIONS INCREASE CASE DETECTION IN PURSAT PROVINCE, CAMBODIA

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In Cambodia, malaria risk is highest in forest or forest fringe areas, linked to the movement of people going into the forest. To better target high-risk groups working in these areas, USAID | PMI Cambodia Malaria Elimination Project (CMEP) supports the Cambodia National Malaria Program (CNM) to train mobile malaria workers (MMWs) where volunteers are selected from these at-risk groups to provide early diagnosis and treatment to mobile and migrant populations (MMPs). Starting in May 2017, a significant increase in malaria cases in Phnom Kravanh (PKV) and Krakor (KRK) operational districts (ODs) required CNM, CMEP and OD team members to analyze and identify health facilities, villages and hotspots for investigation and response. Based on case numbers, three villages were targeted. Interventions included hotspot investigation and response activities (public announcement, bed net top up, health education, mass screening and treatment). Screening results showed a 0.7% test positivity rate (TPR), with the largest village not identifying any positive cases. These findings suggested that village-based screening was missing the high-risk groups. CMEP and OD teams initiated expansion of MMW pro-active case detection (Pro-ACD) in the forest. Learning where malaria was contracted from ex-patients, 6 touch points (where forest goers interacted) and 22 worksites were identified with 18 volunteers selected and trained as MMWs. From Aug 2017-Feb 2018, 1,708 people were tested and 423 were confirmed and treated in PKV and KRK (TPR 25%). A high TPR (38%, of which 66% were *P. falciparum*) was found by MMWs in the forest in PKV from screening at touch points and work sites. In forest and forest fringe areas, mass screening in villages is not enough to reduce or stem the spread of malaria outbreaks. Expansion of services into hotspots through MMW Pro-ACD at touch points and worksites is effective in identifying reservoirs of malaria burden among MMPs, particularly forest goers/workers. A scale up of malaria services into the forest may improve the effectiveness of malaria programs. However, active multi-stakeholder collaboration and coordination is required.

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INDIVIDUAL MOVEMENT PATTERN ANALYSIS USING GPS TRACKERS TO FOREST WORKERS IN ACEH, INDONESIA

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Human movement is a known contributor to sustained malaria transmission in low endemic areas. Many methods have been explored to measure human mobility and its impact of movement to malaria transmission, for example, census data, travel diaries, mobile phone usage data and GPS trackers. Our study used commercially available GPS data trackers to identify specific potentially high-risk locations visited frequently by forest workers in the district of Aceh Besar and Aceh Jaya in Aceh Province, the western province of Indonesia. These districts previously reported the presence of zoonotic malaria species *Plasmodium knowlesi* among forest workers. Beginning in April 2017, we recruited forest workers who were recent malaria cases, as well as individuals from their peer networks, to be followed for a period of two months each. Following consent, individuals were asked to wear an "i-gotU" (i-gotU model GT100) GPS tracking device continually during all of their activities over a two month period; location data were captured every 15 minutes. As of April 2018, 62 individuals had been recruited into the study, 38 of whom completed the study or dropped out before the end of the 2 month data collection period. Data collection for an additional 24 individuals is ongoing and participant recruitment will continue through September 2018. To date, participants have contributed 1,691 days of GPS data collection with over 236,000 data points. More comprehensive analysis on the individual movement patterns to inform interventions that target for mobile forest workers will be presented.

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THE IMPACT OF MALARIA CONTROL INTERVENTIONS ON MALARIA BURDEN IN THE HISTORICALLY HIGH MALARIA TRANSMISSION DISTRICT OF TORORO, UGANDA: A META-ANALYSES OF LONGITUDINAL STUDIES FROM 2007 TO 2017

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There is limited evidence of reductions in malaria burden in high transmission areas of Africa. Understanding the impacts of malaria control interventions is critical to guide strategies for malaria control in hyper-endemic regions. Over a 10-year period (2007-2017), we measured the impact of various malaria control interventions in cohorts of children enrolled in 7 clinical trials and observational studies in Tororo, Uganda, a historically high transmission area with a baseline annual EIR of 310. Interventions included individually assigned chemoprevention regimens, population level universal LLIN distribution in November 2013 and repeated rounds of IRS starting in December 2014. All children received LLIN at enrollment and standardized approaches were used to follow cohort participants including prompt treatment for malaria with ACTs. We measured the incidence of malaria using passive surveillance and parasite prevalence by microscopy at regularly scheduled routine visits. Meta-analyses of a combined data set were performed to estimate the protective efficacy (PE) of the various control interventions. A total of 2148 children were followed over 5136 person years resulting in16698 treatments for malaria, including only 116 (0.7%) treatments for severe malaria and 2 deaths with malaria. Considering observation time between 0.5-2 years of age, in the absence of chemoprevention and before the implementation of IRS (reference group), the incidence of malaria was 5.0 episodes per person year (PPY) and parasite prevalence was 15.2%. The most dramatic declines followed the implementation of IRS (malaria incidence 0.47 episodes PPY, 90% PE; parasite prevalence 3.9%, 74% PE) and especilally among children receiving chemoprevention with monthly DP (malaria incidence 0.03 episodes PPY, 99.5% PE; parasite prevalence 0.9%, 94% PE). In our cohorts, where we provided LLINs and prompt treatment with ACTs, the risks of severe malaria and death were extremely low. In a historically high malaria burden area of Uganda, the combination of IRS and chemoprevention with monthly DP has almost eliminated malaria in young children.

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APPLICATION OF PARASITE GENETIC RELATEDNESS TO IDENTIFY TRANSMISSION PATTERNS FOR MALARIA ELIMINATION EFFORTS IN RICHARD TOLL, SENEGAL

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Senegal has three distinct malaria transmission zones ranging from relatively high incidence rates in the South (> 250/1000) to very low incidence rates (< 2/1000) in the North. The majority (~85%) of clinical malaria cases in the northern Senegal district of Richard Toll are found among individuals with a travel history and are therefore classified as representing imported infection, as previously reported. We compared genetic relatedness between parasite infections and travel history to test the hypothesis that local infections occur in Richard Toll. Previous studies using genetic relatedness reveal increased proportions of highly related parasites in low transmission settings, with evidence of clonal parasites that persist across multiple transmission seasons[2]. We successfully genotyped 646 RDT-positive samples collected in Richard Toll district between 2012 and 2015 using a molecular barcode tool that assesses 24-single nucleotide polymorphisms. The majority (471/646 = 73%) of these represented monogenomic infections (expected with low transmission intensity), with increased polygenomic infections among those with travel history (p = 0.03). Consistent with local transmission, we detected eight persistent clonal lineages in multiple years from 2012 and 2015, mainly among individuals with no travel history. We also observed clusters of genetically identical infections within households with no travel history (p = 0.03). Despite limited data from across Senegal, we were able to identify (6%) parasites that were identical or closely related to parasites being actively transmitted in Dakar, Thies, Kaolack, and Touba. These results demonstrate connectivity with other parts of Senegal and receptivity for transmission within Richard Toll. These findings reveal indigenous transmission is likely in this very low prevalence region, and underscores the utility of genomic data to resolve malaria transmission patterns to stratify interventions for successful malaria elimination.

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IMMUNITY AND MEMORY AGAINST MALARIA: AN ATLAS OF THE MOSQUITO IMMUNE SYSTEM AT SINGLE CELL RESOLUTION

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Malaria is a deadly, worldwide disease. Every year the *Plasmodium* parasite is responsible for an estimated 214 million cases of malaria and over four hundred thousand deaths. In order to infect humans, *Plasmodium* parasites must complete their life-cycle in mosquitoes. The mosquito in turn relies on both humoral and cellular innate immune divisions to defeat invading pathogens, coordinated by the hemocytes. Hemocytes are the equivalent of vertebrate innate white blood cells, circulating in the hemolymph within the insects' body cavity. However, basic hemocyte cell biology and immunological effector mechanisms are largely unknown, mainly due to the small number of immune cells in each mosquito. Developing an in depth understanding of the mosquito immune system will help clarify malaria transmission dynamics and prove useful in developing novel vector control and transmission-blocking strategies. Here we profile 5,292 individual *Anopheles* mosquito hemocytes in baseline, blood-fed and malaria-infected conditions with single-cell RNAseq. We combine scRNA-seq data with low input bulk RNAseq, FISH, electron and confocal microscopy to identify previously unknown cell types and uncover their lineages and transcriptional signatures. Among the novel mosquito immune cells discovered are specialised immune-regulatory, phagocytic, and anti-microbial peptides secreting cell subtypes. We also report shifts in the activation level and composition of the immune cells repertoire of mosquitoes infected with malaria parasites. Analysis shows the existence of three polarized immunological states: suppressed, primed, and malaria-activated. We also report networks of immune regulation centered around SRPNs proteins specific for each immune cell subtype. This is the first comprehensive study of an invertebrate innate immune system at single-cell resolution. Far from being static, the mosquito immune system is revealing a surprising degree of complexity that we are only beginning to explore.

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THE FEMALE REPRODUCTIVE PROTEIN (MATING-INDUCED STIMULATOR OF OOGENESIS, MISO) REGULATES TOLERANCE TO *PLASMODIUM FALCIPARUM* INFECTION IN *ANOPHELES GAMBIAE*

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In *Anopheles gambiae*, male-female molecular interactions following mating are important determinants of female reproductive fitness - a key component of vectorial capacity. Sexual transfer of the steroid hormone 20-hydroxyecdysone (20E) induces a large-scale transcriptional response that permanently reshapes female physiology and also impacts blood feeding-induced processes through interaction with the female protein Mating-Induced Stimulator of Oogenesis (MISO), increasing expression of lipid transporters and boosting fecundity. Furthermore, the factors modulated by the 20E-MISO interaction post-mating are the same that *Plasmodium falciparum* parasites utilize for their own development. Here, we present data demonstrating that in *A. gambiae,* 20E transferred during mating confers female tolerance to *P. falciparum* infection through *MISO*, as silencing of this gene leads to decreased egg production in infected females relative to uninfected and infected controls. Immunofluorescence assays, qRT-PCR, and Western blotting reveal that MISO is produced in the reproductive tissues, including the ovaries and atria, as well as in the trachea oxygenating the ovaries. Correlation network and transcriptional analysis suggest MISO is involved in conserved molting and oxygen-sensing signaling pathways, providing new insight into molecular processes important for egg development in *A. gambiae*. Importantly, species that lack sexually-transferred 20E, including the Central American vector *A. albimanus*, suffer a fitness cost to *P. falciparum* infection, suggesting that divergent evolutionary trajectories of female post-mating physiology across anopheline species may have differentially affected their ability to support *Plasmodium* transmission. The results of these studies elucidate new molecular pathways critical to *A. gambiae* vectorial capacity that may have been shaped by millions of years of pressure from *Plasmodium* parasites.

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GLOBAL MAPPING OF MIRNA-MRNA INTERACTIONS IN THE MALARIA MOSQUITO *ANOPHELES GAMBIAE*

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Mosquito-borne infectious diseases are resurging and emerging threats to human health. Female mosquitoes transmit the causative pathogens while taking blood from vertebrates to acquire nutrition for egg production. Decoding the gene regulation that governs mosquito reproduction and innate immunity will potentially open new avenues to develop effective strategies for disease control. Recent studies have suggested that mosquito miRNAs contribute to the tightly controlled stage-specific gene expression before and after blood feeding. However, the molecular targets of these miRNAs have not been explicitly identified in mosquitoes. The lack of widely applied rules for functional miRNA-target pairing makes it difficult to accurately predict the miRNA targets. Moreover, an increasing body of evidence from other animals indicates that many miRNA-mRNA interactions are context-dependent. In this study, we used an experimental approach to perform a global analysis of the miRNA-mRNA interaction network in *Anopheles gambiae*. By ligating miRNA and target mRNA in the Ago1-RNA complexes using the CLEAR-CLIP method, we uncovered tens of thousands of miRNA-mRNA interactions in the mosquito. After identifying the common motifs shared by the targets of individual miRNAs, we were able to determine how each miRNA recognized their mRNA targets. The interactions comprised canonical seed-based pairing with diverse patterns of auxiliary pairing and seedless targeting. Interestingly, some miRNAs (e.g. miR-276-3p) have shown to use various regions to recognize their targets at different developmental stages. Comparison of the miRNA-mRNA interactomes at multiple time points revealed that the dynamic miRNA-mRNA interactions coordinate the gene regulation in a stage-specific manner. This study identified a subset of miRNAs that are implicated in metabolism, energy homeostasis, and egg maturation, shedding light on future functional studies.

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NEW INSIGHTS INTO THE AMMONIA METABOLISM OF BLOOD-FED *AEDES AEGYPTI* MOSQUITOES

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Mosquitoes are responsible for the transmission of serious diseases including malaria, dengue hemorrhagic fever, chikungunya fever, and Zika virus. Many of these infections prove fatal. To resolve this devastating worldwide problem, we strive to identify new metabolic targets or regulators to implement more effective strategies for vector control. Ammonia, one of the metabolic by-products of blood meal digestion, cannot be stored inside cells and therefore nitrogen metabolism in blood-fed mosquitoes must be tightly regulated to prevent ammonia toxicity. Because the majority of the carbon skeletons of amino acids (AAs) derived from protein meal digestion are completely oxidized for energy production, we hypothesized that the carbon skeleton of glucose supplies the keto acids necessary for the synthesis of AAs and other compounds during ammonia detoxification. To test that hypothesis, we fed *Aedes aegypti* females with a blood meal supplemented with [1,2-$^{13}C_2$]-glucose, and monitored the kinetics of 43 compounds and 272 isotopologs during a time course. We carried out the experiments using low- and high-resolution liquid chromatography mass spectrometry (LC/MS) techniques. Interestingly, only 11 metabolites exhibited [^{13}C]-atom incorporation from [1,2-$^{13}C_2$]-glucose. We discovered that the carbon skeleton of glucose supports ammonia detoxification through the synthesis of specific AAs, AA derivatives, and organic acids including uric acid. We also found that the synthesis of these compounds occurs through a complex interplay between glycolysis, pentose phosphate pathway, decarboxylation of pyruvate, Krebs cycle, and pathways involved in ammonia fixation, assimilation, and excretion. In summary, we discovered a metabolic link at the carbon atomic level in ammonia metabolism of blood-fed *Aedes aegypti*. The mass spectrometry techniques optimized in this study can be applied to investigate the metabolic regulation of nitrogen and carbon metabolism in mosquitoes and in other vectors of diseases, and ultimately could lead to the discovery of metabolic targets or regulators for vector control.

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THE ROLE OF BIOFILM FORMATION IN THE MOSQUITOCIDAL ACTIVITY OF *CHROMOBACTERIUM* SPECIES FROM PANAMA

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In the face of increasing resistance in *Anopheles* mosquitoes to our frontline insecticides, new tools are desperately needed for malaria vector control. We have isolated a *Chromobacterium* species from Panama

*(C.*sp_P*)* that possesses antiparasitic, antiviral, and entomopathogenic properties. We investigated the adulticidal activity of *Csp_P* to female *Anopheles* mosquitoes in order to identify the mechanism of *C.*sp_P's entomopathogenicity as well as to evaluate its viability as the basis for a new vector control tool. *C.*sp_P was cultured for 72 hours at 30°C in either planktonic (shaking) or biofilm (stationary) conditions. We observed differential mortality patterns in *An. gambiae* treated with biofilm culture compared with those treated with planktonic culture, with the biofilm causing faster mortality in a higher proportion of mosquitoes. This indicated to us that the formation of a biofilm may be related to the entomopathogenic activity of members of the *Chromobacterium* genus. We investigated the effects of different biofilm inhibitors on the adulticidal activity of *C.*sp_P as well as *Chromobacterium violaceum*, which forms a more identifiable biofilm through its production of purple violacein pigment. Adulticidal activity of these bacteria appeared related to both biofilm formation and the number of live bacteria in the biofilm culture preparation. Many *Chromobacterium* species proteins are only expressed under quorum-sensing conditions, such as chitinases, which could be involved in lethal damage to the mosquito after ingestion. Ongoing investigation addresses whether or not *C.*sp_P and *C. violaceum* are capable of forming a biofilm within the gut of the mosquito when fed at high enough concentrations, and what factors are produced by quorum-sensing *Chromobacterium* that lead to mosquito death, and therefore could be leveraged into tools for reducing malaria burden in endemic regions.

1458

NADPH IS CRITICALLY NEEDED FOR FECUNDITY AND INSECTICIDE DETOXIFICATION IN *ANOPHELES GAMBIAE*

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Redox reactions are ubiquitously involved in various biochemcal activities. It is vital to maintain redox homeostasis in response to the fluctuation of redox shift in various biological contexts. NADPH-dependent reducing capacity is one of the key factors contributing to the redox homeostasis. To understand the redox capacity and its impact on mosquito fecundity and susceptibility to insecticides in *Anopheles gambiae*, we examined the dynamics of oxidative state via induction by paraquat (PQ) and the inhibition of NADPH regeneration by 6-aminonicotinamide (6AN). In naïve conditions, inherent oxidative state varies between individuals, as measured by GSSG/GSH ratio. A high GSSG/GSH ratio was negatively correlated with fecundity. Both PQ and 6AN feeding increased GSSG/GSH ratio and elevated protein carbonylation, a marker of oxidative damage of proteins. Both pro-oxidants lowered egg production. Co-feeding the pro-oxidants with antioxidant lycopene attenuated the adverse effects on fecundity, implying that oxidative stress was the cause of this phenotype. Pre-feeding with 6AN or co-application with PQ resensitizes DDT resistant mosquitoes to insecticides. These results indicate that redox state is delicate in mosquitoes, manipulation of NADPH pool may adversely affect fecundity and insecticide detoxification capacity. Pentose phosphate pathway (PPP) is a major source for NADPH regeneration. Genetic variations in the pathway genes may affect the functionality. Ribose-5-phosphate isomerase (RPI) isomerizes ribulose-5-phophate to ribose-5-phosphate in the PPP. We identified an Arg24Trp variant in the RPI gene in wild *An. gam*biae populations. Interestingly, the variant is overrepresented in kdr positive populations. Further studies are required to gain further understanding of the redox capacity and insecticide detoxification.

1459

IRON TRANSPORTERS AND THEIR ROLES IN *AEDES AEGYPTI*

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Aedes aegypti is the major vector of viral pathogens, such as dengue, Zika and chikungunya. Despite their impacts on public health, only one vaccine against dengue, whose utility remains to be established, is currently available for prevention. Therefore, to control the transmission of these viruses, intervention of mosquito vectors is one of the most effective strategies. We focused on mosquito's blood iron utilization since blood contains potentially fatal amount of iron along with its high protein content that they use for reproduction. Using our newly developed cell-based screen, we identified three genes as putative iron transporters from ZIP and ZnT families, whose members were previously known to transport iron or divalent metals. Among these, AaeZIP13, an ortholog of *Drosophila* iron transporter dZIP13, was prominently up-regulated after blood meal. RNAi gene silencing of AaeZIP13 delayed transport of iron out of the midgut. Manipulation of these genes and other iron transporters may disrupt the life cycle of the vector mosquitoes and reduce transmission of the viruses.

1460

PIGGYBAC-MEDIATED STABLE INTEGRATIVE TRANSFECTION OF THE HUMAN FILARIAL PARASITE *BRUGIA MALAYI*

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The human filarial parasites cause diseases that are among the most important causes of morbidity in the developing world. The elimination programs targeting these infections rely on a limited number of drugs, making the identification of new chemotherapeutic agents a high priority. The study of these parasites has lagged due to the lack of reverse genetic methods. The slow progress in the development of reverse genetics in filaria can be attributed to the biology of these organisms, which are obligate parasites that cannot be cultured outside of their hosts. Here, we report the development of a novel co-culture method that results in developmentally competent infective larvae of one of the human filaria (*Brugia malayi*) and describe a method to efficiently transfect the larval stages of this parasite. We describe the production of constructs that result in stable integrative transfection using the *piggyBac* transposon system and a secreted luciferase that can be used as a selectable marker to identify transgenic parasites. We describe the production and use of dual reporter plasmids containing both a secreted luciferase selectable marker and fluorescent protein reporters that will be useful to study temporal and spatial patterns of gene expression. The methods and constructs reported here will permit the efficient production of transgenic filarial parasite lines, allowing reverse genetic technologies to be applied to the human filarial parasites for the first time.

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CONTROLLED HUMAN INFECTION WITH SINGLE-SEX *SCHISTOSOMA MANSONI* CERCARIAE

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Controlled human infections (CHI) provide a core platform to accelerate the development of novel drugs and vaccines for infectious diseases and reduce the risk of failure in downstream clinical development. For schistosomiasis, no such model exists. To develop a CHI model for schistosomiasis we have taken the important conceptual step to produce single-sex *Schistosoma mansoni* cercariae according to regulatory requirements for human use. Single-sex worms cannot deposit eggs and consequently pathology is avoided when infecting healthy volunteers. We now report the final data of a dose-escalating clinical trial infecting 17 healthy volunteers to assess the safety, tolerability and infectivity male *Schistosoma mansoni* cercariae in a controlled human infection model. We exposed groups of three to eight volunteers each to 10, 20 and 30 male *Schistosoma mansoni* cercariae and present the safety data of these groups. No serious adverse events occurred. Infection with 10 cercariae was well tolerated with mild adverse events mainly related to the skin penetration of the parasites. Infection with 20 or 30 cercariae was associated with Katayama syndrome in two volunteers. Serum circulating anodic antigen (CAA) levels were measured in weekly samples and peaked at 6-8 weeks following infection. All volunteers were treated with 40mg/kg praziquantel and CAA levels were followed over time. In conclusion, we have established a male *Schistosoma mansoni* controlled human infection model. We show that a limited number of volunteers will suffer from Katayama syndrome after infection with 20-30 male *Schistosoma mansoni* cercariae. We conclude that this model is safe and leads to detectable CAA levels in serum which can be followed over time. Future studies will focus on establishing a female controlled infection model as well as performing reinfection studies.

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SCHISTOSOMES DO NOT AND CAN NOT OXIDIZE FATTY ACIDS

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Blood flukes of the genus *Schistosoma* cause schistosomiasis that affects over 200 million people in (sub)tropical areas. Recently, fatty acid oxidation was suggested to be an attractive target for drug development, as inhibitor studies suggested that fatty acid oxidation was essential for egg production by female schistosomes. This observation was surprising as the energy metabolism of adult schistosomes was always thought to be strictly dependent on carbohydrates and glycolysis, as adult schistosomes are known to mainly degrade external glucose to lactate. To address these opposing reports on the energy metabolism of adult schistosomes, we performed a comprehensive metabolic study using [^{14}C]-labelled fatty acids. These experiments demonstrated that adults, eggs and miracidia do not oxidize fatty acids, as [^{14}C]-labelled carbon dioxide was never produced. Detailed reanalysis of the *S. mansoni* genome revealed that the postulated genes for fatty acid catabolism (beta-oxidation) were incorrectly annotated. Our new analysis revealed that schistosomes actually lack the capacity to oxidize fatty acids, as the genes for the corresponding enzymes are not present in their genome. Therefore, they cannot oxidize fatty acids in any life-cycle stage. However, uptake and metabolism of fatty acids is indeed crucial for egg production, which fits with the earlier observations that fatty acid starvation and inhibition of fatty acid processing blocks egg production. The adult female schistosome needs to take up and handle large quantities of fatty acids, because the secreted immature eggs comprise a large amount of fatty acids that are present as triacylglycerol in lipid droplets. These stored fatty acids are subsequently used by the developing larvae for anabolic processes, such as phospholipid biosynthesis, required for membrane synthesis in the growing miracidium. In conclusion, fatty acids are essential for adult schistosomes for anabolic processes but our results show that fatty acids are not used for ATP production by beta-oxidation as schistosomes do not and cannot oxidize fatty acids.

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PYRUVATE IS ESSENTIAL FOR *WOLBACHIA-BRUGIA MALAYI* SYMBIOSIS

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Human lymphatic filariasis is caused by the parasites *Wuchereria bancrofti, Brugia malayi* and is the second leading cause of global disability, affecting more than 120 million people worldwide. *B. malayi* has evolved a mutualistic association with *Wolbachia* (*w*Bm), its obligate intracellular bacteria. *Wolbachia* are crucial for worm fertility and viability, and have been identified as drug targets for new anti-filarial treatments. While the bacteria and parasite are known to share essential metabolic and biosynthetic processes (glycolysis, nucleotide biosynthesis, iron metabolism), the involvement of the glycolytic pathway in the bacteria-host relationship hasn't yet been determined. The *w*Bm genome is missing two key glycolytic enzymes, and potentially can't convert glucose to pyruvate independently. We have shown that *B. malayi* glycolytic enzymes are anchored to the surface of bacteria, indicating that host glycolysis provides pyruvate to the endosymbiont. In this study, we confirmed that pyruvate is essential for *w*Bm and that glycolysis performed by *B. malayi* is likely a major source of the pyruvate. Incubation of *B. malayi* in medium containing a high amount of pyruvate increased the *w*Bm loads in worms. Moreover, the bacterial genes responsible for encoding enzymes that utilize pyruvate in bacterial metabolic reactions (gluconeogenesis (wBm0209), TCA cycle (wBm0207)) were over expressed by more than 2-fold during pyruvate treatment. Conversely, specific inhibition of the host glycolytic enzymes resulted in a decrease in the *w*Bm load and a reduction in worm fertility and viability. The fitness of worms and the number of *w*Bm could be restored by supplementing the treated worms with extra pyruvate in the culture medium. We discuss whether other intracellular sources of host pyruvate, such as lactate, could be used by the endosymbiont as an alternative source of pyruvate. We hypothesize that the local clustering of host glycolytic enzymes together with LDH at the surface of *w*Bm helps to regulate the local concentration of glycolytic products, including pyruvate, to be used by the endosymbiont as an energy source or for gluconeogenesis.

1464

EXTRACELLULAR VESICLES RELEASED FROM FILARIAL PARASITES ARE ENRICHED IN MTOR REGULATORY MICRORNAS

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We have previously shown that the microfilarial (mf) stage of *Brugia malayi* can inhibit the mammalian target of rapamycin (mTOR; a conserved serine/threonine kinase critical for immune regulation and cellular growth) in human dendritic cells (DC) and we have proposed that this mTOR inhibition is associated with the DC dysfunction seen in filarial infections. Because extracellular vesicles (EV) contain many proteins and nucleic acids such as microRNAs (miRNAs) that affect a variety of cellular pathways, we hypothesized that EV secreted from mf are enriched in miRNAs that target and downregulate the mTOR pathway in human DC. Thus, EV, purified from mf of *Brugia malayi* and confirmed by morphology and size through electron microscopy and Nanosight analysis, were assessed by miRNA microarrays and shown to be enriched (>2fold, p-value<0.05, FDR<0.1) for miR100, miR71, miR34, miR7, and let-7. After confirming their presence in EV using qPCR for these miRNA targets, web-based target predictions (using MIRPathv3, TarBAse and MicroT-CD) demonstrated that miR100 and let-7 targeted mTOR and its downstream regulatory protein 4E-BP1 respectively. After confirming the internalization of mf-derived EV by DC using confocal microscopy, we were able to demonstrate through western blotting, that mf EV downregulate the phosphorylation of 4E-BP1 in human DC to the same degree that rapamycin (a known mTOR inhibitor) does. Our data collectively suggest that mf EV are enriched in miRNAs that regulate mTOR; studies with miRNA inhibitors in mf EV-exposed human DC are ongoing.

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USING METABOLIC NETWORKS TO CHARACTERIZE THE SYMBIOSIS BETWEEN *WOLBACHIA* AND FILARIAL NEMATODES, AND IDENTIFY NOVEL DRUG TARGETS

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Filarial nematodes represent one of the leading causes of disability in the developing world. Many filarial worm species, including *Brugia malayi*, one of the causative agents of lymphatic filariasis, have an obligate endosymbiotic relationship with the alpha-proteobacteria *Wolbachia*. To better understand the molecular interplay between these two organisms, we profiled the transcriptomes of *B. malayi* and *Wolbachia* across the life cycle of the parasite using dual RNA-seq. This allowed us to pinpoint functional pathways that are putatively involved in this essential symbiotic relationship provided by the co-expression of nematode and bacterial genes. We are currently focusing on molting of the worm from L3 to L4, the molt which marks the establishment of infection in the human host and is characterized by a large expansion of the *Wolbachia* population. We have profiled the co-expressed pathways associated with molting as well as how those pathways are affected by either blocking molting of the worm or blocking bacterial expansion. In parallel, we used these transcriptomic data in combination with genomic data to characterize the endosymbiotic relationship at the metabolic level using Flux Balance Analysis to identify choke points that could be exploited for therapy. To better inform our metabolic model, we have also profiled the metabolome of both *Brugia malayi* and *Wolbachia* over the life cycle of the worm. By creating a draft metabolic network for *B. malayi* and *Wolbachia*, and using *in silico* knockouts, we have identified pathways that are possibly necessary for development and fitness and examined how these pathways are influenced by the presence of *Wolbachia*. These predictions are now being tested using RNAi and small molecule inhibitors in order to validate those that can be further developed as novel drug targets.

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TRIATOMINE VECTORS, *TRYPANOSOMA CRUZI* STRAINS, AND CARDIAC ABNORMALITIES ASSOCIATED WITH NATURALLY-INFECTED DOGS ACROSS THE US

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In the southern US, triatomine vectors maintain *Trypanosoma cruzi* in sylvatic cycles with occasional spillover to humans. Infection with *T. cruzi* may be asymptomatic or lead to heart disease and death in humans and animals, with no identified prognostic markers. We analyzed the relationships between markers of exposure, circulating parasite infection, strain type, and cardiac abnormalities in populations of naturally-infected dogs. Additionally, we collected triatomine vectors from the canine's environment. Given that dogs can serve as sentinels of human risk, knowledge of locally-circulating strains is critical from a one health perspective. The Department of Homeland Security trains thousands of working dogs to provide security functions nationwide; the high level of outdoor exposure of these dogs makes them an ideal study system for vector-borne diseases. In 2015-17, we sampled 1,660 working dogs from 43 states using indirect fluorescent antibody tests and immunochromatographic assays to detect an overall anti-*T. cruzi* antibody prevalence of 7.3% (CI: 6.1-8.6%). RT-PCR detected parasite DNA in three canine blood samples (0.2%), including strain type TcI and TcIV. Two species of triatomine (n=75) collected from canine environments showed 49.3% infection prevalence (16:8:6 ratio of TcI:TcIV:mixed TcI/TcIV), and molecular bloodmeal analysis (n=13) revealed vector feeding on dogs, wildlife, and humans. We applied 24-hour, ambulatory ECG monitors to 24 *T. cruzi*-infected and 18 uninfected dogs and measured serum concentrations of cardiac Troponin I (cTnI), a biomarker of cardiac injury. We found that 75% of seropositive and 5.6% of seronegative dogs had one or more ECG abnormalities (p<0.001). Further, the average cTnI levels of seropositive dogs (0.071 ng/mL) was significantly higher than those of the seronegative dogs (0.017 ng/mL, p=0.015), indicating that cardiac injury was more severe in positive dogs. Understanding the epidemiology of *T. cruzi* infection could not only assist canine health, but also assist in protecting human health.

1467

GENDER BIAS IN VISCERAL LEISHMANIASIS: NATURE OR NURTURE?

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Visceral leishmaniasis (VL) is a parasitic disease which is fatal if not treated timely. Gender bias leading to unfavorable clinical outcomes in women has been documented earlier in Bangladesh. Barriers to access health care for women are also a factor in India and Nepal, where a majority of patients that are diagnosed with VL are male. However, our hypothesis is that this gender difference is not totally explained by discrimination of women but also attributable to biological factors that make males more vulnerable to VL. We triangulated multiple data sources to compute VL incidence rates by gender in the region. First, we analyzed the official VL

case data collected through the government reporting system of India and Nepal between 2014-2017. Secondly, we exploited population-based data sets obtained in two longitudinal studies (1. Kalanet and 2. Health & Demographic Surveillance System in Bihar, India). Both data sets are based on an exhaustive household census and include data on seroconversion in antibody tests to *L.donovani* as well as VL incidence based on active house-to-house surveys, minimizing hence the gender difference in access to diagnosis and underreporting. Routine health system data show that men have an overall VL incidence rate which is almost twice that of women (female/male Incidence Rate Ratio (IRR) is 0.67 in India and 0.48 in Nepal). This difference becomes progressively more important after the age of 15 years and further increases with age. In the population-based cohort data VL incidence rates for women are 40% lower than those of men (IRR 0.57 after controlling for age group and study site – 95% CI 0.40-0.81). This study shows that the gender differences observed in VL case load cannot be explained entirely through a gender difference in access to health care alone, as in the demographic surveillance site underreporting was not an issue. Differences in exposure could partly explain findings (analysis in progress). However, the increasing gender difference in VL incidence after the age of puberty seems to suggest a major role for other biological factors such as sex hormones in the development of this disease.

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QRS SCORE DISTRIBUTION AMONG CHAGAS CARDIOMYOPATHY PATIENTS OF VARIOUS SEVERITIES

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Chagas is a neglected tropical disease caused by the parasite *Trypanosoma cruzi,* and is responsible for a large amount of morbidity and mortality in Latin America. Chagas cardiomyopathy is the most severe effect of Chagas disease and is seen in 20-30% of infections. Early diagnosis and treatment have been shown to reduce morbidity and mortality. Previous studies have shown a relationship, in Chagas patients, between cardiac severity and their QRS score, determining myocardial scar size however, this distribution has yet to be compared to that of non-Chagas. We conducted a cross-sectional analysis from a cohort study, in a public hospital in Santa Cruz, Bolivia. Participants who tested positive by at least two assays were considered to have Chagas. QRS scores were determined for each subject by measuring amplitude and duration of Q, R and S waves on the EKG reading. Subjects were classified by cardiomyopathy progression (Stages A, B, C, or D) based on their results on an echocardiogram. The population consisted of 1071 patients, where the prevalence of Chagas was 89%, mostly women. Cardiac severity distribution, in the entire population, shows that stages A and B are more prevalent. The QRS score median in Chagas patients is 2.6 which was not statistically different from non-Chagas patients. No significant differences were observed between the median QRS scores in the non-Chagas patients, within any specific cardiomyopathy severity stage; however, within the Chagas patients, QRS scores increased with progression of cardiomyopathy severity, with significant differences occurring between Stage A and Stages B, C, and D, as well as between Stage B and Stages C and D. Interestingly, QRS score across cardiac disease severity in Chagas patients differs from non-Chagas patients. The median QRS scores increases in Chagas patients by one point across each stage compared to non-Chagas patients where the median scores increased by one point only between A to B. Since QRS score can be statistically different across cardiac severity, it could be used as an inexpensive method of diagnosis in lower income countries where access to echocardiograms is limited.

1469

IGG SUBCLASSES AND CONGENITAL TRANSMISSION OF CHAGAS DISEASE

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Chagas disease is a neglected tropical disease caused by *Trypanosoma cruzi*. Tropical areas with high poverty have higher rates of *T. cruzi* infection. Bolivia has the highest rates of Chagas disease in the world. The mechanism of vertical transmission is poorly understood. In this study, we evaluated IgG subclasses in Chagas-seropositive population in order to find an associated factor that helps us to predict the congenital transmission of Chagas disease. A case-control study was conducted in a public hospital in Santa Cruz, Bolivia. A total of 89 Chagas-positive, pregnant women at day of birth were included. Among these, the case group included 30 mothers who transmitted Chagas to their newborn and the control group included 59 mothers who did not transmit Chagas. ELISA was standardized in order to evaluate Chagas-specific IgG1, IgG2a, IgG3 and IgG 4 levels. Quantitative PCR results and demographic factors were also measured. IgG1 levels were 2.27 times higher in the mothers who transmit Chagas disease ($P < 0.01$), 44% higher values of IgG2a involved in congenital transmission ($P < 0.01$), 7.89 times higher in parasitemia mothers ($P < 0.01$), and 79% lower in mothers who had recently received a blood transfusion ($P = 0.05$). However, IgG3 was not associated with congenital transmission and IgG4 was unmeasurable. We therefore demonstrate that IgG subclasses and parasitic load are important factors associated with the congenital transmission of Chagas disease and thus has the potential for future use in screening for mothers at high risk for transmission of Chagas to their newborn.

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COMPARISON OF TWO APPROACHES TO ACTIVE CASE FINDING OF SLEEPING SICKNESS IN TWO DISTRICTS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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As the number of Human African Trypanosomiasis (HAT) cases is steadily declining over the past decade through intensive active screening campaigns in endemic areas, the elimination as a public health problem of this deadly disease now appears within reach. In the Democratic Republic of the Congo (DRC), several endemic HAT pockets remain though, where the elimination target has not been achieved yet. As elimination efforts are stepped up, reaching those most at risk with the screening program becomes increasingly important. In two health districts in DRC, we have compared the uptake of a traditional approach of mass screening (truck-based teams) with an innovative approach of door-to-door screening with so-called 'mini teams.' Data was captured through a mobile application. We recorded demographic data as well as results of diagnostic tests. We analyzed the sex- and age distribution of people screened in each approach and compared these "population pyramids" to the age and gender distribution of HAT cases recorded

by the national HAT Control Program. The sex-and age pyramid of the population screened show that the young adults (between 20-40 years), and mainly men, are underrepresented. The age and gender distribution of documented HAT cases show that these are the groups most at risk. The underrepresentation of young adults is much less pronounced in the output data obtained from the mini-teams. The door-to-door approach of the mini-teams appears to have a positive influence on the coverage of the people most at risk (adults between 20 and 40). We assume they participate more readily because of reduced waiting times and lesser opportunity costs when screening is done by door-to-door home visits.

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DISTRIBUTION AND ENVIRONMENTAL RISK FACTORS ASSOCIATED TO CHAGAS CARDIOMYOPATHY IN BOLIVIA

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Chagas disease is a neglected tropical disease caused by *Trypanosoma* cruzi that produces a high rate of morbidity throughout Latin America. In 2015, one study showed that the prevalence among adults in rural Bolivia was up to 80%. In 20-30% of the cases, Chagas progresses into cardiomyopathy with high rates of mortality. The distribution and associated factors of Chagas cardiomyopathy are not yet well understood in Bolivia, where the rates of mortality are highest. Early diagnosis and treatment can mitigate the risk of death caused by this disease. We conducted a cross-sectional analysis from a cohort study, in a public hospital in Santa Cruz, Bolivia. Participants underwent three serological tests for Chagas; patients who tested positive on at least two assays were considered to have Chagas. Echocardiography was used to classify cardiomyopathy progression from stage A to the final stage D. Geographic information system (GIS) was used to find distribution among stages of cardiomyopathy. Environmental information was used to evaluate risk factors, such as ecological changes, altitude, normalized difference vegetation index, presence of vector and incidence rate per community. The population consisted of 1,117 patients, 97% of which are stages A and B. We compared the distribution of cases across Bolivia with incidence rate per community, finding a significant association. Cardiomyopathy stages A and B had the same distribution across Bolivia regardless of incidence rate. However, stages C and D were significantly distributed only in communities with a medium and high incidence rate. Altitude, use of land and presence of the vector were significant associated to the progression of Chagas cardiomyopathy. Chagas cardiomyopathy distribution and future environmental changes measured by serial GIS can be used to help predict emerging regions at high-risk for advanced cardiomyopathy. Early detection could help reduce morbidity and mortality rates caused by Chagas disease.

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IMPACT OF HOUSING COMPOSITION ON DOMESTIC TRIATOMINE EXPOSURE RISK: EPIDEMIOLOGIC INVESTIGATION IN WEST TEXAS REGION ALONG THE UNITED STATES - MEXICO BORDER

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Residents of Trans-Pecos region of west Texas along the U.S. - Mexico border have reported a high rate of exposure to bites of triatomine insects, or kissing bugs - the vectors of Chagas disease (*Trypanosoma cruzi* infection). We performed an epidemiologic investigation into the potential for *T. cruzi* vector-borne transmission resulting in human disease in this area of Texas. Residential assessments included housing conditions, socio-economic and environmental factors, and vector evaluation. We enrolled 85 participants residing in either Terlingua, TX or the Panther Junction area of Big Bend National Park (BBNP), and tested them for antibodies against *T. cruzi*. Residents of 55 homes completed self-reported house infestation questionnaire, with 85% (47 out of 55) documenting history of triatomine bites. Fourteen homes were inspected by our study team revealing considerable variance in home structures, which is believed to be associated with increased triatomine exposure. Kissing bugs, predominantly *Triatoma rubida*, were collected by active surveillance and by citizens submissions from three distinct locations: Terlingua, Panther Junction area, and a nearby K-Bar station at the BBNP. *T. cruzi* DNA was detected in 32 out of 88 triatomines (36%), and proportion of positive insects ranged from 0 to 68%, depending on the collection location. Parasites from all 32 positive kissing bugs were determined to belong to discrete typing unit I genotype. While all of the study participants tested negative for *T. cruzi* antibodies, reports of frequent exposures to triatomines, in combination with presence of *T. cruzi*-positive insects, indicates a risk for acquiring *T. cruzi* infection exists in the investigated area.

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IMPACT OF EXTRINSIC INCUBATION TEMPERATURE ON ZIKA VIRUS POPULATION DIVERSITY IN *AEDES* MOSQUITOES

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Arthropod-borne viruses (arboviruses) continue to emerge as major threats to public health. In part, the process of arbovirus emergence has been driven by the emergence of new virus genotypes that alter key transmission phenotypes. The conditions that lead to new virus genotypes are, however, not fully understood. Accordingly, we sought to (1) define the impact of temperature on transmission dynamics of ZIKV and (2) determine whether temperature affects virus population structure during extrinsic incubation (EI) in the mosquito. In particular, we exposed *Aedes aegypti* and *Aedes albopictus* mosquitoes to a bloodmeal containing a Puerto-Rican strain of Zika virus and held them for 7 and 14 days at 25°C, 28°C and 35°C. We also exposed them to fluctuating temperatures that varied from 25-35°C diurnally. We found that transmission of ZIKV in both *Aedes* mosquitoes is most efficient at 28°C (P-value <0.05). However, viral load in the midgut and hemolymph of these mosquitoes is highest at 35°C (P-value <0.05). Using RNAseq to characterize virus genetic diversity, we have identified two consensus changes found in both *Aedes* mosquitoes associated with high temperature (28°C & 35°C) and show that EI temperature increases, so does the number of consensus changes to the ZIKV genome (25°C = 7, 28°C = 20, 25°C-35°C = 14, & 35°C =

18). We then assess the variant frequency during systemic ZIKV infection of four minority variants (frequency of 0.12-0.35) found in the input ZIKV population, and show that there are temperature and mosquito dependent affects to intrahost variant frequency. Last, we use population genetics analysis to investigate how the intrahost ZIKV population diversity in *Aedes* mosquitoes is shaped by the EI temperature. Collectively, our data indicate that temperature has multiple impacts on ZIKV biology within mosquitoes.

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INTRAHOST DIVERSITY OF ZIKA VIRUS IN BLOOD, URINE AND SALIVA OVER TIME IN AN INDEX CLUSTER STUDY IN NICARAGUA

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The Americas recently experienced a major epidemic of Zika virus (ZIKV) infections that have been linked to congenital birth defects and Guillain-Barré Syndrome. To better understand the mechanisms underlying the persistence of this virus in human bodily fluids, a longitudinal study was conducted from August 31 to October 21, 2016, in Managua, Nicaragua. We enrolled 33 RT-PCR-confirmed Zika index cases and their household members (109 contacts) and collected samples on days 0-1, 3-4, 6-7, 9-10, and 21, collecting serum/plasma, urine and saliva specimens. In order to glean as much information as possible about viral genetics from this cohort, we used a novel viral RNA enrichment strategy coupled with Next Generation Sequencing (NGS) that functions without any a priori assumptions about the target genome. This hybridization-based enrichment strategy, consisting of ZIKV-specific 120-nt, biotinylated oligodeoxynucleotides to capture ZIKV genomic material, addresses the principal problem encountered when employing NGS directly on patient samples; namely, the high ratio of host to viral RNA. The strategy developed here allowed us to enrich ZIKV genomic material over 5,000-fold relative to unenriched material. Importantly, by sequencing directly from the sera/plasma, urine and saliva of the infected subjects, we were able to measure the intrahost genetic diversity of the virus in each fluid type. Our ongoing study has utilized the viral variant data to assemble probable haplotypes emerging within each of these compartments and characterized the evolution of these haplotypes over time. Our results indicate that over the course of an infection in a single person, there is variation between compartments and over time. Given that current molecular epidemiology is based on the viral sequences derived from blood and urine interchangeably, we suggest that this compartment-based genomic diversity needs to be taken into consideration.

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A ZIKA VIRUS MUTATION THAT ASSOCIATES WITH FETAL DEATH IN RHESUS MACAQUES REDUCES TRANSMISSION BY *AEDES AEGYPTI* MOSQUITOES

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Fetal microcephaly and death are now recognized as severe forms of congenital Zika syndrome; however, it is still unclear whether recent Zika virus (ZIKV) mutations contribute to this phenotype. We identified a single intra-host variant in the ZIKV NS2B protein (NS2B^{M1404I}) from a rhesus macaque (RM) fetus that died after experimental ZIKV inoculation in the first trimester. Targeted deep sequencing flanking NS2B^{1404} in subsequent cohorts of RM mothers and their fetuses identified NS2B^{M1404I} at minority frequency and sometimes at consensus levels in 100% (7/7) of dead or stillborn RM fetuses and/or the plasma of their mothers and in 26% (4/15) of RM mother and fetus pairs whose fetuses survived to near term or were born alive. By examining sequence data from recent epidemics, we found that NS2B^{M1404I} occurs rarely (5/500, 1%) in consensus human ZIKV genomes. We also deep sequenced ZIKV genomes from non-pregnant human adults, infants, and *Aedes aegypti* from the epidemic and observed that NS2B^{I1404} was more often present at intra-host levels in humans compared to mosquitoes. Since the primary ZIKV transmission cycle is human-mosquito-human, viral mutations that arise in one host must be maintained in the alternate host to be perpetuated. We therefore hypothesized that ZIKV NS2B^{M1404I} may not be efficiently transmitted by *Aedes aegypti* mosquitoes, explaining its low frequency in humans during outbreaks. We engineered NS2B^{M1404I} into a ZIKV infectious clone and examined vector competence in *Ae. aegypti* from Puerto Rico. Although infection and dissemination rates were not different, we found that Ae. *aegypti* did not transmit ZIKV-NS2B^{I1404} as efficiently compared to ZIKV-NS2B^{M1404} 5 [7/20 (35%) versus 10/20 (50%), p>0.05] and 7 [3/20 (15%) versus 13/20 (65%), P<0.001, Chi-squared] days post-feed. The poor transmissibility of this potentially human/primate adaptive ZIKV mutation identified here may explain its low frequency in febrile humans. Furthermore, our data highlights the evolutionary complexity during arbovirus transmission cycles and suggests that some pathogenic mutations are not likely to spread in epidemics.

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INTERNATIONAL TRAVELERS AND VIRUS GENOMES REVEAL 'HIDDEN' ZIKA VIRUS TRANSMISSION DURING THE EPIDEMIC

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The recent Zika virus disease epidemic in the Americas was unprecedented in both its scale and severity. Despite the attention that the epidemic drew, we still do not have a very accurate accounting of its emergence patterns. Specifically, we previously identified significant temporal surveillance gaps between the true introduction of the virus and its first detection within a region. There are likely many reasons for this gap, which may include (1) overlapping symptoms between Zika virus and other viruses, (2) its rapid spread, and (3) the fact that low income areas were often hit the hardest. One mechanism to help alleviate this reporting problem is to monitor the millions of returning US travelers who visited these regions during the epidemic. We used international travel-associated Zika virus infections reported by the Florida Department of Health and air travel

volumes to calculate travel incidence rates when coming from a specific region. We found a strong correlation between the travel incidence rates and local reported rates when there was a high travel volume from the region and consistent local reporting. From some regions with inconsistent local reporting but high travel volumes, our data suggests that there was undetected or unreported Zika virus transmission extending into 2017, when the epidemic was believed to be waning. To further investigate these transmission patterns, we sequenced Zika virus genomes from infected travelers. Our data from Cuba, for example, suggest that (1) Zika virus entered the country multiple times during 2016, when transmission was at its peak in nearby regions, which (2) resulted in a large unreported outbreak in 2017. Overall, we demonstrate how infection patterns of international travelers can help to reveal 'hidden' outbreaks and that Zika virus transmission may be still occurring in underreported regions.

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ZIKA VIRUS OF BOTH AFRICAN AND ASIAN LINEAGES CAUSE FETAL HARM IN A VERTICAL TRANSMISSION MODEL

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Congenital Zika virus (ZIKV) infection was first linked to birth defects during the American outbreak. It is not clear whether American isolates are unique in their ability to cause congenital Zika syndrome (CZS), but some genetic mutations unique to the American-lineage have been proposed to explain, at least in part, the ability of American ZIKV to cause CZS. Recent studies identified a number of mutations in ZIKV infecting humans that arose around the time of the outbreak in French Polynesia and were stably maintained during subsequent spread to the Americas. Some of these mutations have been proposed to contribute to the sequelae associated with ZIKV infection during pregnancy. For example, it has been postulated that a single amino acid substitution identified in the transmembrane protein (prM; S139N) in American-lineage viruses may play a key role in the development of microcephaly. However, the functional role of this mutation has not been evaluated using a natural vertical transmission model. We engineered the reverse mutation (N139S) into the Puerto Rican ZIKV isolate PRVABC59 genome and evaluated its capacity to cause pathogenic outcomes in pregnant mice lacking type I interferon signaling (*Ifnar1-/-*). *Ifnar1-/-* females were crossed to wild type males to produce heterozygous fetuses to resemble the immune status of human fetuses. Maternal inoculation with 10^3 PFU of ZIKV-N139S (mutant) or ZIKV-S139N (wildtype) at embryonic day 6.5 (E6.5) both resulted in fetal demise and severe intrauterine growth restriction (IUGR). We also inoculated dams at E6.5 with 10^3 PFU of the low-passage West African isolate Dakar 41524 (ZIKV-DAK). Infection with ZIKV-DAK resulted in overt clinical signs for the dams, as well as fetal demise, reduced litter size, and severe IUGR. Collectively, our data suggest that ZIKV's capacity to infect and harm the fetus existed prior to the American outbreak. These preliminary results highlight the need to better understand the pathogenicity of Zika viruses circulating in both Africa and southeast Asia.

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ANTI-ZIKA VIRUS ANTIBODIES MODULATE VIRUS TRANSPORT ACROSS PLACENTAL CELL MONOLAYERS AND SUBSEQUENT INFECTION OF FC-GAMMA RECEPTOR-BEARING CELLS

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Zika virus (ZIKV) is a mosquito-borne flavivirus that caused major epidemics throughout the Americas in 2015-2016, and ZIKV infection during pregnancy has been associated with devastating fetal birth defects. The placenta separates maternal circulation from the developing fetus and is the site at which maternal antibodies are transported to the fetal blood stream. Here, we are studying the role of anti-ZIKV antibodies in facilitating virus transport into human placental tissues and in modulating infection of cells in anchoring chorionic villus explants. Briefly, ZIKV alone or in the presence of anti-ZIKV monoclonal antibodies (mAbs) as immune complexes (ICs) were added to the apical side of cultured human chorionic trophoblast (JAR) and umbilical vein endothelial cell monolayers in a transwell system. Then, the amount of free ZIKV and ZIKV-ICs collected in the basolateral chamber of transwells was separated and quantified by qRT-PCR after a protein G-agarose pull-down. We found that low levels of free ZIKV crossed through polarized placental cell monolayers in the absence of antibodies, but the presence of anti-ZIKV mAbs resulted in greater transport of virions in the form of ICs. We show that ZIKV that crosses these cell monolayers infects human monocytic U937 cells expressing the attachment factor DC-SIGN plated in basolateral chambers. We also show that certain anti-ZIKV mAbs directed to the E protein can either neutralize or enhance infection of cells in the basolateral compartment. Ongoing experiments are using ZIKV-specific mAbs and anti-ZIKV mAbs that are cross-reactive to the related dengue virus with either high and low neutralizing potency to understand the role of antibodies in mediating neutralization or enhancement of FcγR-expressing cells in the basolateral chamber after transport across placental cell monolayers. Additionally, we are studying ZIKV infection of fetal macrophages and other cells in the presence and absence of anti-ZIKV antibodies in human *ex vivo* anchoring villus explants. Overall, these studies should improve our understanding of the role of maternal antibodies in ZIKV dissemination during gestation.

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MATERNAL DENGUE ANTIBODIES AND RISK FOR CONGENITAL ZIKA SYNDROME-ASSOCIATED MICROCEPHALY

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It remains unknown whether the presence of antibodies to DENV virus confer resistance or susceptibility to clinical outcomes of Zika virus (ZIKV) infection. We performed a case-control investigation to determine whether maternal DENV neutralizing antibodies were associated with acquisition of congenital Zika syndrome-associated (CZS) microcephaly among infants whose mothers had serological evidence of ZIKV exposure. Active surveillance identified infants with CZS microcephaly, defined as head circumference <-2 SD and clinico-radiological findings, among live births at a maternity hospital in Salvador, Brazil between November 2015 to January 2016. We determined plaque reduction neutralization titers (PRNT) to ZIKV and DENV serotypes for sera from the mothers. Among infants of mothers who ZIKV neutralizing antibodies (PRNT ≥1:40), we selected case infants who had CZS microcephaly and control infants who did not have microcephaly and or had microcephaly without CZS criteria. Logistic regression analyses were performed to evaluate the independent

association of maternal DENV $PRNT_{90}$ and CZS microcephaly. Among 422 live births during the study period, 40 (9.4%) had CZS microcephaly. Among 36 mothers of infants with CZS microcephaly, 35 (97%) had ZIKV neutralizing antibodies, whereas 31 (56%) of 55 mothers of infants without CZS microcephaly had ZIKV neutralizing antibodies. Analysis of 35 case and 31 control infants identified female gender (OR 6.50, 95% CI 1.59-37.97) and ZIKV IgM in cord blood (OR 36.09, 95% CI, 7.06-308.90) as significant risk factors. High neutralizing titers (≥320) to DENV2 was independently associated with decreased risk (OR 0.16, 95% CI 0.02-0.84) for CZS microcephaly. In contrast low neutralizing titers (40-160) to DENV2 was associated with increased risk (4.97 (1.01-32.86) for CZS microcephaly. These findings indicate that the prior history of dengue exposure in mothers influences the risk of severe birth defects among their infants who were exposed to ZIKV *in utero*, and suggests that quantity of DENV antibodies contributes to either resistance or susceptibility to these outcomes.

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SAFETY, IMMUNOGENICITY AND EFFICACY OF THE FIRST INJECTABLE, GENETICALLY ENGINEERED MALARIA VACCINE PFSPZ-GA1 VACCINE

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Immunization of adults with radiation- or chemo-attenuated *Plasmodium falciparum* (Pf) sporozoites (SPZ) (Sanaria® PfSPZ Vaccine or PfSPZ-CVac) has shown to protect >90% of vaccinees against controlled human malaria infection (CHMI) and to protect against naturally transmitted Pf in Africa for at least 6 months. Genetically engineered PfSPZ have potentially improved safety and potency, and consequently significant costs of goods savings. We engineered genetically attenuated rodent and human malaria parasites by deleting two genes (Δ*slarp*Δ*b9*) and have demonstrated full attenuation in rodents, primary human hepatocytes and humanized mice. In addition, mice can be protected from challenge after repeated immunizations. PfΔ*slarp*Δ*b9* PfSPZ (Sanaria® PfSPZ-GA1 Vaccine) was manufactured in compliance with current Good Manufacturing Practices and released for human trials in the EU under a conditional release GMO license. In a dose escalating phase 1 clinical trial, the vaccine demonstrated an excellent safety profile, administered as single doses of 1.35x10e5, 4.5x10e5 and 9.0x10e5 sporozoites (n=3, 3 and 13 subjects respectively). All adverse events related to the vaccine were mild (grade 1). Diagnostic Pf PCR was performed on blood samples collected every day from day 6-21 and on day 28 after immunization and all were negative, indicating full genetic attenuation. Forty-eight subjects were subsequently enrolled in a randomized double blind trail and receive three doses at 8 week intervals of either 9.0x10e5 or 4.5x10e5 PfSPZ-GA1 Vaccine, or 4.5x10e5 PfSPZ Vaccine or saline placebo. Again, the vaccine had an excellent safety profile and no breakthrough parasites were detected. Subjects underwent CHMI with 5 mosquito bites 3 weeks after final immunization. Efficacy results from this challenge phase will be presented. In conclusion, PfSPZ-GA1 Vaccine is the first injectable genetically attenuated malaria vaccine assessed in humans. The accomplishment to manufacture, obtain regulatory approval and demonstrate an excellent safety profile is unprecedented and holds a promise for PfSPZ vaccines with increased potency.

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SAFETY, FEASIBILITY AND EFFICACY OF RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITE (PFSPZ) VACCINE ADMINISTERED BY DIRECT VENOUS INOCULATION IN A PHASE 2 TRIAL IN INFANTS IN WESTERN KENYA

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PfSPZ Vaccine is a pre-erythrocytic malaria vaccine composed of radiation-attenuated, aseptic, cryopreserved *Plasmodium falciparum* (Pf) sporozoites administered by direct venous inoculation (DVI). PfSPZ Vaccine showed vaccine efficacy (VE) of 60% for 14 months in malaria-naïve adults in the US against controlled human malaria infection and 52% by time-to-event and 29% by proportional analysis for 6 months against Pf infection by natural exposure in adults in Mali. To assess VE in Africans with less lifetime Pf exposure, we conducted a double-blind, placebo-controlled trial in 5–12-month-old infants in western Kenya. Infants were randomized 1:1:1:1 to receive 3 doses of 4.5, 9.0, or $18.0x10^5$ PfSPZ or placebo 8 weeks apart from January–August 2017; safety, feasibility, and VE against naturally transmitted Pf were assessed. Incident parasitemia was recorded during clinic and home visits for 6 months after last vaccination. 336 infants received 979 vaccinations, 95.2% by a single injection; 317 infants received all 3 vaccinations. There were no obvious safety signals in blinded safety data. 13.1% (2 Grade 3) and 9.2% (1 Grade 3) of infants had potentially related, solicited local and systemic adverse events (AEs) respectively during 7 days post vaccination. 37 unrelated serious AEs occurred in 35 infants. 58% of controls developed Pf infections during follow-up. Preliminary VE estimates by time-to-event analysis for Pf infection were 13% (95% CI: -32%, 43%), 2% (95% CI: -48%, 35%), and 32% (95% CI: -5%, 55%), in the 4.5, 9.0, and $18.0x10^5$ PfSPZ dose groups, respectively. VE by proportional analysis (any Pf infection vs. none) was 1% (95% CI: -27%, 22%), -7% (95% CI: -35%, 15%), and 13% (95% CI: -13%, 34%) respectively. Administration of PfSPZ Vaccine by DVI to Kenyan infants was safe and feasible; no VE results were significant at the $p<0.05$ level. Lower-than-expected VE may have reflected immunological immaturity, suggested by a recent study in Tanzania, in which infants immunized with PfSPZ Vaccine did not make significant T cell responses although older age groups did. Antibody and cellular immunological data from Kenya will also be presented.

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MODELLING THE RELATIVE ROLES OF ANTIBODY TITRE AND AVIDITY IN PROTECTION FROM *PLASMODIUM FALCIPARUM* INFECTION FOLLOWING RTS,S VACCINATION IN A HUMAN CHALLENGE STUDY

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Vaccination with the RTS,S/AS01 pre-erythrocytic vaccine has previously been demonstrated to induce antibody titres shown to be associated with protection from infection in both challenge studies and subsequent Phase II and Phase III field trials. In a recent challenge study in which the immunisation schedule was altered by delaying the third dose by 5 months and delivering it at 20% of the full dose (delayed fractional dose, Fx017M), vaccine efficacy increased to 86.7% compared to 62.5% under the original dosing schedule in malaria naïve adults. There was no significant difference in antibody titres between the arms; however, the antibody avidity was significantly higher in the Fx017M arm. We extended an existing biologically motivated model of sporozoite infection to characterise how both antibody titre and antibody avidity relate to protection in this challenge study. The model was fitted to immune data from the 46 malaria naïve adults in the Fx017M study. We found that a model incorporating both antibody titre and avidity provided a better fit to the data than a model incorporating antibody titre alone. Categorising both antibody titre and avidity into three levels (low, medium, high) we estimate vaccine efficacy to be 94.2% in those with high titre and avidity, 76.9% in those with high avidity but low titre, 61.6% in those with low avidity and high titre and 38.3% in those with low avidity and low titre. Higher avidity levels were therefore found to be more important in protection than high antibody levels in these individuals, with high avidity required for protection across all but the highest antibody titres. This suggests that the quality of the induced antibody response is likely to be more important than the quantity alone in the development of highly efficacious pre-erythrocytic vaccines against malaria.

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TOWARDS GENERATING A GENETICALLY-ENGINEERED REPLICATION-COMPETENT WHOLE *PLASMODIUM FALCIPARUM* PARASITE VACCINE THAT CONFERS BROAD AND DURABLE PROTECTION AGAINST INFECTION

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Previous studies in models of rodent malaria provide conclusive evidence that a late liver stage-arresting genetically attenuated *Plasmodium* parasite (GAP) vaccine acts as a superior immunogen compared to one that arrests early in the liver, thereby laying the translational foundation for an efficacious late-arresting GAP vaccine against human malaria. Two such LA-GAP candidates include *plasmei2*, the *Plasmodium* ortholog of the eukaryotic RNA-binding protein (RBP) Mei2, and *lisp2* (liver specific protein 2), that are conserved across the *Plasmodium* species and exclusively transcribed in liver stages. We have previously shown that the *P. yoelii plasmei2$^-$/lisp2$^-$* (*Py plasmei2$^-$/lisp2$^-$*) double knockout (KO) parasite is a synthetic lethal that arrests late in liver development and immunizations with *Py plasmei2$^-$/lisp2$^-$* confer complete sterile protection against sporozoite challenge. Herein, we report for the first time the successful generation of a LA-GAP in *Plasmodium falciparum* by gene deletion of *Pf plasmei2*. We found that *Pf plasmei2* is dispensable for asexual blood stages, for gametocyte formation and for developmental stages in the mosquito vector. Infection of human liver-chimeric FRG huHep mice with *Pf plasmei2$^-$* sporozoites does not impair either initial liver stage invasion or growth in cell size late into liver stage development. However, *Pf plasmei2$^-$* KO liver stages have discernible defects in schizogony, and do not form exo-erythrocytic merozoites. When FRG-huHep mice infected with *Pf plasmei2$^-$* KO sporozoites were infused with human red blood cells (RBCs) on Day 7 after infection, they did not become blood stage patent, in contrast to all mice infected with WT NF54 sporozoites and subsequently infused with human RBCs that transitioned to blood stages. These results strongly qualify *Pf plasmei2$^-$* KO as a candidate for a late liver-stage arresting GAP vaccine. Characterization of the *Pf lisp2$^-$* single KO and *Pf plasmei2$^-$/lisp2$^-$* double KO are currently in progress. Generation of such a replication-competent late liver stage-arresting GAP is a significant leap towards generating an efficacious anti-malarial vaccine.

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EFFECT OF DELAYED AND FRACTIONAL DOSING ON THE TITERS, FINE SPECIFICITY, AVIDITY AND BIOLOGICAL ACTIVITY OF RHESUS ANTIBODIES AGAINST A CIRCUMSPOROZOITE PROTEIN BASED VACCINE FMP013

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Circumsporozoite protein (CSP) is present on malaria parasite sporozoites, and it contains NANP)n repeats flanked by N-terminal and C-terminal regions. A vaccine containing repeat and C-terminal regions, RTS,S (GlaxoSmithkline), showed <50% efficacy against natural *P. falciparum* infection. We produced a nearly full-length CSP vaccine FMP013 and formulated it in a liposomal adjuvant ALFQ that contains immune-modulators 3D-PHAD^{TM} and QS21. Here we report Rhesus study results on safety, toxicology and immunogenicity of FMP013 + ALFQ. Five groups (n= 6) received three vaccinations on 0-1-2 or 0-1-6 month schedule. Local site reaction, blood count and serum chemistry data showed a mild fever, a transient increase in WBC and neutrophils and a rise in creatine kinase level; however these normalized by day-7 post vaccination. Overall, the vaccine was safe and very well tolerated. The repeat titers were highly dependent on antigen and adjuvant dose. Overall, the boosting profile of equivalent low antigen doses in full human dose of ALFQ, on a 0-1-2 month schedule, was most desirable for high titer. Increasing antigen or reducing adjuvant dose negatively impacted antibody boosting. Compared to identical doses, fractional 3rd dose on 0-1-6 or 0-1-2 month schedule showed less boosting of C-terminal and no boosting of repeat titers. A trend towards the fractional dose on 0-1-2 schedule inducing higher titer than the delayed fractional group was noted. Most importantly, delayed and fractional schedule significantly increased avidity and delayed boosting was found to be the key driver of higher avidity, not antigen fractionation. ALFQ induced a balanced Th1:Th2 type response in primates and purified IgG strongly inhibited sporozoite development *in vitro*. We reproduced and further elucidated the findings from a delayed and fractional dose regimen of RTS,S in humans. Together these observations strongly support the evaluation of the FMP013+ALFQ vaccine in humans.

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A PHASE IA/B STUDY TO ASSESS THE SAFETY AND IMMUNOGENICITY OF PLACENTAL MALARIA VACCINE CANDIDATE: PRELIMINARY RESULTS OF THE PRIMALVAC TRIAL

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Adhesion of *Plasmodium falciparum*-infected erythrocytes (PEs) to placental chondroitin-4-sulfate (CSA) has been linked to the severe placental malaria (PM) outcomes. Evidences strongly support the VAR2CSA variant surface antigen mediating PEs CSA-binding phenotype as the leading candidate for a PM vaccine. This study was conducted to assess the safety and immunogenicity of 3 different dosages (20µg, 50µg and 100µg) of the recombinant VAR2CSA protein (PRIMVAC), formulated with Alhydrogel or GLA-SE administered at days 0, 28 and 56 A randomized double-blind phase Ia/Ib dose-escalation vaccine trial was conducted in healthy adult women. Within 4 sequential cohorts, volunteers were randomized to 2 arms (PRIMVAC adjuvanted with Alhydrogel or GLA-SE) in the first phase conducted in France and then to 3 arms (PRIMVAC with Alhydrogel or GLA-SE or placebo) in Burkina Faso. Enrolled volunteers were observed for at least 1 hour following each vaccination then seen at 1 day and 7 days later for safety evaluations. Serious adverse events (SAE) were recorded throughout the study duration. Routine clinical laboratory safety analyses were performed prior first injection and at each subsequent visit. A total of 68 subjects were recruited in the four study cohorts No SAE was reported in any of the cohort A volunteers and enrolment in cohort B started. A Data Safety Monitoring Board (DSMB) reviewed the safety data for cohorts A (20µg) and B (50µg) before the trial was initiated in Burkina Faso. The DSMB also reviewed the safety data in Burkina to authorize the progression from the cohort C (50µg) to cohort D (100µg). Last vaccination of the last subject occurred in September 2017. This was the first placental malaria vaccine phase Ia/b clinical trial conducted in France and Burkina Faso. No serious adverse events have been recorded. Preliminary safety and immunogenicity results will be presented.

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TOLERABILITY, SAFETY AND PROTECTIVE EFFICACY OF DIRECT VENOUS INOCULATION OF CONDENSED VACCINATION REGIMEN OF PFSPZ VACCINE IN TANZANIAN ADULTS INCLUDING HIV INFECTED VOLUNTEERS

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PfSPZ Vaccine, composed of radiation-attenuated (non-replicating), purified, cryopreserved *P. falciparum* (Pf) sporozoites (PfSPZ), has been extremely well tolerated and safe in adults, children and infants (5 to 12 months). In Tanzania, 6/6 healthy HIV-negative adults who received 3 doses of $9x10^5$ PfSPZ of PfSPZ Vaccine administered by direct venous inoculation (DVI) at 8 week intervals were completely protected against homologous controlled human malaria infection (CHMI) at 3-11 weeks after immunization (100% efficacy). In Malian adults under high malaria exposure, vaccine efficacy (VE) of 29% and 52% by proportional and time to event analysis respectively was demonstrated against naturally transmitted malaria. Despite these promising results higher VE is desired for more impact in the field. Recently, an accelerated vaccination schedule of 0, 2, 4, 6 days with a 16 week boost provided a better magnitude and duration of protection, including protection against heterologous CHMI, in malaria-naïve adults. As this vaccine is intended for mass vaccination programs (MVPs) the condensed schedule and higher efficacy would facilitate its deployment in the field. Furthermore MVPs would target the entire population including those who are HIV infected. For this reason, demonstrating safety in those living with HIV-1 infection (HIV positive) is an important objective to enable successful deployment of PfSPZ Vaccine in MVPs. The present trial in Tanzanian adults (18-35 years) will determine whether immunization with PfSPZ Vaccine will predispose HIV positive individuals to more severe adverse event profiles. Moreover, we will determine if immunization enhances the progression of HIV disease or conversely if HIV co-infection might adversely affect the immunogenicity of PfSPZ Vaccine and consequently its ability to protect against malaria. The trial began in February 2018 and is expected to end in July 2018. The complete un-blinded results of tolerability, safety, immunogenicity and protective efficacy of this condensed vaccination regimen with a single boost at 28 days and spectrum of HIV exposure status will be presented.

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HOMOLOGS OF HUMAN DENGUE-RESISTANCE GENES, FKBP1B AND ATCAY, CONFER ANTIVIRAL RESISTANCE IN *AEDES AEGYPTI* MOSQUITOES

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Dengue virus (DENV) is transmitted by mosquitoes and is a major public health concern. While chemical and genetic mosquito control strategies have been at the forefront for controlling DENV transmission, the basic biology underpinning mosquito vector competence for DENV remains

poorly understood. The study of innate mosquito defense mechanisms against DENV have revealed crucial roles for Toll, IMD, Jak-STAT and RNAi pathways in mediating DENV dissemination in the mosquito. Often overlooked in such studies is the role of intrinsic cellular defense mechanisms that we hypothesize to work in concert with the classical immune pathways to effect organismal defense. Our understanding of the molecular interaction of DENV with mosquito host cells is limited and we propose to expand upon the recent results from a human cell line study. A comprehensive genome-scale RNAi screen identified 22 human dengue/West Nile virus (DENV/WNV) resistance genes that may be independent of known innate immune mechanisms. We hypothesized that of the 22 human genes identified, a subset would be functionally conserved in *Aedes aegypti* mosquitoes against flaviviruses. We identified 12 homologs of the 22 human genes in the *Ae. aegypti* genome. To evaluate their role in cellular resistance/antiviral defense against DENV, we used RNAi silencing in mosquitoes infected with DENV2. Among the 12 homologs, the silencing of two candidates, FKBP1 and the ATCAY, were associated with a significant increase in DENV titers as compared to the control groups. Depletion of the genes significantly increased dissemination of virus through the mosquito at 14 days post-infection. These data suggest that silencing of FKBP1 or ATCAY increased mosquito susceptibility to DENV replication and dissemination. Our results demonstrated that *Ae. aegypti* FKBP1 and ATCAY genes mediate resistance to DENV infections akin to what has been described for their orthologs in humans. In addition, we will discuss molecular profiling and characterization of the cellular and viral interacting partners of the two DENV-resistance genes.

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A GLOBAL POPULATION GENOMIC SURVEY OF *AEDES AEGYPTI* VARIATION

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The *Aedes aegypti* aegypti (Aaa) mosquito is a highly competent vector of Zika, dengue virus, yellow fever and chikungunya; much of this competence derives from its preference for feeding on humans and ovipositing in urban habitats, and its global distribution. In contrast, the ancestral *Aedes aegypti* formosus (Aaf) subspecies is found only in sub-Saharan Africa, is less anthropophilic, and occurs in forested environs. Yet both subspecies can be found in sympatry in East Africa and are capable of producing fertile hybrids. With the improved reference genome of *Aedes aegypti* aegypti released earlier this year, we have conducted the first whole-genome resequencing-based global population study of this important vector. We have carried out deep Illumina sequencing of linked-read (10X Genomics) libraries for 24 individual mosquitoes in order to perform comprehensive variant discovery in both subspecies, including SNPs, insertions/deletions (INDELs), polymorphic mobile element insertions, and large structural variants such as inversions. We have characterized all of these polymorphism classes in multiple individuals from eight mosquito colonies founded from geographically disparate locations, including sympatric Aaa and Aaf populations (as defined by genetic clustering with African vs non-African populations). Examining signals of selection and diversity we find little evidence of restricted gene flow between sympatric Aaf and Aaa populations, but identify distinct founder effects for the non-African Aaa samples. Despite a reduction in diversity resulting from the expansion of Aaa to countries outside Africa, we also identify plentiful haplotypic and structural diversity in this mosquito which could facilitate its high phenotypic adaptability. We identify genes exhibiting evidence of positive selection in one or both subspecies. This work is a key step in the development of population genomic studies in this species of great public health importance; we hope that it will provide the foundation to subsequent studies of Aedes population structure and the potential for disease transmission by this vector.

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STARR-SEQ IN *ANOPHELES*: QUERYING NON-CODING DNA FOR FUNCTION

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The vast majority of phenotypic variation in animals (>90% estimated from studies in Drosophila and human) is controlled by genetic variation in non-coding regulatory DNA, while genetic variation of protein-coding sequence contributes relatively little to phenotypic variation. Description of non-coding regulatory elements in mosquitoes, and their influence on mosquito biology and malaria transmission, remains little studied. Gene enhancers are an important class of non-coding regulatory elements. Enhancers influence gene transcriptional levels independent of position or orientation in the genome, even at a distance. Enhancers cannot yet be reliably identified by sequence signatures, but rather require functional assays for comprehensive detection. The availability of next-generation deep-sequencing has led to new strategies to screen non-coding DNA for functional enhancers. We have successfully modified and employed STARR-Seq (Self-Transcribed Active Regulatory Region Sequencing), a method first used on D. melanogaster, in *Anopheles coluzzii*, a prominent vector of malaria in West Africa. As proof of principle we have identified candidate enhancers near genes involved in insecticide resistance, innate immunity, and enhancers of developmental genes with good Drosophila orthologues. One of these Drosophila orthologues, an enhancer of Apterous, a gene important in wing development, was identified in *A. coluzzii* and functionally verified by luciferase assay. Results from other candidate enhancers will be discussed. Due to our unique experimental design we are not only able to query for enhancers in *A. coluzzii*, but have the power to examine how segregating genetic variation within species impacts enhancer activity and individual phenotype.

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THE HI-C APPROACH IMPROVED GENOME ASSEMBLIES AND REVEALED PRINCIPLES OF 3D GENOME ORGANIZATION IN MALARIA VECTORS

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The spatial organization of the genome plays an important role in cell function. The main principles of the 3D chromosome folding in eukaryotes have been discovered using Hi-C - a groundbreaking technology that exploits *in vivo* chromatin proximity information. This method can also yield dramatically improved genome assemblies. The main goals of this study were 1) to apply the Hi-C approach to improving the fragmented genome assemblies for *Anopheles* species and 2) to understand the main principles of spatial genome organization in medically important malaria vectors. We performed a Hi-C protocol on 15-hour *Anopheles* eggs to generate 10 Hi-C libraries, including 2 biological replicas for 5 *Anopheles* species, which were sequenced with Illumina 150-bp paired-end sequencing. Using the combination of previous genome assemblies, physical chromosome mapping, and our Hi-C data, we have obtained a new accurate chromosome-level genome assembly for *Anopheles albimanus*, *Anopheles atroparvus*, *Anopheles coluzzii Mopti*, *Anopheles merus,* and *Anopheles stephensi*. We have detected scaffold orientation errors and misassemblies within the existing genome references and made the necessary corrections. The updated genome assemblies will improve

quality of genome annotation for the malaria vectors. We have identified species-specific patterns of short- and long-range chromatin interactions and inter-chromosomal contacts for all studied species. Our study revealed that: 1) anopheline genomes are structured into chromatin compartments, loops, and topologically associated domains (TADs) similarly to that in *Drosophila*; 2) the TAD/interTAD composition appears to be partially corresponding to the bands/interbands pattern of polytene chromosomes of malaria mosquitoes. In sum, our results have demonstrated the common fundamental principles of 3D genome organization in dipteran insects providing new facts for understanding of how architectural genome folding carries into effect within the nuclear space in malaria vectors.

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20-HYDROXYECDYSONE (20E) ACTIVATES MOSQUITO CELLULAR IMMUNITY AND LIMITS *PLASMODIUM* OOKINETE SURVIVAL

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Mosquito blood feeding behavior is driven by the requirement of a nutrient-rich blood meal for egg development, which as a result, can potentially expose mosquitoes to blood-borne pathogens. Shortly after a blood meal, increased levels of the hormone 20-hydroxyecdysone (20E) stimulate yolk protein production in a process known as vitellogenesis through a heterodimeric receptor comprised of the ecdysone receptor (EcR) and ultraspiracle (USP). Reaching peak levels 24 hours after blood feeding, 20E expression levels coincide with the timing of malaria parasite invasion. While well-studied with respect to insect development, mating, and reproduction, little is known about the influence of 20E on mosquito immune response. Here we report the effects of 20E on the innate immune system of *Anopheles gambiae*, further defining the role of 20E in priming mosquito immunity for pathogen challenge. We demonstrate blood feeding can increase the phagocytic ability of mosquito hemocytes, an effect that can be reconsitituted through the injection of 20E alone. In addition, flow cytometry experiments demonstrate that 20E treatments promote shifts in hemocyte populations, suggesting 20E activates mosquito cellular immunity. When mosquitoes were primed with either the injection of 20E or the topical application of the 20E-mimicking insecticide, Halofenozide, malaria parasite numbers were significantly reduced. Upon further experiments, we confirm that 20E priming elicits immune responses limiting *Plasmodium* ookinete invasion. Preliminary experiments to understand the mechanisms of 20E immune priming by targeting the canonical EcR/USP heterodimeric receptor by silencing USP show no effect on parasite survival, suggesting an unknown receptor may mediate 20E-regulated immune responses. Together, these results suggest increased levels of 20E following blood feeding prime mosquito immunity to prevent development of pathogens present in the blood meal. With potential translational applications to reduce malaria transmission, these studies offer important insights into the contributions of mosquito hormones in shaping vector competence.

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DUAL ACTION LARVICIDAL-ADULTICIDAL SIRNA INSECTICIDES FOR BIORATIONAL MOSQUITO CONTROL

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Although mosquito control is the primary means of combating vector-borne illnesses, the current pesticide repertoire is faced with great challenges to sustainability. Our recent screens in *Aedes aegypti* and *Anopheles gambiae* identified hundreds of small interfering RNA molecules (siRNAs) that function as mosquito larvicides. It was hypothesized that a subset of these siRNAs target genes required for viability at multiple stages of the mosquito life cycle and could therefore function as dual action pesticides with both larvicidal and adulticidal activity. To test this hypothesis, larvicidal siRNA molecules were screened for their ability to kill adult mosquitoes. The adult screens were conducted by delivering siRNAs to adult mosquitoes through both microinjection as well as through attractive sugar bait capillary feeding assays. ~20% of the larvicidal siRNAs screened to date, including several with target sites that are conserved in *Aedes, Anopheles,* and *Culex* mosquito species, were found to function as adulticides. Six of the most highly lethal siRNA molecules were down-selected for further characterization, including confirmation of the proposed modes of action for these pesticides in the *Aedes aegypti* nervous system. siRNA pesticide efficacy is being evaluated in simulated field and semi-field trials in which the most effective formulations are being assessed in container breeding sites, ovitraps, and attractive toxic sugar bait stations. It is anticipated that interfering RNA pesticides, which can be seamlessly integrated with existing control strategies, represent a new biorational means of controlling human disease vector mosquitoes.

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A WHOLE-GENOME TAXONOMIC SURVEY OF DIVERSE SOUTHEAST ASIAN MALARIA VECTORS

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With the emergence of drug-resistant parasites in Southeast Asia, understanding the role of diverse vector species and populations in the complex malaria transmission dynamics in this region is crucial to reduce and eliminate malaria. Genome sequencing could provide important insights, but as yet no population genomic studies have been performed on Southeast Asian anophelines. We describe results from a pilot study using Illumina deep whole-genome sequencing of 110 wild-caught individual Anopheles mosquitoes representing 27 different species, collected from Cambodia. We used these data to investigate major gaps in currently available reference data and analytical methods that need to be filled. In particular, we investigated the suitability of available reference genomes, finding that for only 14 out of the 27 species studied was there a reference genome that was sufficiently close and complete to enable standard methods for variant calling and population genomics to be applied. We also investigated the validity of current molecular techniques for identifying vector species. To provide an independent view of the taxonomic relationships between the individuals sequenced, we used three approaches: (1) de novo assembly of mitochondrial genomes; (2) alignment of reads to a small set of sequences that are highly conserved across all currently available reference genomes; and (3) a whole-genome reference-free analysis based on k-mer composition. Finally, we investigated the feasibility of studying genetic variation and recent selection in genes likely to be involved in insecticide resistance. These data will be publicly available as part of the MalariaGEN Vector Observatory, an open access resource of genome sequence data. Based on our experiences with these data, we discuss the next steps and technical foundations that require investment in order to unlock the full potential of genome sequencing as a tool for studying the geographical structure, ecology, and dynamics of the vector populations transmitting drug-resistant parasites in Southeast Asia, and for identifying genes conferring insecticide resistance and vector competence.

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REGIONAL FORUM ON *CANDIDA AURIS*: BUILDING PUBLIC HEALTH PARTNERSHIPS FOR THE PREVENTION, DETECTION AND RESPONSE OF EMERGING MULTIDRUG-RESISTANT FUNGAL INFECTIONS IN THE AMERICAS

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Candida auris and other emerging multidrug-resistant fungal infections (ERFs) have been recently identified as causes of serious healthcare-associated infections (HAI). In the Americas, four countries have reported *C. auris* HAI outbreaks since 2016. To address timely detection and control of *C. auris* and ERFs, the Council of Ministers of Health of Central America and Dominican Republic convened a regional forum for government public health leaders and subject matter experts in August 2017. CDC, in collaboration with PAHO, facilitated lectures and roundtable exercises to improve laboratory and surveillance capacity to detect *C. auris* and other ERFs and review antimicrobial resistance (AMR) policies. A fungal diagnostic capacity survey and an analysis of strengths, weaknesses, opportunities and threats were discussed to define short and mid-term action priorities. Colombia, Costa Rica, Dominican Republic, El Salvador, Guatemala, Honduras, Nicaragua, Panama, the United States and Venezuela were represented by 35 senior public health officials. Attendees included national directors of epidemiologic surveillance departments and public health laboratories (27%), AMR-HAI program coordinators (20%), healthcare epidemiologists and clinical infectious diseases specialists (31%), and laboratory specialists (22%). About 70% of laboratories and healthcare institutions from participating countries reported a low capacity to detect ERFs and report antifungal susceptibility patterns. Action priorities included integration of ERFs into national AMR surveillance systems, public health workforce training, strengthening fungal laboratory capacity and adoption of guidelines for diagnosis, management and surveillance. The *C. auris* regional forum provided an opportunity to increase antifungal resistance awareness and emphasized the need for closer collaborations to implement immediate public health actions. Further expansion of public health partnerships and a continued follow-up of identified action priorities are critical steps to the advancement of ERF surveillance and antifungal stewardship programs in the region.

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ADAPTING THE POPULAR OPINION LEADERS (POL) MODEL TO PROMOTE FAMILY PLANNING AND ZIKA PREVENTION BEHAVIORS AMONG ADOLESCENTS IN SANTA ANA, EL SALVADOR

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The arrival of the Zika virus and its established link to congenital malformations has produced a serious global health challenge. El Salvador has been among the countries most heavily impacted by Zika, forcing the government to officially recommend delaying pregnancy until 2018. In a country where one out of every three pregnancies occur in adolescents, there exists a clear need for outreach education and Social & Behavior Change Communication (SBCC) programming on family planning and Zika prevention. In 2017, PASMO El Salvador in collaboration with PSI designed a study to test the impact of an SBCC intervention using the Popular Opinion Leaders (POL) model on adolescent knowledge, attitudes, and practices regarding Zika prevention. POL is a community-based intervention designed to reduce risk behavior through the use of credible opinion leaders who diffuse information and model behavior to their social networks, it has been cited as a high impact intervention by the CDC. Formative research was completed to identify the target population, social networks, opinion leaders, and Zika baseline knowledge on prevention-related norms. A representative sample of student opinion leaders was identified based on social characteristics and a total of 272 students were trained on Zika prevention, family planning, risk reduction methods and effective and communication strategies to change peer norms. Baseline and first line end line surveys were completed by students within the target population to measure impact in 2017, with a further second end line surveys recently completed in January 2018. Preliminary analysis indicated some significant positive results. Regarding knowledge and attitudes about safe sex, 5/6 of indicators showed improvement. Additionally, 3/6 of Zika-specific knowledge indicators showed significant improvement. This study will help to validate that a peer-to-peer channel is a viable channel for increasing knowledge and Zika prevention behaviour amongst adolescents. This study will be used to advise the scale-up of Zika-education activities in other areas of El Salvador and the region.

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CHALLENGES OF ACHIEVING EFFECTIVE CONTRACEPTION WOMEN OF REPRODUCTIVE POTENTIAL IN MALARIA VACCINE TRIALS IN EQUATORIAL GUINEA

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Past studies have shown that the knowledge and use of contraception among women of reproductive potential in Equatorial Guinea is limited. Additionally, the national family planning program discourages the use of hormonal methods of birth control for females under the age of 18, and instead recommends the use of abstinence or barrier methods. Since a new law established in 2016 banned school attendance for pregnant students, pregnant teens have increasingly sought out induced abortions, which are illegal in the country. A recent clinical trial of PfSPZ Vaccine, which included females age 6 months to 65 years, revealed the difficulties of achieving effective contraception among volunteers who were inexperienced in the use of birth control. Of the 15 female volunteers of

childbearing age enrolled in the trial, 5 (33%) chose oral contraceptives, 5 (33%) chose a medroxyprogesterone injection, 3 (20%) chose abstinence + condoms, and 2 (13%) chose abstinence alone. Three pregnancies occurred among these volunteers during the trial. This experience highlighted the pressing need to expand sex education and contraceptive availability in the country, as well as provide more reliable forms of birth control such as medroxyprogesterone to all trial participants.

1497

DIAGNOSTIC LANDSCAPE FOR OUTBREAK DISEASES - AN OVERVIEW OF THE CHALLENGES

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The 2014-2016 Ebola outbreak in West Africa and 2016-2017 Zika outbreak in the Americas led to an unprecedented response by the global community to rapidly develop new tools for prevention and control. However, challenges remain for developing tools to prepare for future epidemics and pandemics, hindered by limited surveillance data for outbreak-prone pathogens, lack of interest from in-vitro diagnostic companies to invest in products with episodic and small markets, limited resources in both clinical and reference laboratories, and difficulty in predicting the next epidemic or pandemic. To better prepare for future outbreaks, the WHO is leading the R&D Blueprint initiative, which works on the basis of identified priority diseases and collaborative R&D roadmaps, with the goal of accelerating the development and evaluation of medical countermeasures to enable effective and timely emergency response. To support the R&D roadmaps, we conducted diagnostic landscapes for 5 priority pathogens to understand the current technological gaps in affected low and middle income countries (LMICs). Cross-cutting gaps for all pathogens include: the lack of validated and regulated commercial assays that cover all relevant clades/lineages; the need for affordable point-of-care (POC) or near patient (NP) nucleic acid tests to rapidly diagnose and confirm infection; the need for more sensitive POC and NP antigen and antibody-specific immunoassays to support clinical and vaccine trials; the need for screening algorithms and multiplex syndromic panels to differentiate etiologies for acute febrile or respiratory illnesses; and the need for access to well characterized samples and external quality controls for test validation. Shifting from a reactive to proactive response must occur, as diagnostics are essential to building sustainable laboratory capacity that can be leveraged for an accelerated outbreak response, particularly in endemic settings. Understanding diagnostic gaps and en user needs will enable partners working in this area to prioritize product development that builds diagnostic capacity for both endemic and pandemic pathogens.

1498

MATHEMATICAL MODEL FOR QUANTIFYING THE IMPACT OF CONTROLLING DIABETES ON THE SPREAD OF TUBERCULOSIS

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With the epidemiological transition, populations are now seeing remarkable increases in chronic diseases such as diabetes mellitus (DM). While it is known that tuberculosis (TB) progression is hastened and treatment outcomes worsened with the co-morbidity of DM, other interactions between the two remain to be elucidated. With the global push towards ending TB, it is necessary to broaden our scope of TB control by examining other areas that could be targeted. The goal of this project is to inform policy and programs in effective uses of resources, by focusing cost-effective interventions on targeted populations. Therefore, we have developed a mathematical model to determine the impact of adequate DM control on the progression and development of drug resistance. Our ordinary differential equation model population divides into risk groups based on severity of diabetes and level of diabetes treatment. By simulating the reduction of uncontrolled diabetes and performing sensitivity analysis of the model, we will quantify the impact of controlling diabetes on the spread and resistance of TB. Preliminary results are promising, but the very nature of this project precludes predictions at this point. Up to date results will be presented. By providing a model that can be adapted to individual populations or sub-populations, we hope to enable and enhance the global end-TB strategy.

1499

THE EFFECTIVENESS OF FILM-BASED EDUCATION AND OUTREACH FOR MONKEYPOX IN THE CONGO BASIN

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Monkeypox (MPX) is a zoonotic disease endemic to Western and Central Africa. Handling and preparation of bush meat is a known risk factor for human disease; however, the reservoir species is unknown. In the Congo Basin, cases typically occur in remote areas with limited resources to health care. Films about MPX disease recognition, including an emphasis on healthcare and prevention, are shown as part of an outreach program in 49 villages within the Tshuapa Province, in the Democratic Republic of Congo (DRC) in 2011, 2013 and 2015. Educators evaluated the messages in the films by administering a survey to a proportion of participants before and after the films. In total, 1,353 participants completed the survey. 94.1% of respondents reported that they would seek hospital care for MPX after viewing the films, compared to the 77.8% before. There was also significant results of participants being able to identify more than one symptom of MPX, from 34.5% to 63.7% after viewing the film. Behaviors related to primate carcasses showed significance as well. A majority of respondents (90.4%) reported having eaten a primate before, with less than 2.6% of the participants stating they would continue to consume the primate. A similar film-based outreach program was also presented in the Republic of Congo (ROC), with comparable results for healthcare seeking behavior and the ability to better identify signs of disease in humans. However, in ROC, respondents did not report that they would change behaviors that involved consuming primates. Overall, the results suggest there is an improvement of disease knowledge and awareness of high-risk factors associated with MPX with the film based outreach model.

1500

BUILDING BETTER GLOBAL HEALTH DELIVERY: THE EXECUTIVE NURSE LEADER

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Nurses provide over 90% of primary care and constitute 30% of all health workers globally. They are natural change agents and the backbone of health care systems. As frontline health workers, nurses are often the first to identify emerging outbreaks. As demonstrated with the Zika virus in Haiti, nurses were at the forefront leading epidemiologic investigations,

developing protocols and establishing systems to optimize patient care. Although highly resourceful and systems-thinkers, nurses continue to be severely underrepresented at decision making tables. An inter-professional approach inclusive of nurses at decision-making tables is vital to strengthening health systems and combatting future emerging infectious disease and outbreaks. To begin to address this gap, Partners In Health (PIH) created the PIH Nightingale Fellowship, providing nurse leaders with the knowledge, tools, and confidence to lead at the highest level. The first cohort of Nightingale Fellows consists of four fellows from Haiti, Liberia, and Rwanda. The fellows participate in a one-year executive nurse leadership program. The fellowship includes two intensive in-person boot camps, online learning modules, mentorship from executive nurse leaders, and application of leadership concepts through leadership projects at their home institutions. The first cohort will complete their fellowship in June 2018. Preliminary results have demonstrated improvements in leadership practices, managing others, quality and safety, and strategic planning. All four fellows have become more engaged in the larger global nursing community including presenting at the Global Nursing Caucus, initiating academic partnerships, and actively participating in online global nursing forums. Three out of four fellows have been promoted to higher leadership positions. Fellows have self-reported increased confidence in their leadership abilities to represent nursing at all levels. Because of the preliminary success of this program, a new cohort of PIH Nightingale Fellows will begin in 2019 at the University of Health Equity in Rwanda-opened to nurses beyond PIH.

1501

THE GLOBAL BURDEN OF HELMINTH POLYPARASITISM: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Multiple human helminthiases are ubiquitous in the global South and epidemiological reports suggest polyparasitism is the norm rather than the exception. Impoverished communities exposed to multiple helminthiases are often also reported to be burdened with intestinal protozoan infections, malaria, tuberculosis, and human immunodeficiency virus (HIV). The objective of this study was to quantitatively evaluate the commonality of human helminth and helminth-protozoa polyparasitism; additionally, mixed infections of helminths with malaria, tuberculosis, and HIV were evaluated due to their extensive overlap in geographical endemicity with the global distribution of human helminthiases. In this ecological meta-analysis, we searched the PubMed and Web of Science databases for studies reporting the prevalence of single and multiple infections of helminth-only, helminth-protozoa, helminth-malaria, helminth-tuberculosis, and helminth-HIV. We used meta-analysis models based on resampling techniques to calculate the mean difference between the prevalence of polyparasitized and monoparasitized individuals. Polyparasitism was found to be significantly more prevalent than monoparasitism among helminth-only (difference 14% [95% CI: 5-23]) and helminth-protozoa infections (difference 15% [95% CI: 5-24]). Additionally, mixed infections of helminths with malaria, tuberculosis, and HIV were found to be common. These findings advance our understanding of the extent to which polyparasitism exists in the global South and the potential factors that could underlie this phenomenon. These findings support the call for more comprehensive disease control strategies that integrate treatment protocols or seek to redress the underlying determinants common across such infections.

1502

REACHING THE UNREACHABLE-LEVERAGING LESSONS LEARNED FROM MALARIA SERVICE DELIVERY PROGRAMS TO EXPAND INTEGRATED COMMUNITY CASE MANAGEMENT IN REMOTE AREAS OF PAPUA NEW GUINEA

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Access to care remains a challenge for those living in Papua New Guinea (PNG), many of the aidposts are not functional and quality services for malaria, pneumonia and diarrhoea are not always available. The Home-based Management of Malaria (HMM) program managed by Population Services International (PSI) attempted to bridge the malaria service gap in three provinces (East Sepik, East New Britain and Sandaun) using volunteer community-based distributors (CBD). Provinces were selected based on malaria incidence, access to health services and buy-in from the Provincial Health Administration (PHA). The HMM program covered an estimated population of 616,559 with 1,000 active CBDs. In 2016, PSI conducted an evaluation using a household survey, the first of its kind PNG, to compare treatment seeking behaviour for fever among caregivers for children under 5 in HMM and non-HMM communities. To explore potential for integrated community case management (iCCM), the study assessed knowledge, attitudes and treatment seeking practices for diarrhoea and pneumonia. PSI also conducted 13 qualitative interviews with CBDs to understand their experience during the HMM project. Survey results indicated a positive impact of HMM in most rural provinces. In Sandaun, availability of CBDs correlated with fewer caregivers first treating at home and CBDs were their first choice of external care. In East Sepik and Sandaun, CBDs were the preferred source of care by caregivers because they were 'close by or easy to reach'. Results in East New Britain were different, due to increased use of hospitals and less use of CBDs. The results found a high prevalence of diarrhoea, but only 7% of caregivers knew correct treatment. Pneumonia was less reported and understood- 50% of caregivers could not name the main symptoms. Interviews with CBDs indicated a positive perception of HMM and their desire to continue serving. Given the success of HMM in rural areas, we should consider using this channel to roll out iCCM. Discussion on how to ensure continued quality should be considered as well as the operating model with the PHA to deepen integration into the public-sector service provision.

1503

ROLE OF HEALTH-SEEKING BEHAVIOR AND NON-ADHERENCE IN ELIMINATION AND ON DISEASE DYNAMICS OF VISCERAL LEISHMANIASIS IN BIHAR, INDIA

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Visceral Leishmaniasis (VL) is one of the neglected vector-borne tropical diseases with an estimated 40,000 cases and 20,000 deaths annually worldwide. Of these almost 45% cases are from India in which more than 90% are from the Bihar state. In 2005, the governments of India, Nepal and Bangladesh in collaboration with the World Health Organization (WHO) launched a regional initiative to eliminate VL. Elimination is defined as reducing incidence to less than one per 10,000 inhabitants per year at sub-district level so that it would cease to be of public health importance. Elimination was not achieved in spite of multiple target efforts and years (2015 and 2017 successively); WHO has now set the target to year 2020. The national strategy for elimination, which is in line with the regional strategic framework for Southeast Asia, includes early case detection, integrated vector management, surveillance, strengthening human resource capacity, control program management etc. However, these efforts fail to monitor hidden population of patients initiating treatment but not following it properly. Thus, the current challenges in controlling VL in Bihar include (may not be limited to) delay in seeking healthcare, treatment non-adherence, immunodeficiency due to protein malnutrition,

supply and availability of drugs and poor surveillance. This study highlights the critical mechanisms like health-seeking behavior of infected individuals, their treatment non-adherence and the role of self healing individuals as potential for disease elimination via data-driven dynamic mathematical models. These mechanisms are crucial because they act as hindrance in the elimination process delaying the achievement of the target. This work also assesses the combined effects of improving efforts in this direction along with enhanced integrated vector management programs. We conclude that, although it is important to put efforts in decreasing delay in health-seeking and increasing perfectly adherent cases, the highest impact would be caused by completely treating temporarily recovered non-adherent individuals who act as the hidden reservoirs for infection.

1504

CONNECTING GLOBAL AND LOCAL PUBLIC HEALTH: WHY GLOBAL HEALTH SECURITY MATTERS TO LOCAL JURISDICTIONS IN THE U.S.

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Global health security (GHS) requires public health capacity to identify health threats, stop outbreaks, and save lives. In addition to health, there are many other ways in which GHS benefits the US. To assess global and local interconnectivity, we examined ways GHS relates to health and economic threats at the state and local level throughout the U.S. We assessed potential connections of GHS to domestic concerns, namely: state and local preparedness, travel, tourism, education, exports, jobs, agriculture, and partnerships. Publicly available data were gathered from seven US Government Departments (Agriculture, Commerce, Defense, Health and Human Services, Homeland Security, State, and Transportation) and from all 50 state government websites. A total of 22 data points were abstracted from these sources. Case examples of human imported diseases, zoonotic disease outbreaks, global health partnerships, and economic impact of outbreaks were drawn from news media and US government reports. The nature of interconnectivity varied by state, highlighting the importance of different facets of GHS across the country. For example, Texas has been the lead state exporter since 2010, with $264.1 billion in goods exported overseas in 2017 and over 900,000 export-supported jobs. Comparatively, poultry is the top sector of Georgia's top industry (agriculture), with 1.4 billion broiler chickens produced in 2016, making avian influenza a major concern. For smaller states, data helped underscore that GHS's relevance is not limited to states with a high volume of travelers; statistics about state residents overseas and companies with global ties further made this connection. Challenges in assessing interconnectivity using public domain data include the need for rigorous validation of data and reputable sourcing. However, data aids in making clearer the benefits of stopping outbreaks overseas and shows linkages to states. Although there are differences across states, using local data may illuminate the links between domestic issues and GHS. The number and variety of connections between GHS and states may make GHS more relevant to US stakeholders.

1505

PREGNANT WOMEN AND VACCINES AGAINST EMERGING PATHOGENS: ETHICS GUIDANCE ON AN INCLUSIVE AND RESPONSIVE RESEARCH AGENDA AND EPIDEMIC RESPONSE

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Zika virus, H1N1, and Ebola have called attention to how infectious disease outbreaks can severely - and at times uniquely - affect the health interests of pregnant women and their offspring. These examples highlight the critical need to proactively consider pregnant women and their offspring in vaccine research & development (R&D) to combat emerging infectious diseases. New vaccines are rarely designed with the specific needs of pregnant women in mind, and evidence about their safety and efficacy in pregnancy is often limited and late in coming. Significant questions remain about what is required to ensure that pregnant women and their offspring are adequately protected and fairly accounted for in vaccine development and deployment for emerging epidemic threats. To develop guidance, we convened an expert working group comprised of ethicists, public health practitioners, vaccine researchers, and maternal and child health specialists. We also conducted consultations with more than 70 leading experts in vaccine science, maternal-fetal medicine, public health preparedness and response, regulatory affairs, and ethics oversight. Literature reviews supplemented findings from consultations and working group meetings. The guidance highlights a vision in which: pregnant women and their offspring have safe, effective, and accessible vaccines to protect them against emerging epidemic threats; pregnant women and their offspring can benefit from advances in vaccine technologies and are note left behind as new products are developed; and pregnant women are not unjustifiably denied opportunities to participate in vaccine trials from which they may benefit solely because they are pregnant. A set of strategic objectives and concrete recommendations are put forth, focusing on: (I) Public Health & Science Preparedness, (II) Vaccine R&D, (III) Vaccine Deployment During Outbreaks. Adequately addressing the interests of pregnant women in the development and deployment of vaccines against emerging pathogens is essential to mitigating the potential harms they and their offspring face in epidemics. It is also a matter of justice and respect.

1506

INTERNATIONAL BIOLOGICAL REFERENCE PREPARATIONS FOR EMERGING INFECTIOUS DISEASES: VITAL TOOLS IN EPIDEMIC PREPAREDNESS

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Recent years have seen unprecedented investment in research and development (R&D) for countermeasures for high threat pathogens, with specific and ambitious objectives for development of diagnostics, therapeutics, and vaccines. Biological reference materials (BRMs) are materials, such as antigens, antibodies and nucleic acids, with carefully standardised biological activity. The inadequate availability of BRMs for high-threat diseases with epidemic potential poses a genuine obstacle in pursuit of R&D objectives, and the lack of a comprehensive and equitable framework for developing BRMs is a weakness. BRMs are essential for calibration of bioassays and standardisation of vaccines and bio-therapeutics, allowing for data comparison and harmonisation. In 2016, the WHO launched the R&D Blueprint, an initiative that seeks to enhance global health security by building upon the successes, and addressing the gaps identified during the 2014 Ebolavirus disease (EVD) epidemic. Central to the blueprint is a list of priority diseases and a roadmap for each disease that encompasses diagnostics, vaccines and therapeutics. During the 2014 EVD outbreak the lack of Ebola RNA, antigen and antibody BRMs hampered the development of accurate diagnostics and vaccines. This is echoed in the recent MERS-CoV and Zika virus epidemics. Interim antibody reference standards for Ebola were approved in 2015, otherwise no international serology BRMs exist for any other disease on the R&D Blueprint. Availability of BRMs for these diseases prior to occurrence of outbreaks would facilitate product development and research activities. In this presentation we outline the need for internationally standardized BRMs for high threat pathogens, as a core element of global health security. We outline key components of a structured framework to address this deficiency, with the aim of standardising approaches to sourcing and acquiring material for BRM development for priority high-threat pathogens

to allow for production of BRMs in advance of outbreaks, or the early initiation of sample acquisition and BRM development, in the event that an outbreak occurs.

1507

IMPROVING PRECISION OF HEALTH CAMPAIGNS THROUGH AN API FOR IDENTIFYING RESIDENTIAL BUILDINGS

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Health programs focused on delivering services at the household level, such as vaccination or indoor residual spraying of insecticide campaigns, can benefit from accurate maps of residential buildings. Most open building footprint data lack information as to whether buildings are residential or not. While previous work has demonstrated how building data from OpenStreetMaps (OSM) can be classified as residential and non-residential by means of machine learning techniques, many field workers do not have the capacity to implement these algorithms. Here we present the development of an Application Programming Interface (API) where we make these models available to users online. By defining a polygon of interest, the user can receive a geojson file of all OSM buildings with their predicted residential/non-residential classification. The results of the query can be downloaded and integrated into the health program workflow if needed.

1508

MULTI-LEVEL CORRELATES INFLUENCING PROVIDER DEVIATIONS FROM ESTABLISHED PEDIATRIC TREATMENT GUIDELINES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Household surveys from 39 countries indicate that many acutely ill children are not provided necessary evidence-based interventions upon presentation at health facilities; half of children with suspected pneumonia receive antibiotics, one-third of children with diarrhea receive ORS, and <1% of children with diarrhea receive zinc. Additionally, evidence from multiple settings indicates that training alone does not improve adherence to guidelines. The purpose of this systematic review is to identify the multi-level correlates that influence provider deviations from established pediatric treatment guidelines. This systematic review followed PRISMA guidelines and included studies indexed on Medline and Embase. Eligible studies included pediatric populations (ages 10 and under) and were conducted in low-and-middle-income settings. Studies included randomized designs, quasi-experimental designs, and observational studies. Of the 2,896 titles retrieved, 339 studies were identified for full-text review, and 41 full-text articles met inclusion criteria. 16 full-text articles remain under review, pending disaggregated data contributions from study authors. It is estimated that all data will be made available by May 2018 and meta-analyses will be completed in July 2018. Included studies identified patient-, provider-, facility-, intervention-, and guideline-level correlates of guideline adherence and deviation. Thus far, 18 distinct correlates have been identified, with provider training, perceived disease severity, and patient age emerging as the most commonly studied. We will conduct meta-analyses to derive pooled estimates of the relative influence of each correlate on guideline adherence or deviation. Interim results indicate that there is extensive research on factors associated with training and provider experience and that there is less research on multi-level factors influencing practice behaviors, above and beyond the providers themselves. Identification and appreciation of multi-level factors will be important for identifying health systems solutions for the complex drivers of guideline deviation.

1509

USING PARTICIPATORY WORKSHOPS TO ASSESS COMMUNITY ALIGNMENT OR TENSION FOR CHILD MORTALITY SURVEILLANCE INVOLVING MINIMALLY INVASIVE TISSUE SAMPLING

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The Child Health and Mortality Prevention Surveillance (CHAMPS) Network aims to address gaps in knowledge about under-5 mortality by determining the causes of death in seven countries through multiple methods of data collection, including minimally invasive tissue sampling (MITS). Adapting earlier methods of community health asset mapping, we developed a novel community entry activity designed to introduce child mortality and pregnancy surveillance activities. Workshops for community members and community leaders were conducted to elicit perspectives related to child death and dying; autopsy; medical/clinical care and antenatal care; and alignments/tensions between CHAMPS and community priorities. In total, 72 workshops were conducted with 1,579 participants in five CHAMPS sites - Bangladesh, Ethiopia, Kenya, Mozambique, and South Africa - between October 2016 and March 2018. In the aggregate, 80% of participants' responses reflected alignment between mortality surveillance (including MITS) and community norms. However, there was tension in the context of certain cultural factors, most notably religious beliefs. 57% of participants agreed that any procedure (including MITS) conflicting with the burial practices of a religious tradition should not be conducted and 48% agreed that care of the body after death was a religious/spiritual issue and the work of God. The majority of those who held such beliefs did not indicate that they would have negative perceptions of others who consented to MITS. However, for a significant minority, such beliefs could support stigmatizing attitudes toward anyone who participated: 21% of all participants said that no parents should consent to MITS and 10% stated a general belief that tissue removal was always wrong. The results underscore the importance of engaging communities to understand cultural and religious beliefs that may influence the feasibility, including acceptability and gain appreciation for CHAMPS post mortem procedures.

1510

FOCUSING BEHAVIOR CHANGE EFFORTS TO MAXIMIZE USAID-LED ZIKA PREVENTION EFFORTS IN CENTRAL AMERICA AND THE CARIBBEAN: LESSONS FOR FUTURE PUBLIC HEALTH EMERGENCIES

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Since the Zika outbreak in 2015, there has been a proliferation of behavior change messages and campaigns, with over 30 specific preventive behaviors being promoted one year after the initiation of USAID's response. This large number of behaviors presents a challenge to the potential effectiveness of social and behavior change (SBC) programming efforts to prevent Zika at the household and community level. Clear, specific, and concise messages that can be repeated multiple times are necessary to effectively promote behavior change. This situation prompted USAID and partners to develop an evidence-based strategy to select focus behaviors and harmonize messaging across the USAID response. The process began with a literature review of all behaviors being recommended, with five excluded due to lack of strong evidence for efficacy. Two behaviors were also excluded from the prioritization process because USAID was not procuring the required materials. Lastly, the focus was on individual or household level behaviors, those whose locus of control remained within individuals in the household. The literature review included all peer-reviewed journal articles published between 2010 and 2018, including any reports or seminal publications prior to 2010, regarding any *Aedes aegypti* spread diseases. Grey literature or unpublished data provided by implementing partner organizations was also collected through the Zika Communication Network. The findings were compiled into an evidence matrix combining evidence on efficacy and effectiveness of behaviors. Subsequently, four criteria were developed to aid in the prioritization: 1) efficacy of the behavior to prevent negative pregnancy outcomes; 2) potential to reduce Zika transmission at population level; 3) Frequency required to be effective; 4) Ease of access to materials required. Criteria were ranked high, medium or low for each behavior through a combination of evidence review, and discussions with key informants and implementing partners. The goal is to harmonize Zika response efforts around a shorter and more specific set of key prevention behaviors to reduce the risk of Zika transmission.

1511

THE USE OF A CAMPAIGN INFORMATION MANAGEMENT SYSTEM FOR RAPID AND EFFICIENT MASS DISTRIBUTION AND MONITORING OF LONG-LASTING INSECTICIDAL NETS IN AN URBAN SETTING OF BIOKO ISLAND

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It is well established that long-lasting insecticidal nets (LLINs) can be used as a core vector control tool to reduce malaria transmission in endemic countries. LLINs provide both personal and community protection against malaria. For LLINs to provide community protection, universal coverage of every household having at least one LLIN for every two people in a given population is recommended. Ensuring universal coverage during mass distribution campaigns, and monitoring the use and durability of nets in urban settings characterized by high population density and mobility, particularly where houses are not enumerated, poses a major challenge. The Bioko Island Malaria Control Project (BIMCP) developed an Open Data Kit (ODK) and Geographical Information System (GIS) based Campaign Information Management System (CIMS) in 2014 for efficient and rapid household enumeration, LLIN distribution, and campaign monitoring on Bioko Island. The CIMS continues to be used in 2018 to carry out a mass LLIN distribution campaign on Bioko Island. Approximately 175,000 LLINs will be distributed door-to-door to a population of about 330,000 people in 85,000 households during a five-month period by a team of 50 enumerators and 100 volunteers. This system helps to identify enumerated households for field teams, facilitates revisiting closed or rejecting households to increase coverage, and can track nets distributed at the household level. Household information can be captured and analyzed in near-real-time to estimate coverages within defined geographic areas and used to allocate and mobilize additional resources if desired coverages have not been reached. The use of the CIMS tablet application has required substantial training for enumerators, but has ultimately increased operational efficiency and programmatic integrity, and has been adapted for use in multiple concurrent large-scale campaigns.

1512

FINE-SCALE MAPPING OF LOCALITIES HOUSEHOLDS TO PLAN, IMPLEMENT, MONITOR AND EVALUATE MALARIA CONTROL CAMPAIGNS ON BIOKO ISLAND, EQUATORIAL GUINEA

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The Bioko Island Malaria Control Project (BIMCP), in collaboration with the National Malaria Control Program (NMCP) of the Ministry of Health and Social Welfare (MOHSW) of Equatorial Guinea, has been implementing malaria control activities on Bioko Island for at least 14 years. Like many developing nations, the urban environment of Bioko Island is characterized by informal housing, a lack of a household address system, and the absence of formalized geospatial administrative units. In 2011, the BIMCP established a Geographic Information System (GIS) to enumerate households to track household interventions and has collaborated with the Ministry of the Interior to delineate geopolitical administrative units. The GIS mapping efforts have allowed the BIMCP and the Equatorial Guinea Malaria Vaccine Initiative (EGMVI) to plan, mobilize, monitor, evaluate interventions at the household level, and aggregate results at different spatial scales depending on their needs. The system has been possible largely due to considerable support from project donors, collaboration with the government of Equatorial Guinea, and also due to the size of the island (2,017 km2), with less than 100 km2 of which is populated. These factors have made it possible to map populated areas at very fine-scales within small geopolitical units undergoing rapid change due to the country's economic development. Fine-scale urban mapping has been a challenge on Bioko, as 95% of the inhabitants live in a densely populated urban core representing more than half of the island's total populated area. High-resolution mapping of localities and households has enabled the BIMCP to track household interventions in near-real-time, and the outputs have empowered local authorities and leaders to take action in their communities to increase intervention coverage and individual uptake.

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ASSESSING IRS PERFORMANCE AND BARRIERS IN A GENDER-INTEGRATED VECTOR CONTROL PROGRAM ON BIOKO ISLAND, EQUATORIAL GUINEA

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Due to social, economic and physiological barriers, women have historically been underrepresented in spray programs across Sub-Saharan Africa. The Bioko Island Malaria Control Project (BIMCP) has implemented IRS as one of the primary vector control strategies for malaria control on Bioko Island since 2004. This study aims to assess the performance and barriers for engaging women to become IRS operators and assume supervisory positions using data from 2012 to 2018, where approximately 35% of all spray operators have been female. In part, this gender balance has been achieved through guidelines such as equal pay for men and women,

ensuring physical work environment provides safe and private areas for women, pregnancy testing for female operators before every round for safety reasons, and continued financial support for women during maternity leave. The BIMCP collects routine IRS data for every sprayer, assesses the quality of spraying techniques and guidelines through direct observation, and quantifies the insecticide sprayed on walls with high-performance liquid chromatography (HPLC) for assessing sprayer quality control. This study will evaluate the gender policies in place to assess the engagement of women in vector control. It will evaluate not only the productivity of male and female counterparts, comparing attendance and the average number of households and rooms sprayed daily; it will also evaluate the quality of insecticide coverage achieved by male and female operators using results from the HPLC studies and the number of performance infractions recorded by supervisors. As it enters Phase IV of implementation, the BIMCP hopes to continue to engage female spray operators and encourage them to seek higher positions as supervisors and team managers.

1514

IMPACT OF POPULATION MODELING CHOICES ON EPIDEMIOLOGICAL ESTIMATES: IMPLICATIONS FOR INTEGRATED DISEASE CONTROL

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Epidemiological analyses rely on accurate population counts. Increasingly, in locations with scarce or infrequent census data, modeled population estimates based on satellite data or demographic surveys are used as the denominator for infectious disease prevalence estimations and intervention targeting, in place of empirically determined population counts. While these modeled population datasets serve a useful function, there have been few studies evaluating how the selection of population data impacts the resultant disease prevalence estimates or other relevant public health measurements. This project uses malaria control in the Democratic Republic of Congo (DRC) as a case study to examine how the choice of population dataset impacts how underlying spatial distributions of human populations are understood, and therefore how the selection of population data effects the calculated estimates of malaria prevalence and unmet bednet need. The DRC is a particularly salient study site for this work since there has not been a comprehensive national census since 1984, and health studies in the country frequently draw on modeled population estimates. Using spatial methods, this study compares three widely available modeled population datasets to identify the health zones and provinces in the DRC displaying significantly high variability in population size across different population data sources. Of the 515 health zones in the DRC, 263 exhibit a difference in modeled population size of 50,000 or more between the LandScan and WorldPop population datasets. Health zones with the highest variability between population datasets are examined to identify environmental, demographic, and infrastructure characteristics that correlate with low consistency in population estimation. The variability in population estimates in each health zone is applied to understanding variation in the burden of malaria among key population groups in the DRC and targeting bednet distribution campaigns. Implications for selecting sites for future microcensuses to guide malaria interventions are discussed.

1515

UPTAKE OF CONTRACEPTIVES AFTER ABORTION AMONG ADOLESCENT GIRLS AT QUEEN ELIZABETH CENTRAL HOSPITAL IN BLANTYRE, MALAWI

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In Malawi, contraceptive use among adolescents is low, resulting in early childbearing with increased risk of obstetric complications and maternal death. However, there are opportunities to provide contraceptive counseling even after an initial pregnancy has occurred. This study, therefore, explored factors influencing uptake of contraceptives among adolescent girls receiving post abortion care at a large urban facility, Queen Elizabeth Central Hospital, in Blantyre, Malawi. We employed a descriptive cross-sectional quantitative study, randomly sampling 97 adolescent girls meeting the inclusion criteria. Participants were interviewed using a structured questionnaire. Data were entered on EPIDATA and analyzed via SPSS version 20.0. We analyzed socio-demographic statistics, chi-square, and fisher's exact tests to explore associations between socio-demographic characteristics and uptake of contraceptives. Over half 58 (59.8%) of 97 adolescent girls who came for PAC services accepted a contraceptive method. Fifty-six (96.6%) of those that chose to use contraceptives were between 16 and 19 years old with the most common method of choice being the oral contraceptive pill. The main source of information on contraceptive use and sexuality were peers, and the most common reason for refusing contraceptives was fear of side effects. Privacy and confidentiality were the main facilitating factors in accepting contraceptives while parental disapproval was found to be the main hindering factor. Contraceptive uptake was significantly associated with education (fisher's exact p=0.022), religion (fisher's exact p=0.034), and significantly associated with age (fisher's exact p=0.044). Based on the results, we encourage strengthening youth-friendly services at PAC sites, family planning outreach to very young teens, programs that focus on retaining young women in school, and collaboration with local youth agencies to potentially avert unwanted pregnancies and unsafe abortions in teens.

1516

HOUSEHOLD HEALTH EXPENDITURES FOR BIRTH IN BLANTYRE, MALAWI

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Health financing is one of the seven priority areas identified in the Malawi National Health Policy, noting that the nation struggles with inconsistent domestic funding, and inefficient and inequitably allocated resources. These larger issues filter down to impact households in paying for normal life events. In this study we examined health expenditures for births in Malawi, recruiting 388 participants using purposive sampling, collecting data via structured questionnaires on expenditures incurred while planning for an impending birth. We then asked participants to make discrete choices on funding future births. Data analysis was conducted using SPSS version 20.0. Statistics were presented using frequencies and percentages. The results indicate that 97% of the sample paid for birth services of which 33% of participants had been asked to pay informal fees while accessing birth services at public health facilities, the site of most births (87%). Fees were requested for services or commodities such as medicines, ultrasound services, and pregnancy tests. More than 58% of the costs were paid out of pocket as only 10% of participants had Health Insurance as a mode of prepayment. Other expenses such as transport, medicines from private pharmacies, food, and materials that hospitals require laboring women to provide (tarp, sheets, etc) contribute additional oop costs. Participants rely on public health facilities for birth because they offer "free "services. Yet, in most cases the cumulative payments associated with birth and the perinatal period can exceed 10% of income in period, constituting a catastrophic cost. When offered the choice, 57% of participants expressed willingness to enroll in a health scheme. When offered five choices for financing, 32.8% of participants chose bank savings, 32.3% health insurance , 21.7 community based, 7.6% group financing, and 5.6% chose to pay solely out of pocket. Malawians in an urban setting expressed interest in alternate means of paying for birth. The government of Malawi could improve access to maternal health services via financial risk protections rather than relying solely on out of pocket financing.

1517

BROAD CONSENT FOR DATA SHARING

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There is now increasing expectation from research funders, regulatory agencies, and journals for greater sharing of individual-level data from health research. Broad consent appears to be the preferred mechanism to obtain consent for such sharing. There is a paucity of empirical data on broad consent especially in tropical medicine research. The present study seeks to understand what aspects of data sharing are considered most important to inform participants about in the context of broad consent, and the best ways to explain complex and abstract topics relating to data sharing. This was a qualitative study conducted in Bangkok and at the Thai-Myanmar border town of Mae Sot. We conducted 18 semi-structured interviews and four focus group discussions with a total of 37 participants from three stakeholder groups. Our study identified the type of information that participants thought was important to provide: that data sharing has potential benefits, the data will be de-identified, that mechanisms are in place to minimize potential harms to participants, and participants will not be inconvenienced by such sharing. There was however, no consensus among participants on how much information about data sharing to provide. Our data suggest that how much information should be dependent on the primary study, the study population, the individual research participant and the context. During the qualitative study, we found that communicating data sharing to participants was challenging. Additionally, participants thought that, in most cases, research participants should be able to take part in the primary biomedical study without consenting to data sharing. Communicating the concept of data sharing to participants was found to be challenging, with the level of understanding of data sharing and views on how best to consent to data sharing varying with context and participant population.

1518

GEOGRAPHICAL ASSESSMENT OF SIX MOST COMMON INFECTIOUS DISEASES IN SEVEN LARGE CITIES IN INDONESIA USING GEOGRAPHICAL INFORMATION SYSTEMS

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Infectious disease is a major burden in tropical countries like Indonesia. To reduce the transmission when certain pathogens are spreading in an area, early case detection and fast mobilization of resources are needed. Maps are commonly used to depict where infectious disease cases most frequently occur. Geographic Information System (GIS) technology, an open source software, has greatly increased the speed and accuracy which spatial information on infectious disease cases can be collected, integrated with other information to obtain the dynamic of pathogen transmission, and easily accessed by users in limited resource setting. To have a better understanding of where the six most common infectious diseases were distributed in 7 large cities in Indonesia and the dynamic of the pathogens, data from acute febrile illness requiring hospitalization (AFIRE) study were used. These data included GPS coordinate of participants' address, date of fever, onset of illness, and probable pathogens, which were identified by blood culture, molecular and serology assays. 823 from 1486 subjects had etiology confirmed with dengue (483 cases), *Rickettsia typhi* (103 cases), *Salmonella spp.* (103 cases), influenza (68 cases), *Leptospira spp.* (44 cases), and chikungunya (37 cases) as the most prevalent pathogens. Each case was grouped by date of illness and plotted based on coordinate of the address using open source GIS. Dengue cases were scattered in all areas of the cities, whereas *R. typhi* and *Leptospira spp.* cases were clustering in the center. Sporadic transmission occurred for three most prevalent viruses, dengue, chikungunya and influenza. Dengue serotypes were distributed through the year, with one serotype dominating for a specific duration before being replaced by the other serotypes. The application of GIS for integration of health surveillance systems proven to be useful tool to provide real-time information regarding the pathogens dynamic transmission in Indonesia. If the health providers aware of the prevalence and spatial distribution of the disease within their communities, an early detection could occur thus improve the health outcomes.

1519

PUBLICATION RATES OF ABSTRACTS PRESENTED AT THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE ANNUAL MEETINGS

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Annual research meetings represent an important opportunity for researchers to share their research and obtain feedback, previous its publication. However, a significant fraction of the abstracts accepted at these meetings is never published. We aimed to determine the publication rates of abstracts presented at the American Society of Tropical Medicine and Hygiene (ASTMH) annual meetings in the last decade. We performed a retrospective cohort study and assessed a random sample of the totality of abstracts presented at the ASTMH Annual Meetings (2008-2014). The study outcome was the publication rate in peer-reviewed journals indexed in Pubmed, Scopus, Embase, or Web of Science using first author last name and the primary medical subject heading term. Covariates of interest included study design, number of authors, first author's affiliation, first author's affiliation' country, year of publication, journals metrics, and institution. A random sample of 2,564 abstracts was obtained (~20%, N=12,875), for an average of 1,839±318 abstracts per annual meeting. Of these, around 25±1% were accepted for oral presentations. The overall publication rate was 46% (95% Confidence Interval, 38-53%) with no tendency to increase (p-value=0.34). This publication rate seems to be positively associated with the study design (Experimental vs. Observational studies), number of authors (more than five authors vs. five or less), and journal (AJTMH vs. Other); but negatively associated with the first author's affiliation (Public vs. non-public), first author's affiliation' country (High-income countries vs. low/middle-income countries), and type of abstract (oral vs. poster). However, at the multivariable model most important predictors of publication rate was an oral presentation (55% vs. 43%, RR 1.3, 95%CI 1.2-1.4) and experimental design (67% vs. 40%, RR 1.7, 95%CI 1.5-1.8). The publication rate of the abstracts accepted at the ASTMH seems to be low and steady in the last decade, which seems to be related to a variety of factors including study's design and oral presentation.

1520

WHAT WOMEN WANT DURING INSTITUTIONAL DELIVERY: EXPLORING THE DIS-UTILITIES ASSOCIATED WITH ATTRIBUTES OF DISRESPECT AND ABUSE DURING INSTITUTIONAL BIRTH

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Achieving universal health coverage, an important part of sustainable development goals, will require not only that health services are

available, but people would want to access the services. Recent studies have highlighted the relationship between disrespect and abuse during institutional delivery and women willingness to subsequently access health care in health facilities. This study explored the values women attached to attributes of respectful care. We conducted a cross-sectional study of women with recent facility birth 0-6months in Gombe state Nigeria. We assessed their preferences for different attributes of respectful maternity care using discrete choice experiment. Women preferences for place of delivery and willingness to deliver in a health facility are strongly and negatively associated with attributes of dis-respect and abuse during institutional birth, including poor communication such as not informing the woman what is being done to her or what to expect during labour, allowed to ask question or being answered to politely during labour and delivery (B – 0.82, p <0.000), denied safe traditional birth practices during labour and delivery (B – 1.70, p <0.00), non-supportive care (B – 0.59, p <0.005). Women attached strong dis-utility to shortage of staff or the likelihood of not meeting a health worker (B – 2.08, p <0.000). In conclusion, our results suggest that women have a clear and strong negative preferences for attributes of disrespect and abuse practices during institutional delivery. Understanding and recognizing these preferences may be particularly useful in designing effective interventions aimed at increasing the demand and delivery of quality and respectful maternity care.

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A QUALITATIVE STUDY OF BARRIERS AND ENABLERS TO THE ESTABLISHMENT OF ROBUST CLINICAL RESEARCH DATA MANAGEMENT SYSTEMS IN LOW AND MIDDLE INCOME COUNTRIES

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Data Management (DM) is an essential component of clinical research, which is however poorly developed, especially in Low and Middle Income Countries (LMICs). Strengthening capacity for DM is essential, so that investigators in LMICs have the skills and resources to organise and analyse their data, and to use the information to guide policy, while also responding to increased demands for data sharing. While the need to understand the barriers and enablers to the establishment of robust clinical research Data Management Systems (DMS) is clear, little empirical research has been performed on this topic, particularly in LMICs. We therefore have conducted a qualitative study, recruiting participants in Dakar, Senegal and Brazzaville, Republic of the Congo. Our objective was to explore the current DM practices and to identify areas where additional training and resources are most needed. We have interviewed eight to twelve clinical research staff involved in data management or quality control at each site. Data from these in-depth interviews were complemented by informal conversations with key informants, and by observations made by the primary researcher (and interviewer) during an immersive fieldwork period of 3-4 weeks at each site. Our findings confirm that running robust DMS requires adequate financing and the existence of strong Information and Technology (IT) infrastructure (the 'hard components' of the DMS). More importantly, they highlight the significance of 'soft components', including good communication and social interactions among those involved in clinical research DM. Our data also emphasise the necessity to establish DM as a recognised and valued professional role in academia and disease control programmes; as well as the need to raise awareness of the complexity of this field and of the critical role of well-trained data managers. We will discuss the contribution of these findings to strengthening clinical research DM capacity in similar LMIC settings.

1522

MULTI SECTORAL APPROACHES TO PREVENT AND CONTROL MALARIA AND ARBOVIRAL DISEASES

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Vector-Borne Diseases (VBDs) including malaria and emerging arboviral diseases account of about one quarter of all infectious diseases. Although some progress has been seen for malaria, other diseases such as those caused by arboviruses such as Dengue, Chikungunya, Yellow fever and more recently Zika, are expanding, with increased number of cases and fatalities.It has become evident that the prevention and control of these diseases have to go beyond health and to include more than a single sector approach since the transmissions patterns are driven by vector-host-pathogen- relationships which dynamically interact with natural conditions, human societies and vector parameters. The importance of multi sectoral collaborations has, therefore, been unanimously been recognized by countries when approving the Global Vector Control Response (GVCR) at the 70th World Health Assembly in May 2017. The GVCR includes multi-sectoral approaches as one the four pillars to develop an appropriate and sustainable response. The Swiss Agency for Development and Cooperation (SDC), the Canada' International Development Research Centre (IDRC), the Swiss Tropical and Public Health Institute (STPH) and the Vector Environment and Society Unit of TDR/WHO have joined forces with the ultimate aim to build a conceptual framework for multi sectoral approaches to prevent and control malaria and emerging arboviral diseases based on available evidences and subsequent implementation case studies. In a first step, five scientific reviews were commissioned to explore the landscape of the multi-sectoral approaches for the prevention and control of vector-borne diseases. In a second step, we now aim to use the available evidences to discuss priorities topics for implementation activities, and - on this basis - to develop a conceptual framework to provide to stakeholders some guidelines on how to develop multi-sectorial VBDs control.

1523

COMMUNITY-BASED SURVEILLANCE OF NOTIFIABLE DISEASES THROUGH THE STRATEGY OF COMMUNITY WATCH AND ALERT COMMITTEE (CWAC) IN SENEGAL

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Early response to an epidemic, depends on early detection, which cannot be done without effective involvement of the community. In Senegal, an external joint evaluation, highlighted shortcomings in the national surveillance system, including those at the community level. Catholic Relief Services, with funding from the CDC, supports the Ministry of Health and Social Services' (MHSS) priority of strengthening community disease surveillance. In Diourbel region, community surveillance of diseases, having epidemic outbreak potential, is managed through the Community Watch and Alert Committee (CWAC) strategy. Each CWAC member investigates suspected cases of 1 of 8 diseases under surveillance (cholera, yellow fever, measles, neonatal tetanus, bloody diarrhea, poliomyelitis, meningitis and Ebola), or any unusual event/rumor, according to community case definitions. Identified alerts are immediately notified by telephone to

the Head of Health Post known as "Infirmier Chef de Poste" (ICP) who will carry out the necessary investigations within 48 hours to confirm or cancel the alert. Once the alert is confirmed, the ICP will follow instructions from the central level for the management of suspected cases, including removal of the suspected case to transport of samples to the reference laboratory for analysis. Program activities include training of community actors, regular supervision, and an evaluation after 6 months of implementation to share the results, and better understand the level of national ownership at all levels of the health system.After six months of implementation, 108 alerts were received. The top 5 disease alerts included: Bloody diarrhea: 50 alerts (42 investigated and 30 confirmed suspected cases); Cholera: 16 alerts (16 investigated and 0 confirmed); Measles: 14 alerts (12 investigated and 5 confirmed suspected cases); Yellow fever: 13 alerts (12 investigated and 10 confirmed suspected cases); and, Poliomyelitis: 5 alerts (4 investigated and 4 confirmed suspected cases). Strengthening surveillance at the community level has enabled the Diourbel Region to improve its performance in epidemiological surveillance.

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STRENGTHENING THE HEALTH INFORMATION SYSTEM IN GUINEA

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Management of health information remains a thorny problem to ensure quality, promptness and completeness of health data for efficient decision making in West Africa. In 2014, the evaluation of the National Health Information System (NHIS) of Guinea, according to the PRISM (Performance of Routine Health Information System Management) approach, revealed the following weaknesses: absence of strategic and normative documents; absence of an electronic tool for data management; only about 20% of health reports were complete; absence of standard and harmonized data collection tools; and, no effective supervisions, all leading to a weak system unable to provide health information in real time. To address these issues and strengthen the health information system, Catholic Relief Services provided support focusing on 3 key areas: 1. Strengthening normative frameworks such as: the 2016-2020 National Health Information System Strategic Plan; developing a catalog of indicators for the health sector; manual of procedures for data management; as well as, monitoring tools focused on the Health Information System. 2. Strengthening routine data collection with the establishment of the DHIS2 national deployment management team. This resulted is 320 managers trained on DHIS2 (7 on Level 1 in Lomé), 260 executives trained in basic computer science and 2 in advanced DHIS2 in Oslo. 3. Capacity strengthening of operations in terms of various equipment. This support enabled the deployment of the DHIS2 and the data capture from 2015 to 2017. The rate of national level supervision on the function and us of the HMIS increased from 0% at the end of 2015 to 80% at the end of 2017, and data completeness improved from 20% to 97% at the end of 2017. The country now has standardized data collection tools, harmonized and available at each level of the health pyramid, and all health entities report in the DHIS2 software. Two challenges remain: the existence of parallel software systems by some of Ministry programs, and the inadequate use of DHIS2 data for decision-making. To address these challenges, discussions are underway to allow programs to migrate to DHIS2.

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MULTI-LOCUS SEQUENCE TYPING OF THE SHEEP TICK *IXODES RICINUS* AND ITS SYMBIONT *CANDIDATUS* MIDICHLORIA MITOCHONDRII ACROSS EUROPE REVEALS EVIDENCE OF LOCAL COEVOLUTION IN SCOTLAND

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Ticks have complex microbiomes, but only a small proportion of the bacterial symbionts recorded from ticks are vertically transmitted. Moreover, co-cladogenesis between ticks and their symbionts, indicating an intimate relationship over evolutionary history driven by a mutualistic association, is the exception rather than the rule. One of the most widespread tick symbionts is *Candidatus* Midichloria, which has been detected in all of the major tick genera of medical and veterinary importance. In some species of *Ixodes*, such as the sheep tick *Ixodes ricinus* (infected with *Ca*. Midichloria mitochondrii), the symbiont is fixed in wild adult female ticks, suggesting an obligate mutualism. However, almost no information is available on genetic variation in *Ca*. M. mitochondrii or possible co-cladogenesis with its host across its geographic range. Here, we report the first survey of *Ca*. M. mitochondrii in *I. ricinus* in Great Britain and a multi-locus sequence typing (MLST) analysis of tick and symbiont between British ticks and those collected in continental Europe. We show that while the prevalence of the symbiont in nymphs collected in England is similar to that reported from the continent, a higher prevalence in nymphs and adult males is apparent in Wales. In general, *Ca*. M. mitochondrii exhibits very low levels of sequence diversity, although a consistent signal of host-symbiont coevolution was apparent in Scotland. Moreover, the tick MLST scheme revealed that Scottish specimens form a clade that is partially separated from other British ticks, with almost no contribution of continental sequence types in this north-westerly border of the tick's natural range. The low diversity of *Ca*. M. mitochondrii, in contrast with previously reported high rates of polymorphism in *I. ricinus* mitogenomes, suggests that the symbiont may have swept across Europe recently via a horizontal, rather than vertical, transmission route.

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ENTOMOLOGIC INVESTIGATIONS OF RE-EMERGING CHIKUNGUNYA OUTBREAKS IN MOMBASA CITY, KENYA, 2018

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Chikungunya virus (CHIKV) is a mosquito borne Alphavirus, primarily transmitted by *Aedes aegypti* and *Ae. albopictus*. The first major chikungunya outbreak in Kenya was in 2004-2005. Since then, no transmissions were reported until a resurgence of the virus in Mombasa city in 2017. Entomologic investigations were conducted in January 2018 to determine mosquito spp. causing the outbreak, identify breeding sites associated with the vectors and estimate vector densities. BG-sentinel and CDC light traps were set during the day and night to collect adult mosquitoes which were processed and screened for CHIKV. Residential houses were stratified and water holding containers inside and outside of dwellings inspected for immatures of *Ae. aegypti*. Breeding habitats and

their locations were determined and container index (CI), house index (HI), breteau index (BI), pupal index (PI) and pupae per person index (PPI) calculated. A total of 6940 adult mosquitoes were collected by BG sentinel (91%) and CDC light traps (9%) identified to species and analyzed for infection. *Culex pipiens* were the most abundant (69%), *Ae. vittatus* (14%) and *Ae. aegypti* (13%). CHIKV was isolated from a pool of *Ae. aegypti* mosquitoes. A total of 131 houses with a population of 547 households were surveyed, representing mean population of 4.2/house. 528 immature mosquitoes were collected comprising *Ae. aegypti* (n=524;99.2%), *Ae. vitattus* (n=2;0.4%) and *Cx. pipens* (n=2;0.4%). *Ae. aegypti* immatures were observed in 48 houses (HI=37) and 70 out of 668 water containers (CI=11;BI=53). 70 pupae were collected representing pupal index of 53 and PPI of 0.53. Jerry cans were the predominant containers inspected (n=437;65%) but plastic drums (n=127) recorded the highest positivity rate (35%) and plastic tanks (n=115;20%) for immatures. Indoor and outdoor containers were positive with no significant difference in positivity (*P<0.05*). The risk of chikungunya transmission in Mombasa city was high and therefore vector control programs should be initiated to reduce vector population. The role of *Ae. vittatus* in CHIKV transmission needs to be investigated.

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SALIVARY AND ANTENNAL TRANSCRIPTOMES OF *CIMEX LECTULARIUS* AND FUNCTIONAL STUDIES OF SALIVARY NITROPHORIN

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Cimex lectularius, the bed bug, was nearly eradicated in the developed world shortly after World War II using DDT and other pesticides. A recent resurgence of bedbug infestations in urban areas of developed countries has caused concern among the public and researchers. Although *C. lectularius* is not known to transmit pathogens to humans, they do elicit a type I hypersensitivity reaction known as cimicosis. *Cimex* salivary proteins, including nitrophorin, have been linked to cimicosis, but the mechanism is still unknown. Using an Illumina-based RNA-seq analysis we characterized the differentially expressed transcripts of the salivary glands of *C. lectularius*. This data offers insight into the major transcripts expressed in the salivary glands of bed bugs and identifies targets for potential knock down screening. Results from RNAi trials indicate notably high silencing efficiency of salivary nitrophorin using double-stranded RNA and ongoing *in vivo* studies aim to to shed light on the immunological connection between *Cimex* salivary nitrophorin and cimicosis. A parallel study is underway to complete the first global analysis of the *Cimex* antennal transcriptome and an update on current progress will be included.

1528

DISTRIBUTION OF TICK SPECIES COLLECTED FROM SEVEN SITES IN NORTHERN AND SOUTHERN AREAS OF GHANA

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Ticks are important vectors and reservoirs for many zoonotic disease-causing pathogens worldwide. Africa has nearly 50 endemic tick species that are noted to infest domestic animals and livestock with the genera *Amblyomma, Rhipicephalus* and *Hyalomma* known to have the health effects on livestock among these endemic species in Africa. Some African countries have reported tick-borne infections like Crimean-Congo haemorrhagic fever including Ghana. However, there is scarce information on the diversity and distribution of the tick vector in Ghana. Identification of tick species and knowledge of their densities is critical for an effective vector control program. The distribution of arthropods is generally known to be influenced by environmental factors such as rainfall, humidity, temperature and vegetation thus ticks were collected from cattle, sheep and dogs within two ecological zones in Ghana (northern sahel savannah and coastal savannah) was assessed. A total of 1,925 ticks were collected from seven sites and morphologically identified. The combined results showed that *Amblyomma* (60.94%)*, Rhipicephalus (30.55%)* and *Hyalomma (8.51%)* genera were predominant in the study sites. 1240 ticks were collected in the northern sahel, which included four sites with Navrongo and Tamale, the species distribution was Amblyomma (60.6%), Rhipicephalus (28.5%) and Hyalomma (10.9%). 685 ticks were collected in the coastal savannah, which included three sites in Accra, Afienya and Shai Hills and the species distribution was Amblyomma (61.46%), Rhipicephalus (34.3%) and Hyalomma (4.24%). The *Amblyomma variegatum (60.94%)* species was the most common in both ecological zones. *Amblyomma variegatum* in addition to having a high impact on livestock health and productivity has been implicated in the transmission of tick-borne pathogens such as Crimean-Congo haemorrhagic fever virus and Dugbe virus, both of which have been isolated in Ghana. The prevalence of these three genera of ticks in the study sites highlight the need for continuous tick surveillance to give a clearer understanding of vector dynamics for that will contribute to a control strategy.

1529

FIRST REPORT OF SAND FLIES NATURALLY INFECTED WITH *LEISHMANIA NAIFFI* AND *BARTONELLA* SPP. IN THE PERUVIAN AMAZON

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In Peru, *Lutzomyia verrucarum* and *Lu. peruensis* are known vectors of cutaneous leishmaniasis and bartonellosis in the Andean region. However, there is limited or no information regarding sand fly vectors of these diseases in the Amazon region. In this study, we carried out sand fly collections from lowland and highland jungle areas of the Peruvian Amazon and tested them for *Leishmania* and *Bartonella* DNA. Sand flies were collected from Madre de Dios (292 m.a.s.l.) in July 2014, San Martin (421 m.a.s.l), and Cajamarca (1,231 m.a.s.l) in January 2015 using CDC light traps and Mosquito Magnet trap. A total of 277 female sand flies from Madre de Dios, 153 from Cajamarca, and 611 from San Martin were pooled by species, site, and trap type (1-10 individuals per pool) for molecular screening. *Leishmania* detection was performed by kDNA PCR followed by nested cytB PCR and sequencing. *Bartonella* screening was performed by ITS PCR followed by nested gltA PCR and sequencing. Seven of 48 pools (0.03 infection rate) from Madre de Dios and 11 of 146 pools (0.02 infection rate) from San Martin were kDNA PCR positive for *Leishmania* spp. One *Lutzomyia hirsuta* pool from San Martin was found infected with *Leishmania naiffi* and one *Lu. carrerai* pool from Madre de Dios with *L. guyanensis*. Two of 48 pools (0.01 infection rate) from Madre de Dios, 17 of 146 pools (0.03 infection rate) from San Martin, and 2 of 43 pools (0.01 infection rate) from Cajamarca were ITS/gltA PCR positive for *Bartonella* spp. One *Lutzomyia nevesi* pool from San Martin and one *Lu. maranonensis* pool from Cajamarca were positive for *Bartonella* DNA closely related to *Candidatus* B. rhondoniensis found in triatomine bugs from French Guiana. Seven *Lutzomyia nevesi* pools and two *Lu. hirsuta* pools from San Martin, and one *Lu. whitmani* pool from Madre de Dios were positive for *Bartonella* DNA closely related to *B. bacilliformis*. Molecular screening with additional markers will confirm *Bartonella* and *Leishmania* species. This is the first report of *Leishmania naiffi* and

Bartonella spp. molecular detection in sand flies from the Peruvian Amazon, suggesting that these pathogens may be circulating in this region of Peru.

1530

PROFILES AND SWARM DYNAMICS IN *ANOPHELES GAMBIAE* MOSQUITOES AT SELECT TARGET MALARIA STUDY SITES

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The *Anopheles gambiae* complex (*s.l.*) is the major Afro-tropical vector of malaria. Innovative methods, including genetic modification and sterile male techniques are being developed to precisely target such malaria vectors. Estimation of the proportion of male and female mosquitoes sampled is also vital for monitoring and control purposes but due to none or limited surveillance in East Africa little is known anopheles swarm. This requires detailed understanding of current mosquito diversity, abundance, sex ratio, mating behaviour and dynamics in different ecological environments. This baseline study was aimed at identifying factors that enhance swarming and collection of *An. gambiae s.l.* male populations at three mainland sites for the target malaria project in Uganda. Mosquito swarms were surveyed by local trained volunteers from March 2017 to date in three villages Kibbuye, Katuuso and Kayonjo in Uganda. Aspiration of house eaves was done in the morning. Swarm markers were identified and sweep nets were used to sample. Swarms GPS location was mapped. A total of 113 *Anopheles* swarms were identified, characterized and mapped. Mean swarm height was 2.6m and common markers included bare ground, roads, abandoned bricks covered with grass and sand. 70% of swarm were collected from bare ground grass thatched compound and there was high number of swarm during rainy season. Aspiration of eaves especially grass thatched houses as a location of swarm in time of low density is very important. The study also hopes to promote open dialogue with community members on existing and promising strategies on identification of mosquito swarms.

1531

SPATIAL ANALYSIS OF TRANSPORT NETWORKS IN NORTHERN UGANDA FOR GUIDING VECTOR CONTROL ACTIVITIES FOR SLEEPING SICKNESS

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Sleeping sickness, also known as human African trypanosomiasis (HAT), is a disease transmitted by the bite of a tsetse fly. Control of Gambian sleeping sickness, the most common form of HAT, is primarily achieved through active and passive surveillance and subsequent treatment plus vector control. A cost-effective method of controlling riverine species of tsetse that transmit Gambian sleeping sickness is to deploy insecticide-impregnated '*tiny targets*' in high transmission risk areas. These targets have been shown to reduce the tsetse population by over 90%, hence significantly reducing the risk of sleeping sickness transmission. In areas where the intervention is implemented, traps are used to monitor the tsetse population at regular intervals to evaluate the impact of the intervention on the tsetse population. As the intervention scales up to cover larger geographical areas, maintaining a cost-effective monitoring programme becomes more challenging. This study therefore focussed on the logistical challenges associated with tsetse monitoring within an historical HAT focus in north-west Uganda. Staff involved in the monitoring programme were requested to record GPS data during their monitoring activities over six weeks which enabled accurate information on travel routes and speeds to be established. Additional travel network data (locations of roads, category of road) were also obtained from OpenStreetMap plus through image classification of high resolution (<3m) remotely sensed images. Using this information, friction surfaces representing the time-cost of travelling along each section of road within the area covered by the monitoring programme were developed. A 'least-cost path' analysis was then undertaken to estimate the travel times between key locations within the study area and current and proposed tsetse monitoring locations. This resource has the potential to improve the planning of monitoring activities within the current intervention area. Further, the methods used to establish the friction surfaces can be extrapolated to assist in the expansion of the tsetse plus other vector control programme to new areas.

1532

CHARACTERIZING WILDLIFE, DOMESTIC ANIMALS, AND HUMANS AS SOURCES OF TRIATOMINE BLOODMEALS: COMPARISON OF SANGER SEQUENCING AND AMPLICON DEEP SEQUENCING

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Knowledge of host associations of blood-feeding vectors may afford novel insight for managing disease systems in order to protect public health. Triatomine insects vector the causal agent of Chagas disease, *Trypanosoma cruzi*. Across the southern US, this parasite is mainly transmitted among triatomines and wildlife, with occasional spillover into humans and domestic animals. We used two methods—Sanger sequencing and amplicon deep sequencing—to characterize the vertebrate host community previously fed upon by these insects. A collection of 119 triatomines from across Texas were dissected, and the amount of visible blood in the hindgut was scored. DNA was extracted from the hindgut, and PCR amplification of a 228bp region of vertebrate cyt *b* was completed. 37 (31.1%) samples produced amplicons of the target size successfully sequenced by Sanger. The frequency of success was significantly higher (OR 4.8, CI 2.0-13.2, $p<0.001$) in recently fed bugs vs. those with desiccated bloodmeals. Sanger sequencing revealed 15 distinct host species, which included domestic animals (e.g., dogs, chickens, cats, cows), wildlife (e.g. rats, raccoons, ringtails, crickets), and humans. Illumina MiSeq amplicon deep sequencing on the same PCR products (average of >172,000 usable reads per triatomine sample) replicated the host community data set found using Sanger sequencing, but also afforded detection of multiple hosts in 5 (13.5%) triatomines, including a triatomine with four detected bloodmeal sources (toad, human, dog and squirrel). Current efforts are underway for amplicon deep sequencing of bloodmeal hosts from 400 additional vectors collected in domestic, peridomestic, and sylvatic environments across the southern states for which *T. cruzi* infection status and parasite strain are already known, affording a robust assessment of host associations in relation to infection parameters. An enhanced understanding of vector-host-parasite networks may allow for integrated vector management programs to target interventions specific to highly-utilized and highly-infected host species.

1533

SURVEILLANCE OF *AEDES AEGYPTI* IN SIERRA LEONE, WEST AFRICA

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The prevalence of arboviral diseases transmitted by *Aedes aegypti* mosquitoes in West Africa has increased dramatically in recent years, with large outbreaks of Dengue, Yellow Fever, and Chikungunya reported

throughout most of the region. However, very little basic entomological information is known about the vector in the region, which limits the ability to prevent and control their spread and hence transmission of these viruses. Therefore, we performed an entomological survey of *Ae. aegypti* in Sierra Leone, where it has not been studied before. Between June and August 2017, we conducted egg, immature, and adult sampling of *Ae. aegypti* in two locations in Sierra Leone (Bombali and Bo Districts). Eggs were counted to determine densities and then used to rear adults in the insectary. Lab-reared females were tested for resistance to six different insecticides as well as for the prevalence of knock-down resistance (KDR) genotypes. Immature sampling was done to identify breeding sites and calculate standard *Ae.* indices. Wild-caught adult females were preserved and tested for arboviruses. The mean eggs per trap was 23.7 while 58 adult females were captured. For immatures, indices were high in general, ranging from 13-17 for Container, 18-61 for house, and 65-153 for Breteau. Tires and bottles were the most common breeding sites, accounting for 44% and 20% (respectively) of all positive container types. Adults reared from eggs in both locations were 100% susceptible to Deltamethrin and resistant to Permethrin (53% mortality). Evidence of higher KDR genotypic frequencies among Permethrin-resistant mosquitoes was found in one of the locations (Bombali). Viral testing on adults for Flaviviruses and Chikungunya Virus was negative, however the sample size was small. A serosurvey conducted in one of the locations (Bo) in 2013 showed a high seroprevalence for Chikungunya, therefore, additional sample collection and testing is warranted. Further recommendations are to focus on tires and bottles for clean-up campaigns, and if chemical control measures begin for *Ae. Aegypti* in either location, they should use Deltamethrin and discontinue use of Permethrin.

1534

THE GEOGRAPHIC DISTRIBUTION OF SUSPECTED LYME BORRELIA IN MONGOLIA

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In Asia, *Borrelia garinii, B. afzelii,* and *B. miyamotoi* are transmitted by the *Ixodes persulcatus* tick and clinically present with a wide range of neurological and arthritic symptoms. *B. garinii* has been reported to cause neuroborreliosis in Europe, Russia and China, but no studies to date have described the clinical manifestations of Lyme borreliosis (LB) in Mongolia. This case series examines 150 cases of suspected Lyme borreliosis in Mongolia from 2007 to 2017 to describe the frequency of symptoms, potential regions of interest, and case demographics associated with suspected LB. Data regarding sociodemographics, dates and origin of reported infection, clinical symptoms, and methods of diagnosis (IIFA and/or ELISA test) were collected from individuals across 13 aimags (provinces). Zavkhan and Selenge, located in northern Mongolia, where vegetation density is high, had the highest percentage of reported suspected cases, with 25% and 20% respectively. Ages ranged from 1 to 78 years old with a mean of 26 years, however 37% of individuals were under age ten. Most frequently reported symptoms include fever, rash, headache, and enlarged lymph glands. Peak months of tick bite and treatment seeking occurred between April and June. More than twice as many women sought treatment as men, and the distribution of men who sought treatment was skewed toward children and the elderly. LB surveillance and monitoring should be expanded in the northern region of Mongolia and be considered in differential diagnosis in those reporting a recent tick bite.

1535

MOLECULAR ANALYSIS OF TICK SPECIES IN COSTA RICA AND PANAMA

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Different tick species may vary in behavior and vector competence, so a correct identification is necessary to understand the epidemiology of tick-borne diseases. Analysis of gene sequences is a useful tool to confirm species and detect possible cryptic species. The aim of this study was to compare ticks from Costa Rica and Panama using the mitochondrial 16S rDNA gene. A fragment of the mitochondrial 16S rDNA gene was amplified (primers 16S+1 y 16S-1; 460 bp) from 93 tick samples from Costa Rica (16 species identified morphologically and unidentified samples) and 15 from Panama (9 species). DNA sequences were compared with sequences in GenBank, and phylogenetic trees were constructed. Results were complemented with amplification, sequencing, and analyses of cytochrome C oxidase subunit I (Cox-1) and internal transcribed spacer 2 (ITS2) gene fragments. Most 16S rDNA sequences obtained were highly similar (>99%) to corresponding sequences in GenBank: *Amblyomma calcaratum, A. geayi, A. naponense, A. nodosum, A. ovale, A. sabanerae, A. mixtum, A. dissimile, A. rotundatum, A. varium*, *Rhipicephalus sanguineus* s.l. (tropical lineage), *R. microplus, Dermacentor nitens, Ornithodoros knoxjonesi*. Sequences from *Amblyomma naponense* and *A. pecarium* from Panama had lower similarity to those available (98.7-99%). Two sequences of *Amblyomma oblongoguttatum* s.l. were 98.7% similar to sequences from Brazil, but another sequence was only 91.9% similar to it. Ticks identified as *A. parvum* and *Ixodes boliviensis* had sequences <94% similar to those from South America. Sequences of *Haemaphysalis yuxtakochi* were only 96.2% similar to a sequence from Uruguay. 16S rDNA sequences for *Amblyomma tapirellum* and *Dermacentor latus* were not available for comparison. Evaluation of Cox-1 and ITS2 gene fragments allowed confirmation of results. Phylogenetic analyses also showed similar relationships. Results showed possible intraspecific variations and cryptic tick species in the region. Detailed morphological and biological studies will be necessary to determine their final taxonomic status.

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MAPPING MALARIA RISK IN PACIFIC REGION OF COLOMBIA USING ENVIRONMENTAL VARIABLES RELATED TO THE INCIDENCE

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Colombia ranks third in Latin America in the number of malaria cases. Currently, the Colombian Pacific is the main endemic region reporting more than 60% of total cases. Although, efforts have been made to map and categorize malaria risk at the municipality level, topographic and environmental complexities suggest the necessity to categorize malaria risk at a finer spatial scale. Therefore, this study aimed to the design a malaria risk map of the Colombia Pacific region and to the determination of the environmental variables influencing malaria incidence. The relationship of the variables NDVI, NDWI, TWI, precipitation and temperature with the annual parasitic index (observed API) was evaluated using a Generalized Linear Model (GLM). An incidence risk map at a spatial scale of 1 Km^2 was constructed through a Bayesian Logistic Regression analysis. A significant correlation was found for the incidence of malaria with the variables precipitation and NDVI (R^2 = 0.98, p <0.05). The moderate to high risk areas were located mainly in central Chocó Department, along the San

Juan and Atrato rivers, and in areas west of the Cauca river and Pacific lowlands of the Andes Mountains. A linear regression used to validate the model showed a statistically significant relationship between the observed and estimated API (R^2 = 0.86588, $p < 0.05$). Finally, the risk map obtained could be used to inform public entities of the malaria risk in particular places of this region, in order to optimize the economic resources allocated for vector control and surveillance.

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COMPARATIVE ANALYSIS OF THE SALIVARY N-GLYCOMES OF THREE NORTH AMERICAN TICK SPECIES

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Ticks are parasitic arthropods of great medical and veterinary importance because of their capability of transmitting various viral and bacterial pathogens. We believe that the prolonged duration of tick feeding, as compared to other blood-sucking arthropods, contributes to their success as vectors. During attachment, there is an ongoing exchange of tick saliva and host blood, where the nutrients for tick growth are derived. We hypothesize that as the tick feeds, the salivary N-glycome is dynamic, possibly to mimic the host glycans and evade detection by the immune system. Tick saliva and salivary gland proteins were isolated from *Amblyomma americanum, Amblyomma maculatum*, and *Ixodes scapularis* ticks and then were reduced, alkylated, and digested with trypsin. Dried glycopeptides were incubated with Peptide-N-Glycosidase A (PNGase A) and PNGase F to release the N-linked glycans, and the glycans were then recovered by passing through a C18 sep pak cartridge. Analysis of the glycans present in tick salivary samples were performed using MALDI-TOF/TOF-MS. The N-linked glycan profiles of these three tick species have revealed a wealth of knowledge concerning the variation of tick salivary protein glycosylation during growth and feeding. We have noticed similarities of the profiles between the antennary structures and glycoforms of all assessed tick species, but the individual composition of each is unique. This research establishes a foundation to better understand the purpose of tick N-glycan synthesis during the blood meal.

1538

"CROSS-VECTOR MONITORING": UTILIZING MOSQUITO EXCRETA/FECES TO SCREEN FOR PATHOGENS VECTORED BY NON-MOSQUITO HOSTS

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By limiting biological mass, the molecular testing of mosquito-derived excreta/feces (E/F) has the potential to increase the throughput of xenomonitoring efforts and expand its utility for the sensitive screening of infectious agents. Equally promising, previous proof-of-concept work has exhibited the capacity for "cross-vector monitoring" as the real-time PCR-based detection of *Trypanosoma brucei brucei* DNA within the excreta/feces (E/F) of mosquitoes has been demonstrated following the provision of a *T. brucei*-positive bloodmeal. However, to date, the capability of this technique to serve as a methodology for the detection of non-mosquito vectored pathogens has not been evaluated using field-collected samples. Accordingly, human bloodspots, mosquitoes, and mosquito-derived E/F were collected from two rural communities in the Northern region of Ghana. Following DNA extraction, real-time PCR testing of mosquitoes and the corresponding E/F from those same mosquitoes resulted in the detection of *Mansonella ssp.* Detection of the same pathogen occurred following similar testing of DNA extracted from human bloodspots collected in the same region. This capacity to detect pathogens vectored by non-mosquito arthropods in the E/F of mosquitoes expands the utility of molecular xenomonitoring and increases the potential for integrated evaluation among various monitoring and evaluation programs. Furthermore, the adaptation of E/F testing to the detection of blood-borne pathogens altogether lacking a vector host should also be evaluated.

1539

RELATIVE VECTOR COMPETENCE OF *AEDES AEGYPTI* AND *AE. ALBOPICTUS* FOR AN EPIDEMIC STRAIN OF ZIKA VIRUS UNDER SIMULATED FIELD CONDITIONS

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The major arbovirus vector *Aedes aegypti* was firmly implicated in the transmission of the Asian lineage of Zika virus (ZIKV) during the pandemic affecting French Polynesia and the Americas. However, the contribution of the invasive mosquito *Aedes albopictus* is more uncertain given varying estimates of the vector competence of this species. Mosquito vector competence depends on factors that include the compatibility of mosquito/virus strains and environmental variation. We determined the vector competence of an Australian strain of *Ae. aegypti* and a strain of *Ae. albopictus* threatening the Australian mainland, for a strain of ZIKV isolated from Joao Pessoa, Paraiba state, Brazil during the pandemic. We maintained mosquitoes under a natural cyclical temperature regime and a standard regimen of constant 28°C and tracked virus dissemination within mosquitoes over time by quantifying viral copy number, performing whole-mosquito immunofluorescence analysis, and testing for live virus in saliva. Overall, infection rates at 14 days post inoculation were 69.2% for *Ae. aegypti* and 90.0% for *Ae. albopictus* and viral dissemination rates to legs and wings were 56.4% and 70.0% for the two species, respectively. However, while 56.4% of *Ae. aegypti* had infectious virus in the saliva, the same parameter of transmission was only 15% for *Ae. albopictus*. Both species supported heavy viral loads indicated by means of $> 10^8$ copies per mosquito at 14 days and a characteristic 'saturated infection' pattern through most body tissues. However, there were significant differences in viral copy number at earlier time points between cyclical and constant temperature regimes. These results indicate the presence of a barrier to virus transmission for this strain of *Ae. albopictus*. However, the lower vector competence of *Ae. albopictus* should be factored in with its greater range when determining its contribution to ZIKV transmission.

1540

CHARACTERISTICS AND TREATMENT PATTERNS OF OUTPATIENTS WITH RICKETTSIAL DISEASES IN A LARGE, COMMERCIALLY INSURED POPULATION, UNITED STATES, 2005-2016

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Rickettsial diseases (RD) include Spotted Fever Group (SFG) Rickettsiosis, Ehrlichiosis, Anaplasmosis, Typhus Group (TG) and Rickettsialpox, among others. Doxycycline is the treatment of choice in all age groups, and early treatment based on clinical diagnosis is important to prevent severe and fatal outcomes. SFG, Ehrlichiosis, and Anaplasmosis are nationally notifiable in the United States, but data on treatment patterns are not collected. We conducted a retrospective analysis using Truven Health MarketScan® Commercial Claims and Encounters databases. We included any individual with an outpatient claim using an ICD-9/10-CM code for RD, who had one year continuous pre- and 3 months post-diagnosis enrollment and pharmaceutical claim data. The first outpatient record with RD was considered the incident diagnosis. Demographics, epidemiologic characteristics, and treatment patterns were summarized for all individuals. In total, 13,909 individuals were included; median age was 45 years (IQR: 28-55 years), 2141 (15%) were under 18 years of age, and 51% were

male. The most common diagnosis was SFG (7332; 53%), followed by Ehrlichiosis (4182; 30%), Typhus (1343; 10%), and other rickettsioses (1016; 7%); 36 individuals had more than one diagnosis. Over half (7512; 54%) received doxycycline within 30 of the index date; of these, 3690 (49%) received it within 14 days. The majority (5572; 74%) of adults received the recommended dose of 100mg; and the same amount were treated for at least 10 days. The proportion with a pharmaceutical claim for doxycycline varied considerably across diagnoses and ranged from 26% for TG to 61% for SFG Rickettsiosis. Among the 6397 (46%) without a claim for doxycycline, 2217 (35%) were prescribed another tetracycline or antibiotic; 3883 (28%) were not prescribed any antibiotic. RD continue to be an important cause of disease in the outpatient population, and yet providers are still only prescribing the recommended treatment to about half of those in whom they suspect the disease. Continued education and increased awareness is critical to prevent severe and fatal outcomes from RD.

1541

SITUATIONAL ANALYSIS OUTCOME OF SCABIES CONTROL PROGRAM IN ETHIOPIA

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Scabies is an infestation of the skin caused by the human itch mite (*Sarcoptes scabiei* var. *hominis*). The microscopic scabies mite burrows into the upper layer of the skin where it lives and lays its eggs. Scabies imposes a considerable economic burden on individuals, families, communities, and health systems. Families in endemic areas spend a substantial portion of income on treatments, re-directing available funds for food and essential commodities. An analysis of the 2010 Global Burden of Disease study estimated the global prevalence to be 200 million cases. Scabies is also the major risk factor for impetigo in tropical countries. Following the inclusion of Scabies on the public health emergency reporting system in 2016, cases of scabies have been reported as a part of the country health reporting system. In 2017/18, cases of scabies were reported from the Amhara, Tigray, Oromia, Benishangulgumuz and Southern Nation and nationalities (SNNPR) regions. More than 704,210 people were affected with scabies in five regions of the country, with prevalence more than 10% in some districts. An in-depth assessment in Amhara region suggested limited knowledge of disease prevention, control and treatment aspects by different levels of health professionals. Absence of a standardized case definition further hinders the number of cases reported to the national level. Scabies has been included in the WHO NTD portfolio. Ethiopia established a scabies control program despite absence of standardized evidence for prevention and control strategies. Though scabies is common in many African countries, there is little evidence on the burden of the disease, treatment of choice, dosing, frequency, relationship with WASH and possibility of elimination. Therefore, attention on mapping protocols, clear case definitions, integrating with WASH activities and high level advocacy is needed for policymakers, donors and pharmaceutical companies in order to ensure universal accesses care coverage and breaking the neglect.

1542

DETECTION OF DUGBE VIRUS FROM TICKS IN ACCRA, GHANA

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Dugbe virus is a negative sense RNA virus which belongs to the Nairobi sheep disease virus serogroup. The virus belongs to the family Nairoviridae and genus *Orthonairovirus*. It is transmitted by tick species of three genera; *Rhipicephalus, Amblyomma* and *Haemaphysalis.* The virus was first detected in domestic livestock from *Amblyomma variegatum* in Ghana. This study aimed to screen field collected ticks for the presence of Crimean-Congo haemorrhagic fever virus (CCHFV). Sampling was done in seven sites within Greater Accra, Northern and Upper East regions. A total of 927 ticks were collected from cattle, dogs, sheep and goats and morphologically identified using the African Ixodidae identification keys. Ticks were pooled by species, gender, study site and animal host. Pooled samples were homogenized using Mini Beadbeater-96 with lysis buffer. Nucleic acid was extracted using QIAamp Viral RNA Kit (QIAGEN, Valencia, CA, USA) following manufacturer's instructions. Extracts were screened for CCHFV using real time polymerase chain reaction. Three of the pools tested positive for CCHFV. The positive tick species were *Rhiphicephalus sanguineus* in Navrongo (sheep), *Hyalomma rufipes* in Shai Hills (cattle) and *Amblyomma variegatum* in Michel Camp (cattle). These pools were further analysed using next-generation sequencing targeted enrichment protocol developed by USAMRIID-CGS. Sequence alignment and phylogenetic analysis was done using MUSCLE and MEGA7 software respectively. Sequencing performed on all three pools failed to confirm presence of CCHFV however, the resulting data from one of the pools from Michel Camp, located outside Accra showed whole genome sequence of Dugbe virus. This pool may have initially tested positive for CCHFV because of the similarity in the S segment of nairoviruses. Phylogenetic analysis of the complete sequence of the Dugbe virus using Maximum likelihood tree algorithm showed a close relationship with the Dugbe strain previously found in Ghana and Nigeria. Further surveillance and characterization studies need to be conducted to determine the prevalence of the virus in Ghana.

1543

TICK-BORNE DISEASE IN SOUTHERN COASTAL ECUADOR: DETECTION OF ANTIBODIES TO SPOTTED FEVER GROUP RICKETTSIA IN FEBRILE INDIVIDUALS IN 2014-2015

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Ticks (family: *Ixodidae* and *Argasidae*) transmit debilitating and often fatal diseases to people in both tropical and non-tropical settings throughout the world. The incidence of tick-borne diseases (TBDs) have been increasing globally due to several factors, including climate change and ineffective comprehensive TBD surveillance and prevention programs. In areas with limited resources, diagnostic testing of TBDs is often unavailable because of the large number of potential pathogens and the complexity and cost of available diagnostic assays. In this pilot study, we analysed human plasma samples from an ongoing arbovirus disease surveillance studies in Machala, a tropical coastal city in southern Ecuador. Samples were collected from individuals with acute febrile illness who attended 4 sentinel clinics operated by the Ministry of Health. Samples were screened for the presence of antibodies to tick-borne Spotted Fever group *Rickettsia*. We used an indirect enzyme immunoassay to detect IgG and IgM antibodies in 222 plasma samples. We found that 60/222 (27%) and 38/222 (17%) of specimens had positive or equivocal IgG responses, respectively. Although testing is still ongoing 15/184 (8%) and 13/184 (7%) samples had positive or equivocal IgM ELISAs respectively. Molecular studies are ongoing to identify the Rickettsia species most likely infecting humans in this region of Ecuador. Our findings suggest there is a potentially significant burden of TBD in this region that has been undiagnosed and untreated. To our knowledge, this is the first study of TBD in this region. The results highlight the importance of surveillance studies to detect TBDs and the need for novel low-cost diagnostics for TBDs.

1544

IDENTIFICATION AND CHARACTERIZATION OF RESISTANCE TO DELTAMETRINE IN TRIATOMINE BUGS FROM RURAL HOUSES OF PAMPA ARGENTINA, CHACO PROVINCE, ARGENTINA

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Triatoma infestans resistance to insecticides has been reported in the Bolivian and Argentina Gran Chaco Region both at the macro and meso scale. The objective of this study was to characterize the current susceptibility to deltrametrine in triatomine bugs from the rural locality of Pampa Argentina, Chaco Province, Argentina. The transversal study was conducted in all the houses of the locality (n=121) were triatomine bugs were captured and flowable deltametrine was applied. Collected bugs were identified and sent to the laboratory for their analysis through a rapid test and a bioassay. The infestation rate obtained was 25.5% and the analysis of pyrethroid susceptibility showed resistance levels higher than 1000 (RR>1000). In a second stage, a community approach was conducted in order to inform the community of the findings, evaluate houses one by one, obtain demographic data through questionnaires and capture triatomine bugs to determine their toxicological status. A total of 106 houses were evaluated and 49 of them were positive for the presence of bugs (46.2%). Analysis of susceptibility to pyrethroids showed a homogenous distribution of resistant bugs. Alternative control strategies currently available are organophosphate insecticides that were rejected by both the local health team and the community. These results show the need of other alternative strategies that are effective and non-toxic for the inhabitants and their animals. The absences of such strategies will increase the abundance of triatomine bugs, promoting the transmission of Chagas Disease to the population and moreover, the dispersal of pyrethroid resistant insects to other localities.

1545

GENETIC DIVERSITY OF *ANAPLASMA* AND *EHRLICHIA* BACTERIA IN MONGOLIAN TICKS

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Ehrlichia and *Anaplasma* are tick-borne bacterial diseases that cause human granulocytic anaplasmosis (HGA) and human monocytic ehrlichiosis (HME). Additionally, these pathogens are responsible for severe illness in animals, potentially having a major impact on economies which rely heavily on livestock, such as in Mongolia. Previous studies of tick-borne diseases (TBDs) in East and Central Asia have focused on areas in China and Russia, leaving the status of TBDs in Mongolia largely undescribed. In this study, ticks were collected, identified, and pooled from three distinct environments across central Mongolia. Tick pools were homogenized for polymerase chain reaction (PCR) analysis of relevant TBD genes. This study focused on sequencing *Anaplasma* sp. gene targets *gltA, msp2,* and 16S rRNA, as well as *Ehrlichia* sp. 16S rRNA and *sodB* genes. *A. phagocytophilum* had a calculated 1.6% (95% CI 0.5-3.7%) maximum likelihood estimation (MLE) infection rate in *I. persulcatus* ticks collected from the Selenge aimag (province). The calculated MLE infection rate of *A. ovis* in *D. nuttalli* ticks ranged from 4.82% (05% CI 2.4-7.7%) in the Dornogovi aimag to 15.2% (95% CI 9.7-22.8%) in the Selenge aimag. Non-specific *Anaplasma* and *Ehrlichia muris* both had MLE rates of 1.2% (95% CI 0.3-3.1%) in *I. persulcatus* ticks from Selenge. A majority of *D. nuttalli* ticks were recovered from livestock and 51% of those pools carried *A. ovis*. Genetic analyses of selected 16S and *gltA* positive amplicons confirmed the PCR results with phylogenetic clustering, indicating genetic relatedness to other species found in China and Asia. Considering the collective economic losses that can result from these pathogens and the potential for spillover to nomadic herdsmen, these results highlight the need for better TBD surveillance and intervention within Mongolia.

1546

BACTERIAL GENERA IN SALIVARY GLANDS AND GUT TISSUE OF *RHIPICEPHALUS MICROPLUS* TICKS FROM LIVESTOCK IN ANTIOQUIA, COLOMBIA

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Since ticks (Ixodida) are obligated blood-feeders have become an increasing problem to people and animals in the world. They are considered to be of medical and veterinary importance because can also transmit many human and animal disease pathogens, which include viruses, protozoa and bacteria. *Rhipicephalus microplus* is important tick specie that economically affects the livestock industry in tropical and subtropical regions of the world, despite its importance, its role like biological vector have been few researched so far. In this study, bacterial

diversity of *salivary glands* and gut tissue of *R. microplus ticks* was characterized by massive parallel sequencing of 16S amplicons. For this, *R. microplus ticks were collected in cattle in two regions from Antioquia, Colombia.* Salivary glands and gut tissue were independently pooled according to the geographic collection sites in five female ticks per pool. *DNA was extracted using PureLink® Genomic DNA Mini Kit and the variable region* V3-V4 of 16S rRNA was amplified using universal primers. Amplicons were sequenced using the MiSeq platform (Illumina) and bacterial diversity analysis were performed with Mothur v1.39 following an OTU-based approach. Sequences were clustered into OTUs with a 97% identity and classified using the Ribosomal Database Project database v9. A total of 5600 OTUs including rare OTUs were detected in salivary glands and gut tissues from *R. microplus.* The number of OTUs per sample ranged from 288 to 1250. Some OTUs identified were classified into the following bacterial genera: *Pantoea, Lactobacillus*, *Gardnerella, Stenotrophomonas, Sphingobacterium, Prevotella, Enterobacter, Pseudomonas, Anaplasma, Coxiella* and *Ehrlichia.* Our results provide an important molecular approach to describe bacterial organisms associated to *R. microplus* from Livestock in Antioquia, Colombia. In addition, pathogenic genera detected may be useful for implement entomological and epidemiological surveillance programs.

1547

TICK-PATHOGEN INTERACTION: CONNECTING THE DOTS BETWEEN INNATE IMMUNITY AND REDOX SIGNALING PATHWAYS

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Dietary selenium, through its incorporation into selenoprotein, plays an important role in immunity and inflammation responses due to its crucial roles in regulating reactive oxygen species and redox status in almost all tissues. In previous studies, it has been shown that selenophosphate synthetase 2 (SPS2), a homologue of selenophosphate synthetase (SelD) identified in mammals, is essential for selenoprotein biosynthesis. Relish, a homologue of nuclear factor-kappa B (NF-kB), in the immune deficiency signaling pathway, regulates the expression of microplusin, an antimicrobial peptide (AMP). In this study, we hypothesize that silencing of SPS2 and Relish will cause an increase in *Rickettsia parkeri* level in infected *A. maculatum* ticks. To define the functional role of SPS2 and Relish in hematophagy and pathobiome colonization, an RNAi approach was utilized to deplete target genes expression in pathogen infected ticks. The transcriptional expression of target genes was confirmed in the knockdown tissues of both SPS2 and Relish. A significant decrease in replete weight, and a marked increase in distress in the host provided evidence for the critical role of target genes during feeding of knocked down ticks. A qPCR and 16s rRNA diversity assays showed that the gene-silenced ticks had significant increase in *R. parkeri* load than the control, proving that SPS2 and Relish play a role in the maintenance of tick pathobiome. Interplay between redox signaling and innate immunity pathways will be discussed in the context of tick-pathogen interactions.

1548

CANINE SENTINELS OF VECTOR-BORNE DISEASE IN TEXAS, USA: HIGH-RISK DOGS INFECTED WITH DIVERSE AGENTS INCLUDING *TRYPANOSOMA CRUZI*, BUT LITTLE EVIDENCE OF *BORRELIA BURGDORFERI*

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Dogs are useful as sentinels for human risk of exposure to vector-borne diseases. In the southern US, while screening is routine for many vector-borne diseases in dogs, exposure to *Trypanosoma cruzi*, agent of Chagas disease, is under-recognized. *T. cruzi* causes fatal heart disease in dogs across the southern US, but autochthonous infection with *T. cruzi* in humans is less common. Additionally, as tick-borne diseases continue to emerge across the US, understanding of the tick and pathogen communities in the southern US remains incomplete. We conducted a repeated cross-sectional study to determine the prevalence of *T. cruzi*; the tick-borne pathogens *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia burgdorferi*; and the mosquito-borne filarial nematode *Dirofilaria immitis* among dogs recently admitted to animal shelters across Texas. These dogs may be considered at high risk for vector-borne exposures due to prolonged time spent outdoors and lack of routine ectoparasite treatment. We also collected ticks from these dogs and tested them for infection with *Rickettsia* spp. and *Borrelia* spp. Among 608 dogs sampled, 110 (18.1%) were seropositive for T. cruzi. Prevalence estimates of the other four vector-borne agents were as follows: *Ehrlichia* spp. 3.6% (22/608); *Anaplasma* spp. 6.9% (42/608); *B. burgdorferi* 0.2% (1/608); and *D. immitis* 16.0% (97/608), with regional variation in exposure. We documented coinfections or coexposures in 41 dogs, but only coexposure to *Ehrlichia* spp. and *Anaplasma* spp. was more frequent than expected ($P<0.001$). We collected 353 ticks from dogs, mostly *Rhipicephalus sanguineus* with few *Ixodes scapularis* and *Dermacentor variabilis*. Three percent of *R. sanguineus* were infected with a *Rickettsia* endosymbiont, and no *I. scapularis* harbored *Borrelia* spp. This study reports exposure to diverse vector-borne pathogens among Texas shelter dogs, including widespread exposure to *T. cruzi* at a prevalence that is comparable to *D. immitis*. Only one dog was seropositive for *B. burgdorferi*, suggesting very low risk of exposure across the state. These data can be used to direct future investigations and prioritize public health campaigns.

1549

THE DILEMMA OF RECENT DISCOVERY, DETECTION AND DIVERSITY OF NEW RICKETTSIAE AND RICKETTSIAL DISEASES

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Rickettsial diseases are associated with well-known pathogens such as *Rickettsia rickettsii* (Rocky Mountain spotted fever), *Anaplasma phagocytophilum* (human granulocytic anaplasmosis), *Ehrlichia chaffeensis* (human monocytic ehrlichiosis), and *Orientia tsutsugamushi* (scrub typhus) and continue to be a disease, social and economic burden to society. This problem is compounded by the recent discoveries of new rickettsial agents within the known arthropod vectors of lice, mites, ticks and fleas, as well as detecting these agents within new invertebrate hosts such as mosquitoes, tsetse flies and leaches. The discovery of new agents causing new diseases is bad enough, but finding new agents for old diseases in new endemic regions (e.g. scrub typhus) is increasing the public health risk of rickettsial diseases in a fashion not previously envisioned.

1550

SPECIFICITY ASSESSMENT OF REAL TIME PCR PROTOCOLS FOR THE DETECTION OF *RICKETTSIA FELIS* AND *R. ASEMBONENSIS* USING RICKETTSIAE STRAINS FROM COSTA RICA

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Rickettsia felis is an emergent pathogen that causes a disease known as "flea-borne spotted fever". Even though *Ctenocephalides felis* fleas are considered the main vectors and reservoirs of *R. felis*, they can also harbor similar rickettsiae that may be considered endosymbionts or

microorganisms of unknown pathogenicity; one of them is *Rickettsia asembonensis*. Some of the described rickettsiae species that have been reported in Costa Rica include *Rickettsia rickettsii*, *Rickettsia amblyommatis*, *Rickettsia bellii*, *R. felis*, and *R. asembonensis*. The purpose of this study was to assess the specificity of two real time PCR protocols that are available for the detection of *R. felis* and *R. asembonensis*, using different rickettsiae isolated in Costa Rica as well as rickettsiae from local fleas. Both real time PCR protocols were tested (presence/absence) using DNA from local cell culture isolates of *R. rickettsii, R. amblyommatis*, and *R. felis*, as well as DNA from flea samples positive for *R. asembonensis* (*Rickettsia* sp. strain RF2125). In addition, fleas from 3 sites in Costa Rica, where *R. felis* and *R. asembonensis* have been previously detected (Tres Ríos, Desamparados, Guápiles), were collected from dogs and tested using both protocols. The real time PCR for *R. felis* and *R. asembonensis* were specific, as they detected the presence of these species, and not other rickettsiae; there were no cross reactions between *R. felis* and *R. asembonensis*. *Rickettsia felis* was detected in 7 of 16 fleas from Tres Ríos, 8 of 17 from Desamparados, and 1 of 12 from Guápiles, although some of the positive fleas seem to have low infections. Preliminary results showed that *R. asembonensis* was also detected in fleas from all sites, although more tests are currently being conducted. These results show that the protocols tested can be used to specifically detect *R. felis* and *R. asembonensis* in fleas from Costa Rica; in addition, they will be useful for determining the possible co-infection of fleas with these rickettsiae, and for evaluating interactions such as competition for niche phenomena.

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THE IMPACT OF SCABIES ON THE HEALTHY HUMAN SKIN MICROBIOTA

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We hypothesize that in the tropics, scabies mite infection plays a significant role in the establishment, proliferation and transmission of opportunistic, pathogenic bacteria and forms a critical precursor of skin-borne infection that can lead to severe illness. The first *in vivo* evidence confirming a scabies-associated microbiome came from a longitudinal pilot study in a scabies porcine model. We demonstrated a substantial impact of mite infection on healthy skin microbiota, causing a shift from commensal to pathogenic staphylococci. To investigate scabies infestations in humans we analysed metagenome data generated from DNA preparations of mite and skin material collected from severe crusted scabies patients located in northern Australia. A remarkable preliminary finding is the high abundance of opportunistic pathogens, including *Staphylococcus argenteus* and *Acinetobacter baumanii*. As the prevalence of these pathogens continues to increase, confirming a precursor role of scabies in these skin and soft tissue infections is adamant. To define the microbiome in skin lesions of patients with common scabies in different geographical locations (remote North Queensland, India and France) we analyse skin scrapings from multiple body sites including control samples from corresponding co- and contra-lateral healthy sites using 16S and ITS1 rRNA amplicon Illumina MiSeq sequencing. The study will provide a more global picture of the microbes associated with scabies. To understand the mite internal microbiome, analysing the metagenome data of washed mites revealed microbial genera reported previously to be symbionts in various arthropods. Among the potential endosymbionts detected, Streptomyces species supply nutrients and antibiotics to support their arthropod hosts, and some species cause chronic subcutaneous infection in humans. To elucidate the role of Streptomyces in scabies we confirmed its presence in situ by FISH analysis, and quantified its abundance in individual parasite stages by species specific ddPCR. Targeting bacterial symbionts could be an alternative approach to scabies control.

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AGENT-BASED MODELLING OF TICK-BORNE DISEASE EXPOSURE IN MONGOLIAN LIVESTOCK AND HERDERS

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Investigating the risk factors associated with tick-borne disease exposure can have significant impacts on global health outcomes - particularly in resource-poor regions. Mongolian herders are at an increased risk of contracting tick-borne diseases because of the close interaction with livestock and landscape features, and provide an illustrative case for testing predictive models. To evaluate the effectiveness of an agent-based model (ABM) of tick-borne disease developed to predict and identify at-risk populations, a general ABM of tick-borne disease transmission was developed and parameterized for Mongolian herders. The model was used to predict seropositivity of herders and livestock in and compared to data collected in 2014 to evaluate model success and fit. Compared to the collected data, the preliminary ABM results suggest that the prevalence may be underestimated using this method, in comparison to differential equation models which may overestimate the prevalence. Utilizing ABM and differential models in tandem may best approximate the collected levels of infection and seroprevalence in at-risk populations. Due to limited diagnostic capabilities at both the local and national level, an increased understanding of the risk for infection with these high consequence pathogens can lead to improved prevention methods and decreased morbidity and mortality.

1553

EFFICACY AND RESIDUALITY OF LARVICIDE IN SIMULATED FIELD CONDITIONS: COMPARATIVE EVALUATION OF TWO PRODUCTS OF PIRIPROXIFEN AT 0.5% G BEFORE *AEDES AEGYPTI*

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In the year 2017, dengue presented an increase of cases, reporting 82 thousand cases. Being, the main strategy of vector control the chemical control. The present study compares the efficacy and residuality in simulated field conditions of two piriproxifen products: PYRILARV® 0.5% G and SUMILARV® 0.5 G, which were evaluated at a concentration of 0.01 mg / L (WHO). Third stage larvae of Ae. aegypti, susceptible strain Rockefeller. Both products were tested simultaneously with 4 treatments: without water exchange and with three water exchanges at 25, 50 and 75% in a total volume of 1 liter. Each treatment was evaluated with five repetitions. In the controls, 4 replicas were used. The effectiveness and residuality of both products was determined through bioassays with 25 larvae in each replication at 1, 30 and 60 days post-treatment, being evaluated until the emergence or death of all the mosquitoes and the percentage of adult emergence inhibition was determined (IE%, WHO 2005). The generic formula PYRILARV® 0.5% G, caused 100% of the IE at 1 day post-treatment); however, its effect decreased abruptly from 100 to 53.2% at 30 days and decreased to <1 to 60 days. On the other hand, SUMILARV®, caused percentages of IE> 97 up to 60 days. The results according to the water exchanges at 25, 50 and 75% show that PIRILARV® 0.5% G presents differences in IE%, where a higher percentage of water exchange generates less IE%, this effect was observed at 30 days and in SUMILARV® 0.5 a proportional decrease in IE% was observed as more volumes of water were exchanged for 60 days. In addition, it was observed that both products generate higher mortality

in pupae (91%). Physical-chemical analysis shows that the analyzed sample of PYRILARV® 0.5% G, lot n° 55120 shows a high variability (DSR> 2%) in the content of pyriproxyfen. In the country, there is a high risk of the increase in viral diseases transmitted by Ae. aegypti, by the use of insecticides of low quality and demonstrated inefficiency in the reduction of mosquito populations by a "limited vector control intervention."

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EFFECTS OF AERIAL ADULTICIDE SPRAYING ON THE RELATIVE ABUNDANCE OF *CULEX TARSALIS* AND *CX. PIPIENS*

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In order to minimize transmission of West Nile virus to humans, mosquito control districts utilize a variety of methods to reduce the abundance of adult and larval mosquitoes. Of the available control options, aerial adulticides are among the most effective tools for immediate reduction of infectious adult mosquito populations during periods of epidemic risk. However, estimates on the magnitude and duration of reductions from aerial adulticides vary spatially and temporally due factors associated with stochastic variation in nightly trapping success like weather and seasonality. Thus, making it difficult to estimate the effects of single applications. We used a decade (2006-2015) of trap collection data from CO_2-baited traps in Sacramento and Yolo counties, California to fit generalized additive models that adjusted for expected spatial and temporal trends in adult female abundance for *Culex tarsalis* and *Cx. pipiens*, the primary West Nile virus vectors in California. Estimates for the magnitude and duration of reductions in relative abundance for each species in urban and agricultural areas were obtained from the models. One-week post-spraying, aerial sprays reduced *Cx. pipiens* by an estimated mean of 78.6% (95% CI: 70.3-84.6) and in urban areas and 90.6% (95% CI: 72.0-96.9) in agricultural areas. *Cx. tarsalis* populations were reduced by a mean of 61.3% (95% CI: 47.1-71.7) in urban areas and 79.7% (95% CI: 51.3-91.5) in agricultural areas. Reductions persisted for both species, with longer population suppression for *Cx. pipiens* compared to *Cx. tarsalis*. Taken together, our results indicate that aerial adulticides are effective for rapid and sustained reductions of West Nile virus vectors, which is critical for preventing outbreaks of disease.

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IMPACT OF VOLATILE METOFLUTHRIN ON *ANOPHELES DARLINGI* BEHAVIOR EVALUATED IN EXPERIMENTAL HUTS IN ZUNGAROCOCHA, LORETO, PERÚ

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New formulations of volatile pyrethroids have behavioral and lethal impacts on mosquito vectors, suggesting a great potential as an alternative mosquito control tool. In this study, we evaluated the effects of metofluthrin emanators (SumiOne™ net) on *Anopheles darlingi* in experimental huts in Loreto, Peru. Two trials were performed, one in closed huts and another one in huts with opened window interception traps. Wild-caught *An. darlingi* females were collected 1d before release in experimental huts (100 females per hut); releases were performed at 1800 for a 5-day period per trial. In the first trial, we evaluated the effects of 1 and 2 emanators on protected human landing counts (HLC; mosquitoes landing on collector's exposed right leg) and mosquito knock down (KD). HLC were performed 10 min before (pre-treatment), and 55 and 105 min after (post-treatment) emanator deployment. In the second trial, we compared an untreated control vs. a 2-emanator treatment. Emanators were deployed at 1800 h; HLC and mosquito KD were recorded at 10, 5 and 120 min after deployment (post-treatment). Window interception traps were checked hourly from 2100 to 0100 h. All trials consisted of 10 reps/treatment; treatment means were compared using Kruskal-Wallis Test. In closed huts, HLC decreased considerably post-treatment relative to pre-treatment with both 1 (2.7±1.7 vs. 0.3±0.4; $p<0.005$) and 2 (1.7±1.2 vs. 0.2±0.4; $p<0.005$) emanators; KD increased with 1 (0.3±0.5 vs 4.2±2.4) and 2 (1.1±6.0 vs. 10.3±6.0; $p<0.005$) emanators. In semi-enclosed huts, HLC in untreated control was 18 times higher than that in the 2-emanator treatment (1.8±1.0 vs. 0.1±0.3; $p<0.005$); KD with was 5 times higher than that of untreated control (4.8±3 vs. 0.9±1.5; $p<0.005$). The number of mosquitoes exiting the hut did not differ between the 2-emanator treatment and control (12±5 vs. 16±6; $p>0.005$). The metofluthrin formulation evaluated had an impact on *An. darlingi* behavior having recorded reduced biting and knock down in experimental huts, supporting potential implementation as a mosquito control method in real, endemic settings.

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FIELD TESTING INSECTICIDE-TREATED BARRIER SCREENS FOR PROTECTION FROM HOST-SEEKING, EXOPHILIC MOSQUITOES IN THE NORTHERN PERUVIAN AMAZON

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Insecticide barriers are an important component of mosquito control programs. Moreover, recent studies have shown that insecticide-treated mesh barriers can reduce foraging mosquito densities, suggesting their potential use as a new mosquito control tool. This study evaluated whether untreated and insecticide-treated screens used as vertical barriers can intercept and reduce densities of host-seeking and resting exophilic mosquitoes in rural areas in the periphery of Iquitos, Loreto, Peru. Mosquito species composition, densities, and human biting rates (HBR) were recorded via protected Human Landing Count (HLC) and direct collection from screens. We first tested two untreated barrier screens (10 m x 2 m) placed 10 m away from a hut in Zungarococha community (March 2-7, 2017). A second trial was performed using an untreated screen and a permethrin-treated screen (10 m x 2 m) placed 10 m away from a dining hall in a military base in Mapacocha (September 5-14, 2017). A total of 3,010 mosquitoes (1,850 by HLC; 1,160 from screens) that comprised 29 species were collected from Zungarococha. *Anopheles darlingi* (40%) and *Culex quinquefasciatus* (18%) were the most abundant species. Baseline HBR (no screen) averaged 20.1 and 32.7 mosquito bites/man/hour intra- and peri-domestic, respectively; untreated screen HBR were 14.4 and 23.1 mosquito bites/man/hour intra- and peri-domestic, respectively (28-29% HBR reduction). In Mapachocha, a total of 2,422 mosquitoes (1,210 by HLC; 681 from untreated screen; 531 from treated screen) that comprised 16 species were collected. *Culex quinquefasciatus* (82%) and *Mansonia indubitans/titillans* (8%) were the most abundant. Baseline HBR (no screen) averaged 8.1 and 12.0 mosquito bites/man/hour intra- and peri-domestic, respectively; treated screen HBR were 5.9 and 8.2 mosquito bites/man/hour intra- and peri-domestic, respectively (27-32% HBR reduction). Results suggest that barrier screens can effectively trap multiple species of foraging, exophilic mosquitoes and potentially reduce exposure to infective mosquito bites in the Amazon.

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TOXIC, ANTIFEEDANT, AND REPELLENT ACTIVITY OF DRIMANE SESQUITERPENES FROM THE MEDICINAL PLANT *CINNAMOSMA FRAGRANS* AGAINST *AEDES AEGYPTI* MOSQUITOES

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Chemical pesticides are often used to control populations of mosquito vectors. However, overuse of these chemicals can lead to resistance in mosquitoes. Moreover, several environmental and health concerns such as accumulation of harmful residues in the environment, and undesirable effects on non-target organisms, including beneficial insects and vertebrates, have been proven to be associated with repeated use of conventional chemical pesticides (e.g., pyrethroids, DDT). These problems highlight the need for discovering safe insecticides and repellents. Recently, compounds derived from botanical sources have received much attention as potentially environmentally-safe bioactive compounds against pestiferous insects. Notably, drimane sesquiterpenes, a class of plant secondary metabolites, are known to elicit antifeedant and toxic effects in insect pests. However, the activities of these compounds against mosquitoes have not been previously characterized. Here we demonstrate that the bark of a plant (*Cinnamosma fragrans*), commonly used in Madagascar as a traditional remedy for a variety of human illnesses, is enriched with an electrophilic drimane sesquiterpene dialdehyde (cinnamodial) that is toxic, antifeedant, and repellent to the yellow fever mosquito *Aedes aegypti,* an important vector of emerging arboviral diseases in humans, such as chikungunya, dengue, and Zika fevers. In addition, we identify other drimane sesquiterpenes from the bark of *C. fragrans* that exhibit toxic and/or antifeedant/repellent activity against *Ae. aegypti* suggesting that the bark contains a 'cocktail' of compounds that are bioactive against mosquitoes. Our data indicate that drimane sesquiterpenes may serve as a valuable chemical platform for the development of next-generation, natural product-based insecticides and repellents for controlling mosquito vectors of emerging arboviruses.

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CO-OCURRENCE OF THE V1,016I AND F1,534C MUTATIONS IN *AEDES AEGYPTI* POPULATIONS RESISTANT TO PYRETHROIDS AND DDT FROM THE COLOMBIAN CARIBBEAN REGION

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Knock-down resistance is conferred mainly by non-synonymous mutations, that reduce the binding between the pyrethroid insecticides and the voltage-gated sodium channel. In 2014, it was reported for first time in *Aedes aegypti* from Colombia the V1,016I mutation and in 2016 the F1,534C mutation in populations resistant to DDT and pyrethroid insecticides. The present study was carried out to determine the frequency and co-occurrence of both mutations in the voltage-gated sodium channel in populations of *Ae. aegypti* and their role in the resistance of pyrethroids and DDT. Nine populations of *Ae. aegypti*, previously characterized as resistant to the insecticides λ-cyhalothrin, deltamethrin, cyfluthrin, permethrin and DDT were used for this study. Genomic DNA was used to determine genotypes by allele-specific PCR for V1,016I and F1,534C mutations. All populations analyzed presented both mutations, with allelic frequencies between 0.07-0.35 for I1,016 and 0.47-0.88 for C1,534. We identified a percentage of co-occurrence of mutant homozygotes I1,016/C1,534 of 5.3%. A significant correlation was found between the knockdown resistance values ($RRCK_{50}$) and the frequency of double homozygous mutants (I1,016/C1,534) for deltamethrin ($r = 0.69$, $P < 0.05$) and cyfluthrin ($r = 0.83$, $P < 0.05$); the frequency of homozygous mutants and heterozygotes (I1,016/F1,534C) for λ-cyhalothrin ($r = 0.91$, $P < 0.05$) and permethrin ($r = 0.70$, $P < 0.05$); heterozygous and homozygous mutants (V1,016I/C1,534) for cyfluthrin ($r = 0.82$, $P < 0.05$) and DDT ($r = 0.76$, $P < 0.05$). In conclusion, the presence of dual kdr mutations in the populations of *Ae. aegypti* from the Colombian Caribbean Region demonstrate a major role in the resistance to pyrethroids and DDT.

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PARALLEL EVOLUTION OF *VGSC* MUTATIONS AT DOMAINS IS6, IIS6 AND IIIS6 IN PYRETHROID RESISTANT *AEDES AEGYPTI* FROM MEXICO

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Presence of pyrethroid resistance in *Aedes aegypti* mosquito populations threatens our ability to control transmission of dengue, Zika and chikungunya viruses in Mexico. A major mechanism of pyrethroid resistance, commonly known as knockdown resistance (kdr) is conferred by amino acid replacements in the voltage gated sodium channel (*vgsc*). Around 11 replacements have been associated with pyrethroid resistance. Interestingly, replacements can vary across geographies and specific combinations of replacements synergize resistance. In Mexico, two kdr replacements (F1,534C and V1,016I) have co-evolved in the last 16 years, eventually reaching fixation across many *Ae. aegypti* populations. Recently, V410L in DI6 segment of *vgsc* was identified in a pyrethroid resistant *Ae. aegypti* strain from Brazil and its effect on *vgsc* activity was confirmed by electrophysiology; nevertheless, V410L was not detected in wild *Ae. aegypti* collections from Brazil. A genome association mapping study in our laboratory identified V410L in *Ae. aegypti* from Mexico and we developed a genotyping system to screen individuals from 25 *Ae. aegypti* historical collections conducted from 2000 to 2016. We tracked the first V410L heterozygote back to 2002. Allele frequencies increased during the last 16 years in close linkage disequilibrium with V1,016I and F1,534C. We also tested the phenotype/genotype association at the tri-locus genotype level showing that the triple resistant homozygote genotype is associated with resistance to both permethrin and deltamethrin.

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EFFICIENT ALLELIC DRIVE DEMONSTRATED IN *DROSOPHILA* WITH POSSIBLE APPLICATIONS IN INSECTICIDE RESISTANCE ERADICATION

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Insecticide resistance is a global problem in a wide range of insect pest species. Various mutations are known to confer DDT/pyrethroid resistance in many insect/pest species including mosquitoes. We want to develop an allelic drive to eliminate such resistance in field population of *Anopheles stephensi*. We have already demonstrated an efficient allelic drive making use of the Notch locus, in which dominant gain-of function and loss-of-function are available. We use a strategy involving a DsRed-tagged genetic insertion that encodes two guide RNAs: the first gRNA ensures the self propagation this insertion, and the second gRNA directs the Cas9

dependent cleavage of a Notch sensitive allele. Another more general approach (copy-grafting) permitting selective inheritance of a desired allele some distance from the gRNA cut site is also developed. We have also observed a phenomenon we refer to as shadow-drive wherein perdurance of non-genetically encoded Cas9/gRNA complexes results in gene-drive associated with lethal somatic mosaicism in the next generation. The combination of efficient allelic-drive and trans-generational lethal mosaic shadow-drive has significant implications for a wide variety of applications and greatly broadens the potential applications of active genetics. In future we will use this strategy to resensitize the resistant field populations of many vectors responsible for various insect-borne diseases. Regarding this we are going to use the model organism *Drosophila melanogaster* to study the polymorphism at two important knockdown resistance (*kdr*) locus i.e. M918 and L1014 in the voltage gated sodium ion channel globally known in *Anophelines spp.*

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INGESTED INSECTICIDE TO CONTROL *AEDES AEGYPTI*: DEVELOPING A NOVEL DRY ATTRACTIVE BAIT STATION (DABS) FOR INTRA-DOMICILIARY USE

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Attractive toxic sugar baits are a promising mosquito control strategy; however, no studies have yet examined how they might be adapted to control *Aedes aegypti*, the main vector of arbovirosis such as dengue, chikungunya and Zika. This study reports on laboratory results of a novel dried attractive bait station (DABS) to control *Ae. aegypti*. DABS were manufactured using foam pieces previously soaked in a sucrose solution laced with boric acid, and allowed to air-dry. To establish the effectiveness of our DABS for killing *Ae. aegypti*, laboratory-reared mosquitoes were introduced into cages with or without a DABS, and mortality was measured. To determine whether the toxic effect was caused by ingestion of the dry bait, we performed "disrupted-feeding" tests in which specimens in experimental groups had their proboscis surgically removed, while control specimens were unaltered. Both experimental and control specimens were exposed to DABS, and mortality was measured. We used electron microscopy to identify any potential damage to midgut tissue caused by ingestion of the toxic bait. Additionally, we tested the persistence of the DABS toxicity for up to 120 days post-manufacturing. Mortality post-exposure to DABS reached 55% within 24 hours, and 100% within 72 hours. Mortality was observed in both starved and blood-fed *Ae. aegypti* females. Furthermore, our results suggest the toxic bait enters the insect´s organism by ingestion, and electron microscopy revealed potential tissue damage in the midgut of specimens exposed to DABS. The killing effect of the devices was maintained for up to 120 days after manufacturing. These results suggest that DABS are effective at killing *Ae. aegypti* in laboratory settings, and prompt the need for further testing these devices under field conditions. DABS can be constructed cheaply, and require no electricity to function. Therefore, if deemed effective under field conditions, the production of these devices may be easily scaled for use in resource-limited settings, potentially becoming a novel and affordable tool for *Ae. aegypti* control.

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SELECTION FOR PERMETHRIN RESISTANCE IN *AEDES ALBOPICTUS* FROM CHIAPAS, MEXICO

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Insecticide resistance and its known mechanisms have been extensively studied in *Aedes aegypti* mosquitoes, the major vectors of Zika, Dengue and Chikungunya viruses. However, another important vector is *Aedes albopictus.* The primary method to control the spread of these vectors and their diseases is by insecticide application. With continued application of insecticides like permethrin, *Ae. aegypti* has developed an observable resistance, requiring increasingly higher concentrations to be killed. Since permethrin is widely used in many countries, it is important to understand if *Ae. albopictus* is also developing resistance and what possible mechanisms are involved. We collected *Ae. albopictus* from Tapachula, Chiapas, MX, and reared them in our lab. We exposed adults from each location to several concentrations of permethrin. The concentration needed to kill 50% of the mosquitoes (LC_{50}) and resistance ratios (RR) were compared to our control colony from New Jersey. Survivors were bred to generate the "La Macha" colony, which were later exposed to the same concentrations in the previous assays. Survivors were kept and bred again. We will continue these procedures for several generations. Another portion of La Macha will be reared separately and remain unexposed to insecticides for later comparison. We observed lower mortality in mosquitoes from Mexico compared to the control, with RR ranging from 1.27 to 2.06, indicating there is a low level of resistance. We predict to see an increase in the RR of La Macha compared to the first assays, however it may take several generations of continued selection to observe this. Additionally, we extracted DNA from survivors of the first assay to be sequenced and screened for known resistance polymorphisms. Alignment of PCR sequences is currently underway to scan for these mutations. Analysis of DNA samples will help improve our understanding of mechanisms associated with the observed differences in mortality of the bottle assays. The results of this study will be important to consider when proceeding with future methods of mosquito control.

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UPDATE ON THE INSECTICIDE RESISTANCE PROFILE OF *AEDES AEGYPTI* FROM CENTRAL AMERICA AND THE CARIBBEAN

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The 2015 Zika outbreak in Latin America and the Caribbean made evident the need to strengthen vector control strategies in the region. The mosquito, *Aedes aegypti,* which also transmits dengue, chikungunya, and yellow fever, is usually controlled by integrated strategies, including the use of insecticides to contain outbreaks and reduce the density of adult mosquitoes. However, in most countries of Central America and the Caribbean, the efforts to understand the levels of susceptibility of the local populations of these mosquitoes have been sporadic and incomplete. Here, a complete screening of the susceptibility of *Aedes aegypti* mosquitoes from El Salvador, Honduras, Guatemala, Dominican

Republic and Haiti by using the WHO and CDC methods is presented. The insecticides tested were the pyrethroids, permethrin, deltamethrin, lambda-cyhalothrin and etofenprox; the organophosphates, malathion and pirimiphos-methyl, and the carbamate, bendiocarb. The susceptibility to the larvicide temephos, the growth regulators methoprene and diflubenzuron, and a commercial formulation of the biolarvicide *Bacillus thuringiensis israelensis* (Bti) was also evaluated. In general, resistance to all pyrethroids is high and widely distributed, tolerance to organophosphates is incipient and there is full susceptibility to the only carbamate tested. The susceptibility to temephos varied depending on the region: in Central America the levels of resistance were high, while in Dominican Republic and Haiti levels were low-moderate. Methoprene and diflubenzuron caused similar lethality to reference and field strains, indicating no resistance. Finally, the commercial formulation of Bti was lethal even below the application dose. The current widespread practice in the region is the use of pyrethroids and temephos to control adult and immature mosquitoes, attributing to the high tolerance levels. These results will contribute to making informed decisions and updating the *Aedes* mosquito control strategies at national and regional levels, as well as stimulate the establishment of national and regional insecticide resistance networks.

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YEAST-ENCAPSULATION OF CITRUS-DERIVED ESSENTIAL OILS AS AN ENVIRONMENTALLY FRIENDLY LARVICIDE

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Larvicides are an integral part of any vector-borne disease control program. Widely used chemical and bacterial larvicides are limited in efficacy and sustainability by cost, toxicity to humans and other non-target species, rapid evolution of resistance, and degradation of the aquatic environment. Recent increases in dengue fever and Zika virus transmission underscore the urgent need for a safe, effective, low cost mosquito larvicide suitable for use in urban areas. Here we report on the production and evaluation of a novel larvicide made from non-toxic, inexpensive food-grade materials (baker's yeast and essential oil). While essential oils (EO) are recognized as cidal to a broad spectrum of mosquito larvae, dispersing EO directly into aquatic environments is problematic due to cost, UV degradation, and environmental disruption. Our approach obviates these limitations by encapsulating EO in baker's yeast, and feeding these yeast microcapsules to larvae. Once ingested, enzymes in the larval gut digest the capsule, releasing EO and killing the larvae. Our presentation describes a method modified to produce yeast microcapsules with 30-40 wt% loading of EO from *Citrus reticulata* (Mandarin orange) and *C. sinesis* (Sweet orange). Laboratory trials with both encapsulated EOs showed dose-dependent killing with an LD_{50} of 30 mg L^{-1} within 24 hours of treatment. Similar results are observed with encapsulated EO components: R-limonene, γ-terpinene, and myrcene. Larvicide treatment is completed without dispersal of EO into the aquatic environment: the yeast microcapsules are produced without surface residues, segregate EO from the aquatic environment, and are directly ingested by the target species. Our presentation includes the results of lab-scale and preliminary field-testing of this novel larvicide, as well as detailed physical and chemical characterization of the microcapsules.

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IMPROVING THE EVIDENCE BEHIND ENTOMOLOGICAL THRESHOLDS FOR INTEGRATED VECTOR MANAGEMENT OF WEST NILE VIRUS

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The vector index (VI) is used to estimate the relative abundance of arbovirus-infected female mosquitoes. For zoonotic pathogens with complex transmission cycles such as West Nile virus (WNV), the VI is being used increasingly to guide vector control programs as an entomological indicator of human infection risk. Despite its frequent use by mosquito control programs, there is no established VI threshold below which transmission to humans would not be expected. To provide better evidence for this threshold, we characterized the relationship between VI and human WNV disease incidence using surveillance data from several vector control programs, each encompassing one or more counties across the state of California. We used Poisson models to identify optimal demographically-adjusted VI thresholds for cities within each county and examined the predictive value of VI at different spatial scales to determine whether VI is capable of providing sensitive and specific predictions of human WNV disease incidence at scales relevant for vector control. We found the relationship between VI and human WNV disease incidence to vary greatly by county, with predicted human incidence for any particular value of VI being higher in agricultural areas than in urban areas. Accordingly, the optimal VI threshold for predicting incipient high-incidence periods in agricultural counties was lower than in more urban counties. Comparisons of predictive performance across spatial scales pointed to the city level as being the smallest spatial unit for which predictions were reliable enough for operational use. We aim to use these results to develop an interactive tool that vector control programs can use to estimate city-specific human WNV risk to inform decisions on when and where to target broad-scale mosquito control measures.

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LOSS OF PYRETHROID RESISTANCE IN *AEDES AEGYPTI* FROM SOUTHERN MEXICO AFTER EIGHT GENERATIONS WITHOUT INSECTICIDE EXPOSURE

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We examined 8 insecticide resistant *Aedes aegypti* mosquito colonies collected from Southern Mexico. Colonies were maintained in laboratory conditions for 8 generations without exposure to insecticides. We screened each generation for the loss of pyrethroid resistance by using PCR-Melting curve to genotype 2 knockdown resistant mutations correlated with pyrethroid resistance in the voltage gated sodium channel; Val1016Ile and Phe1534Cys. Ile1016 allele frequencies ranged from 0.35-0.83 in the F1 generation and 0.31-0.63 in F8. Cys1534 allele frequencies were between 0.67-1.0 in F1 and 0.29-0.99 in F8. We used Fisher's natural selection model for recessive alleles to determine the fitness coefficient for 3 genotypes at each loci which allowed us to predict the evolution of susceptibility for each site. Sites behaved differently since the initial frequencies of the resistant alleles varied. High levels of initial resistance take more generations to become susceptible again. We used bottle assays to evaluate the concentration needed to kill 50% of the population (LC_{50}) for permethrin and deltamethrin in F3, F6 and F8. The resistance ratios (RR) for permethrin ranged from 11.23-49.85 and 1.85-26.18 fold for F3 and F8, respectively. For deltamethrin the RR ranged between 5.79-97.98

and 0.18-36.67 in the F3 and F8, respectively. The RR for both pyrethroids decreased over 8 generations with a stronger effect on deltamethrin. We evaluated F3, F6 and F8 for different life history traits such as number of eggs laid (fecundity), number of hatched eggs, pupation time, adult emergence and sex ratio. Fecundity showed a negative correlation with Ile1016 allele frequencies, Cys1534 allele frequencies and RR of both pyrethroids. This data suggests that there is a fitness cost for resistance, which effects fecundity. However, it is possible to recover susceptibility in resistant mosquito populations. Using Fisher's natural selection model, along with surveillance data, will help improve strategies for mosquito control by calculating the time needed without exposure to insecticides in order to become susceptible for a specific field population.

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NOVEL STRATEGIES TO ANALYZE INSECTICIDE RESISTANCE GENES OF LATIN AMERICAN MALARIA VECTORS

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Malaria is still an important problem for public health in Latin America, and the development of insecticide resistance poses a major threat to the malaria elimination efforts. Monitoring of insecticide susceptibility, combined with the determination of the molecular mechanisms involved in the insecticide resistance, are needed to effectively deploy the appropriate vector control measures. To determine these mechanisms, target site mutations and changes in metabolic enzyme expression have to be analyzed. Novel primers were designed and validated to develop PCR assays that amplify the voltage-gated sodium channel (*VGSC*) and acetylcholinesterase-1 (*Ace-1*) genes in four malaria vectors from Latin America: *Anopheles albimanus, An. darlingi, An. vestitipennis* and *An. pseudopunctipennis*. Also, a quantitative PCR to determine gene expression of the CYP6 genes was implemented using degenerate primers to analyze if cytochrome oxidases are involved on the insecticide resistance of *An. albimanus* populations. The conventional PCR assays and a new qPCR assay were validated to analyze the region of the *VGSC* and *Ace-1* genes associated with insecticide resistance for *An. darlingi*, *An. vestitipennis* and *An. pseudopunctipennis* Quantitative PCR of the P450 CYP6 gene family was developed to assess relative expression in *An. albimanus* laboratory strains. Direct sequencing of PCR products and melt curve analysis confirmed the specificity of the primers to analyzed these resistance mechanisms. Additionally, specific and degenerate primers for the P450 cytochrome families (CYP6, CYP9 and CYP4) were designed for the anophelines vectors to identify possible P450 genes involved in insecticide resistance. The development of these PCR assays presents an important first step in the analysis of molecular mechanisms of insecticide resistance in field populations, allowing for better characterization of insecticide resistance in poorly characterized anopheline species relevant for malaria elimination in Latin America.

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CONSTANT SPATIAL PATTERNS OF HIGH DENSITY OF *AEDES AEGYPTI* EGGS AND THEIR CORRELATION WITH SURROUNDING ROOFED AREA OBTAINED FROM SATELLITE IMAGES

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Vector control of immature forms of *Aedes aegypti* is the main preventive measure of urban arbovirosis such as dengue, Chikunguya and Zika. Identifying and predicting high risk clusters through ovitraps surveillance can improve national strategies aimed at prevention and control. The objective of this study was to characterize the temporal-spatial patterns of egg density of *Ae. aegypti* monitored by ovitraps and to correlate these patterns with surrounding roofed area in Sullana, Peru (pop = 317 443), an endemic area of dengue. Density of *Ae. aegypti* eggs was obtained from the surveillance of 273 ovitraps located in 200 m sided squares monitored weekly in Sullana during 2017. Roofed area was measured in 144.34 m sided hexagons containing at least one ovitrap using satellite images of Sullana from 2017 captured by the RapideEye satellite with a spatial resolution of 5 m on each side, and downloaded from the website www.planet.com (free license). The space-time patterns were defined using Gi * Getis-Ord space-time statistic . The percentage of roofed area between clusters of high and low density of *Ae. aegypti* eggs was compared using T-student test and log-binomial regression implemented with generalized linear models. 186 hexagons with ovitraps were analyzed, 58 of which remained constant clusters in time, resulting in 18 and 40 clusters with high (HDE) and low (LDE) density of *Ae. aegypti* eggs, respectively. The percentage of roofed area between HDE and LDE clusters was different (p = 0.00). The regression analysis showed that, when the percentage of roofed area increased, the prevalence of HDE clusters increased, PR = 1,038 (95% CI 1.005 - 1.071) and the prevalence of the LDE clusters decreased, PR = 0.968 (95% CI 0.951 - 0.985). These findings suggest that roofed area obtained from satellite images is directly associated with the constant presence of a high amounts of *Ae. aegypti* mosquitoes. This knowledge could be useful to identify and predict high risk areas, as well as to design interventions with an effective resources allocation. This methodology might be applicable for the prevention and control of other diseases transmitted by tropical vectors.

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THE GENETIC BASIS FOR OUTDOOR HOST-SEEKING BEHAVIOR IN *ANOPHELES COLUZZII* DURING THE BIOKO ISLAND MALARIA CONTROL PROJECT

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Nationwide vector control programs have been created to reduce malaria transmission across Africa. These programs largely rely on indoor application of insecticides through the use of long-lasting insecticide treated bed nets and indoor residual spraying of insecticides. However, the efficacy of these control tools is threatened by behavioral resistance in which mosquitoes avoid insecticides applied indoors by host-seeking outdoors. One region where increased outdoor host-seeking has been documented is Bioko Island, Equatorial Guinea, where the Bioko Island Malaria Control Project has conducted IRS based vector control since 2004. We examined if behavioral resistance of *Anopheles coluzzii* on Bioko represents an adaptive response to continued IRS. Using a pool-seq approach with large sample sizes (286-1009 mosquitoes/pool) sequenced to a high coverage (average 421-733x/pool), we compared the genomes of indoor and outdoor host-seeking *An. coluzzii* from 2009, 2013/2014. This allows us to identify genetic variation underlying indoor vs outdoor host-seeking. Secondly, we compared pre-intervention *An. coluzzii* collected in 2004 to the post-intervention samples to identify loci under positive selection during the vector control program. Our analyses are

based on identified between 12.6 and 27 million SNPs we identified in our samples. To determine which SNPs have significantly differentiated allele frequencies between groups, we simulated the sampling effects of pool size and coverage level on F_{ST} estimates. We fit these simulated data with a multivariate exponential model which provided the significant threshold for each SNP. Additionally, we are screening for genes containing significantly differentiated SNPs across multiple groups and replicates. Using these methods, we have identified sites associated with indoor/outdoor host-seeking, although a specific mechanism is not currently clear. If outdoor host-seeking does indeed have a genetic basis, ongoing work using this high-powered poolseq approach will identify exophagic alleles responsible for this adaptation.

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SURVEY FOR VIRAL SYMBIONTS IN MOSQUITOES FROM TEXAS, USA AND THEIR INFLUENCE ON VECTOR COMPETENCE OF ZOONOTIC ARBOVIRUSES

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The emergence and re-emergence of mosquito-borne diseases such as Zika, chikungunya and dengue fever remain a global public health challenge that threatens many communities in tropical and subtropical regions of the world. These viruses are driven principally by *Aedes aegypti* mosquitoes and naturally-occurring microbes are intrinsic factors that can influence the ability of mosquitoes to transmit viruses. Additionally, microbial symbionts offer opportunities for innovative vector control strategies such as the way in which *Wolbachia* plays a role in creating populations refractory to the transmission of certain zoonotic arboviruses. Our objective was to identify viral symbionts (i.e. insect-specific viruses) in mosquito vectors from different regions of Texas, USA focusing on: *Aedes aegypti*, *Aedes albopictus,* and *Culex quinquefasciatus*. Our preliminary results showed the presence of cell fusing agent virus (CFAV) and Aedes flavivirus (AeFLAV) in *Ae. aegypti* and *Ae. albopictus* from San Antonio, TX. CFAV was also detected in *Ae. aegypti* populations from the Lower Rio Grande Valley of South Texas. Variation was observed in the minimum infection rate (MIR) according to the different locations reaching up to 178 per 1000 mosquitoes for CFAV and 155 per 1000 mosquitoes for AeFLAV. Additionally, *Culex quinquefasciatus* mosquitoes from College Station, TX were found positive for Culex Flavivirus (CxFLAV), with a low MIR of 0.1 per 1000 mosquitoes. In an effort to understand the interaction of these viral symbionts with human pathogens, we are colonizing populations of these naturally infected ISFs mosquitoes from Texas and conducting co-infection and transmission experiments with arboviruses of human health concern.

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HIGH RISK OF MALARIA TRANSMISSION BY *NYSSORHYNCHUS DARLINGI* IN THE MAZAN DISTRICT, IN THE PERUVIAN AMAZON

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Malaria remains an important public health problem in Peru, where cases have increased since 2011. Over 52,000 cases were reported in 2017, with *Plasmodium vivax* being the predominant species (76%). *Anopheles* (now *Nyssorhynchus*) *darlingi* is the main malaria vector on the Mazan river, Loreto Department. However, in this area little is known about its behavior and how it affects the dynamics of malaria transmission and/or the efficacy of vector control strategies. To assess vector bionomics, five surveys were conducted in four communities in the Napo and Mazan micro-basins, Amazonian Peru in 2016-2017 and seasonal abundance, Human Biting Rate (HBR), Entomological Inoculation Rate (EIR) and Human Blood Index (HBI) were quantified. *Nyssorhynchus darlingi* accounted for 95% of the species collected (6,762/7,118 mosquitoes). Ninety-one specimens (1.6%) of *Ny. darlingi* were positive for *Plasmodium* sporozoites (*P. vivax* VK210/VK247 or *P. falciparum*) of 5,870 tested for sporozoite detection by ELISA assays. The highest EIRs were detected outdoors in March (4.54, Visto Bueno), and June (3.60, Urco Miraño; 3.52, Libertad); and indoors in June (3.05, Visto Bueno). The Mazan river communities (LIB-VIB) showed the highest HBR (294-568) and EIR (3.52-4.54), whereas the highest IR was recorded at the Napo River (URC: 0.14). The HBI ranged from 0.69 - 0.94; humans were the most common blood source, followed by chickens and cows. In addition, 17% of *Ny. darlingi* collected fed on both humans and chickens. In summary, this study found *Nyssorhynchus darlingi* was the only mosquito species incriminated as malaria vector. Moreover, malaria risk is heterogeneous, with higher transmission outdoors than indoors in Urco Miraño (Napo River) but with similar transmission (outdoors and indoors) in Libertad and Visto Bueno on the Mazan River. Taken together, our results underscore the urgent need for alternative malaria vector control methods of local intervention targeting outdoor-biting mosquitoes.

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THE ROLE OF THE MIDGUT BARRIER IN DETERMINING MOSQUITO COMPETENCE FOR ZIKA VIRUS

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During the Zika virus (ZIKV) epidemic the Americas, the vector range of this pathogen was hotly debated. While *Aedes aegypti* was confirmed as the primary vector, conflicting publications on the susceptibility of *Culex quinquefasciatus* called into question the vector-specificity of ZIKV. Understanding which mosquito species are involved in ZIKV transmission, and the mechanisms determining vector competence, is critical to successful vector control. We investigated the susceptibility of *Cx. quinquefasciatus* to ZIKV infection, utilizing multiple mosquito and virus strains, and demonstrated both a laboratory (JHB) and field strain from China to be refractory to infection by diverse ZIKV isolates when exposed via blood meal. However, upon intrathoracic injection of ZIKV, salivary gland infection occurred in JHB strain *Cx. quinquefasciatus*. This demonstrates a midgut barrier to ZIKV infection in JHB *Cx. quinquefasciatus*. We have continued our investigation of this barrier to identify the dynamics of ZIKV in the blood bolus and midgut epithelium of susceptible and refractory mosquito species. *Ae. aegypti* and JHB *Cx. quinquefasciatus* midguts were assayed for ZIKV RNA at 12 hour intervals immediately post infection. Productive viral infection was clear in *Ae. aegypti,* with viral titers dropping quickly after exposure and increasing after an eclipse period. However, no evidence of productive midgut infection was observed in JHB *Cx. quinquefasciatus*. ZIKV RNA was detectable in midguts of this strain early after exposure but quickly dropped below detection. We also detected no infectious ZIKV particles in the blood boluses of JHB *Cx. quinquefasciatus* in the same timeframe, suggesting that the infection barrier occurs rapidly after exposure. Investigation now focuses on the extra/subcellular localization of ZIKV in *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes during this timeframe. Determining the differing virus-vector interactions between susceptible

and refractory mosquitoes may reveal cellular factors critical to virus infection, and improve understanding of molecular mechanisms of ZIKV infection in susceptible vectors.

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MALARIA VECTOR SURVEILLANCE RESULTS IN BURUNDI CALL FOR ENHANCED PREVENTIVE INTERVENTIONS TO CONTROL THE DISEASE

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Malaria remains the leading cause of mortality in Burundi. In 2016, the number of cases reached 8.6 million, out of a population of 11 million. Vector surveillance was implemented in sentinel sites to assess local vector behavior and infectivity to inform the country's vector control program. From December 2016 through November 2017, 12 entomological surveys were conducted in eight sentinel sites. Among the 64,799 human-biting mosquitoes collected, five primary vectors were molecularly identified, *Anopheles arabiensis*, *An. gambiae*, *An. coluzzii, An. funestus* and *An. leesoni*. The biting activity of these vectors significantly increased from 12 am to 5 am in comparison to the first half of the night (from 6 pm to 11 pm) ($p<0.001$). The indoor density per light trap was 12.86 for *An. gambiae* s.l. and 3.53 for the *An. funestus* group. A parous rate of 82.91% was observed for *An. gambiae* s.l. populations. For the *An. funestus* group, the parous rate was 83.66%. The lowest parous rate of 74% was observed at the indoor residual spraying site (Kiremba). The average sporozoite rate observed was 1.81% (66/3648). The monthly variations of entomological inoculation rate (EIR) demonstrated that there are two main malaria transmission seasons in the country. The first transmission season occurred between February and June (range: 5-31 infective bites/person/month) and the second occurred from September to December (range: 7-15 infective bites/person/month). The annual EIR varied among sentinel sites with 0 infective bite/person/year at Matana, 6.90 infective bites/person/year at Kiremba, 14.80 infective bites/person/year at Mabayi, 31.35 infective bites/person/year at Gihofi, 70.63 infective bites/person/year at Vumbi, 80.33 infective bites/person/year at Mpanda, 81.02 infective bites/person/year at Nyanza-Lac, and 265.95 infective bites/person/year at Cankuzo. Significant reduction of the EIR was observed after IRS at Cankuzo (42.58 infective bites/person/month before versus 1.75 infective bites/person/month after IRS; $p=0.038$). These results showed that IRS interventions are having a positive impact on malaria transmission in Burundi.

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SIGNIFICANT REDUCTION OF MALARIA VECTOR TRANSMISSION FROM 2001 TO 2016 IN MALI: A REAL FUTURE FOR NON-SEVERE MALARIA

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Malaria in the Sahel occurs typically over a short period of three months, targeting aestivating mosquitoes could reduce the early *rounds* of vector-human amplifications and potentially reduce malaria transmission exponentially. An integrated fight against malaria since *Plasmodium falciparum* resistance to chloroquine has been undertaken in Mali, to slow down malaria burden. In the current study we compared malaria vector transmission data collected in two endemic areas: Bandiagara and Toukoro. In the first site data were collected prior to the intensive dissemination of ACTs and the policy of large-scale free distribution of long-lasting mosquito treated nets. In the second site, data were collected 15 years after, when ACTs and free MTNs distribution take place. Results comparison show a significant reduction of Entomological Inoculation Rate, EIR from 15 infective bites per man per month in Bandiagara in 2001 to 0.27 infective bites per man per month in 2016 in Toukoro. The reasons for this significant reduction of vector transmission level could be due not only to the political authorities' involvement, but especially because of population's massive adhesion to the global integrative strategy of the fight against this disease. That show positive impact of collaboration between scientists, heath centers, communities and decision makers.

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THE IMPACT OF CO-CIRCULATING PARASITES ON WEST NILE VIRUS TRANSMISSION

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Interspecific interactions between parasites are known to influence population and community epidemiology. *Culex* mosquitoes ingest a variety of viral, protozoan, and macro-parasitic organisms that circulate among avian and mammalian hosts, however, the epidemiological consequences of mosquito co-infection on vector-borne transmission remains largely unknown. Using controlled experimental transmission studies we assessed how avian malaria co-infection impacts vector survivorship and the ability for vectors to transmit West Nile virus (WNV). *Culex quinquefasciatus* mosquitoes colonized from Texas were artificially exposed using membrane feeders to *Plasmodium relictum,* propagated in domestic canaries (*Serinus canaria*), and strains of West Nile virus isolated in Texas in combinations of sequential and concomitant infection. Results from these controlled transmission assays were used to parameterize multi-host, multi-vector compartmental models and assess how avian malaria may impact West Nile virus transmission dynamics at population and community scales. Transmission heterogeneity was evaluated using stochastic simulations and sensitivity analyses employing a Latin Hypercube sampling design. Results highlight the importance of considering co-circulating parasites, such as avian malaria, when evaluating the spatial and temporal risk of WNV transmission and further enrich our broader understanding of mechanisms that drive vector-borne disease heterogeneity.

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A NOVEL APPROACH FOR PRODUCING FIELD-BASED ESTIMATES OF *ANOPHELES GAMBIAE* BITING BEHAVIOR AND DISPERSAL ABILITY

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Mosquito-borne diseases cause a significant burden on human populations in the tropics. Distribution of these diseases is governed by a complex mix

of genetic, environmental and social factors which in turn affect pathogen, vector and host interactions. Different mosquito species show a variety of host biting behaviors. Some species show an extreme preference for human blood over other non-human blood meal sources. As a result, their influence on the transmission of human vector borne diseases is highly significant. However, even the most anthropophilic of disease vectors will source a proportion of their blood meals from non-human hosts. A novel, field-based method to inform the plasticity in the vector's choice of blood-host using strategically located mosquito traps for *Anopheles gambiae* has been developed. We present the first estimates for the remarkably local spatial scale across which this behaviour is plastic and provide novel data on mosquito dispersal behavior post feeding. This novel experimental set up informs two aspects of vector behaviour in the field which are imperative to disease transmission and for which current estimates are lacking for all major vector species'.

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INSERTION OF OIL PALM INTO FOREST IN BORNEO NARROWS MOSQUITO DIVERSITY BUT FAVORS A KEY VECTOR OF SYLVATIC DENGUE VIRUS

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Notable arboviruses including dengue (DENV), chikungunya, and Zika, emerged into human transmission via spillover from sylvatic transmission cycles maintained between non-human primates and *Aedes* mosquitoes in tropical forests. These ancestral sylvatic strains continue to circulate and to spill over into the human population in present day. To forestall emergence of novel virus strains from these sylvatic cycles, it is crucial to identify the environmental factors that shape the likelihood of spillover. Sarawak, Borneo, a known focus of sylvatic DENV transmission, is undergoing rapid land cover change, particularly insertion of oil palm plantations into forests. We hypothesized that this land cover conversion would alter the diversity, abundance, and distribution of mosquitoes and thereby influences rates of spillover. We tested four predictions: (i) mosquito diversity and abundance would decline in plantations; (ii) mosquito diversity would be greatest at the forest edge iii) *Aedes albopictus*, an opportunistic vector of DENV, would occur in equal abundance in plantations and forest, (iv) *Ae. niveus,* a canopy-living vector of sylvatic DENV, would be most abundant in forests. Adult mosquitoes were collected at 34 sites each at 10 distances from interior oil palm to interior forest in Sarawak. In total, 894 mosquitoes comprising 24 genera were collected. Genus-level mosquito richness and diversity increased with distance into forest and sites 100 m into the forest were the most diverse, contrary to our prediction. Mosquito community composition was different between distances sampled, but remained more similar from 10 m up to 500 m. Mean mosquito abundance declined significantly in interior plantation; however, as predicted there was no difference in the mean number of *Ae. albopictus* sampled at any distance. While rare, *Ae. niveus* were only found at least 10m into the forest, supporting our fourth prediction. These data indicate that likelihood of spillover of sylvatic viruses increases with distance into the forest, where more types of potential vector species occur and where mosquito abundance is, on average, higher.

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QUANTIFICATION OF MOSQUITO BITING RATES USING SURVEYS AND THEIR IMPLICATION IN DETERMINING DENGUE VIRAL TRANSMISSION RISK IN THE GREATER NEW ORLEANS REGION

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Over the past decades, *Aedes*-borne viral diseases such as dengue, chikungunya, and Zika have been surging in incidence and threatening to spread to new geographical areas where their mosquito vectors thrive. In order to estimate viral transmission risks in these areas, mathematical modeling is required; and the accurate estimates of the local transmission parameters are essential. One of the most important parameters that determines infection risk is mosquito biting rate. However, this rate is rarely characterized in field settings due to the lack of appropriate and research methods. In this study, mosquito biting rates in two study sites within the Greater New Orleans Region were estimated using a short survey questionnaire to ask study participants about the quantities of mosquito bites they experienced in the past 24 hours. In addition, Human Landing Capture was utilized in the same two areas to estimate the proportions of perceived mosquito bites that belonged to *Ae. aegypti* and *Ae. albopictus*. The results demonstrated significant variations in biting rates between outdoors and indoors and between the two residential areas. Higher exposure to mosquito bites was correlated with the amount of time study participants spent outside during risk periods. There was also a significant effect of study site on bite exposure outdoors, possibly due to the difference in the numbers of host-seeking mosquitoes. An epidemiological compartmental model describing dengue virus transmission was applied to demonstrate how the observed differences in the biting rate of *Ae. aegypti* and *Ae. albopictus* between the two study sites would result in differential dengue transmission risks in these areas. This study highlights a practical research method, the survey questionnaire, that could be used to estimate mosquito biting rates in other locations.

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ACOUSTICS BEHAVIOR OF THE MALARIA VECTOR *ANOPHELES ALBIMANUS*

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Malaria is one of the most important mosquito-borne diseases worldwide. In Colombia, 52,954 cases of Malaria were reported in 2017. *Anopheles albimanus* is one the primary vectors of malaria in Colombia, where containment of the disease is currently focused on the use of insecticides and bed nets. However, due to changes in mosquito behavior and an increase in insecticide resistance, it is necessary to develop new methods of control that exploit the biological traits of *An. albimanus*, potentially allowing for more specific targeting of this disease vector. Reproduction is one of the main mosquito biological traits related to vectorial capacity. However, little is known about *An. albimanus* behavior during courtship and mating, including acoustic behavior. Our goal is to better understand the acoustics of *An. albimanus* in order to develop and/or improve tools implemented in vector control. To gain insight into *An. albimanus* swarming and courtship behavior, we are using methods such as artificial vision and acoustic source separation techniques to dissect the basics of mating in this species under laboratory conditions, focusing on swarming

and mating acoustics. We previously described that the fundamental frequency of tethered *An. albimanus* females is 368.9 ±34.2 Hz and 524.11 ±63.7 Hz in males, independent of individual size. Here, we describe the fundamental frequency of individual, free flying females and males, both of which are significantly higher than when individuals are tethered. When measuring the fundamental frequency of groups of males, we observed a unique tone when males display a stereotypical flight. Finally, we characterized the fundamental frequency of pairs of female and male mosquitoes before copulation or rejection. Taken together, these results contribute to our knowledge of *An. albimanus* mating behavior and will potentially aid in future vector control efforts.

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VECTOR MOSQUITOES AND COMMUNITY KNOWLEDGE, ATTITUDES, AND PRACTICES DURING HURRICANE RESPONSE AND RECOVERY IN THE UNITED STATES VIRGIN ISLANDS

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In recent decades, the United States Virgin Islands mosquito surveillance, control, corresponding research, and community outreach has been limited and sporadic. As a result, little is known about the USVI's vector mosquito populations and the community's knowledge, attitudes, and practices about mosquitoes. Both are important aspects of a hurricane response and recovery and are relevant after recent Hurricanes Irma and Maria hit the U.S. Virgin Islands on September 6, 2017 and September 19, 2017, respectively. Mosquito surveillance for adult *Aedes aegypti* in response to the Zika epidemic began in February 2017 and is ongoing. Gaps in data remain because of the hurricanes and available funding. Preliminary *Aedes aegypti* surveillance results suggest a population peak at the end of April to early May following dry season, but one year of data is insufficient to establish a trend. We conducted Community Assessments for Public Health Emergency Response (CASPERs) in June 2017, November 2017, and February 2018 to provide household-level community information pre- and post-hurricane. CASPERs were conducted with two-stage cluster sampling. Thirty census blocks were selected with probability of selection proportional to the number of households within the cluster. Teams then systematically selected households within each cluster. An adult resident of selected households was invited to complete the in-person questionnaire. According to the November 2017 CASPER, 87% of households experienced an increase in mosquito biting after the hurricanes. In addition, environmental conditions became more favorable for mosquito breeding and feeding from June to November 2017. Inadequate mosquito population trend information hindered the ability to conduct a more thorough response despite evidence of increased mosquitoes. Understanding mosquito population trends before hurricanes in conjunction with community assessments is critical to an effective hurricane response and re-building mosquito surveillance and control capacities in the U.S. Virgin Islands.

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THE ZIKA AIRS PROJECT (ZAP): FIRST YEAR OF ENTOMOLOGICAL SURVEILLANCE AND VECTOR CONTROL USING A BIOLARVICIDE IN COUNTRIES OF LATIN AMERICA AND THE CARIBBEAN

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In 2015 and 2016, the Zika virus spread rapidly across the region. The high incidence of this virus, as well as its link with microcephaly in infants, raised international concern and the World Health Organization (WHO) declared a public health emergency in 2016. In response to this emergency, the United States Agency for International Development (USAID) approved additional funds to implement activities to prevent Zika transmission. The Abt-led initiative, Zika AIRS Project (ZAP), is enhancing USAID's ability to build capacity in Latin America and the Caribbean for vector control, working alongside ministry of health officials to prevent Zika transmission through entomological monitoring and vector control strategies. After its first year of implementation, ZAP outcomes include the reduction of *Aedes aegypti* populations at the household level through regular field team visits to conduct environmental cleanup, ento-monitoring and application of a biolarvicide based on *Bacillus thuringiensis*. Targets for the larviciding campaign were wide-ranging, with the smallest coverage targeting 34,000 houses in Haiti, to the most inclusive in Honduras comprising 280,000 households. Complementary strategies to study local *Aedes aegypti* populations involved sentinel site surveillance of egg density using ovitraps with/without hay infusion in 100 houses, and larvae/pupae surveys in 200 houses. The main breeding sites for *Ae. aegypti* are drums, plastic-containers and tires in Haiti, cement tanks or pilas in Guatemala, El Salvador and Honduras; and metal barrels in Guatemala and El Salvador. Results showed higher percentage of eggs when using infusion, in all countries. Density of eggs, pupae, and traditional *Aedes* indexes had a decreasing tendency in all sites across countries after a few weeks post surveillance, and before and after larvicide application. Breteau indexes reached historic low values (1-5) in El Salvador and Honduras post larviciding campaign. ZAP teams have increased the number of trained personnel and invigorated technical capacity for public health entomology in all target countries.

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HIGH DIVERSITY OF ANOPHELINES IN OUTDOOR COLLECTIONS IN NCHELENGE DISTRICT, ZAMBIA

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Among the major challenges faced in the global drive to eradicate malaria is the existence of previously unrecognized and potential vectors, as well as behavioral plasticity of the major vectors. Because the vast majority of vector interventions are implemented inside sleeping structures, universal coverage of bed nets and indoor residual spray will still leave people vulnerable to residual transmission from exophagic and exophilic vectors. High malaria transmission persists in Nchelenge District of northern Zambia, despite decades of control in the form of artemisinin-combination therapies, indoor residual spray, and bednets. The Southern and Central Africa International Centers for Excellence in Malaria Research (ICEMR) studies this region, as part of efforts to understand the mechanisms underlying persistently high transmission. The major vectors in this region are *Anopheles funestus s.s.* and *An. gambiae s.s.* Very few other species are caught indoors. Outdoor vector activity has not been rigorously studied to date. In order to study outdoor foraging vectors, we used a Latin square design, and rotated households through 3 different 'treatments' on different nights: CDC light traps in adjacent animal pens, CDC light traps indoors near sleeping inhabitants, and CDC light traps with artificial baits adjacent to household entryways. Preliminary analysis has shown that while *An. funestus* was most abundant, a large number of *An. coustani* were also collected in addition to over 10 different other morphological species of anophelines. Ongoing analyses will characterize the transmission

potential of these species in our study area, including CSP ELISA to determine infectious rates and blood meal PCR to determine biting rate and human blood indices.

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VECTOR COMPETENCE OF *AEDES ALBOPICTUS* FOR ZIKA VIRUS

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Much work has been done in recent years to determine the vector competency of *Aedes albopictus* (Skuse) for Zika virus (ZIKV). If competent, *Ae. albopictus* could become a very important vector in the spread of ZIKV to more northerly latitudes as well as a link between sylvatic, rural and urban systems. Recent review of the literature surrounding *Ae. albopictus'* competence for ZIKV, while suggestive, provides no quantitative analysis of *Ae. albopictus'* competence, nor does it address the potentially confounding differences between studies. For our study, we undertook a quantitative meta-analysis of the literature surrounding this topic. We examined infection rates (IR) and transmission rates (TR) at 7 and 14 days post infection (dpi) across 9 studies comprised of 26 sub-studies/treatments using a restricted maximum likelihood (REML) model. Our model examined potentially confounding variables in the studies, including freshness of blood meal, origin of viral strain, log transformed viral dosage and whether the viral strain and mosquito population tested came from the same geographic region. Our results suggest that: 1) *Ae. albopictus* is a competent vector for ZIKV, 2) Freshness of blood meal has a large and significant effect on transmission at 7 dpi, 3) Co-occurrence of viral strain and mosquito population has a significant effect on both IR and TR at 14 dpi, 4) East Asian and Oceanic strains of ZIKV are less infective to and transmissible by *Ae. albopictus* than South American and North American strains of the virus. These results indicate a need to re-examine the literature and to revise methods for vector competence studies.

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GENETIC DIVERSITY OF *ANOPHELES COUSTANI* IN HIGH MALARIA TRANSMISSION *FOCI* IN SOUTHERN AND CENTRAL AFRICA

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Despite ongoing malaria control efforts implemented throughout sub-Saharan Africa, malaria remains an enormous public health concern. Current interventions such as indoor spraying of insecticides and use of insecticide-treated bed nets are aimed at targeting the primary malaria vectors that are majorly endophagic and endophilic. While these control measures have resulted in a substantial decline in malaria cases caused by these primary vectors, and continue to impact indoor transmission, the importance of secondary vectors for malaria transmission has been neglected. One of these secondary vectors, *Anopheles coustani*, is a species previously believed to exhibit mostly zoophilic behavior. Recent studies from across Africa are bringing to light the contribution of this and ecologically similar anopheline species to human malaria transmission. Like many of these understudied species, *An. coustani* has greater anthropophilic tendencies than previously appreciated, is often both endophagic and exophagic, and carries *P. falciparum* sporozoites. These recent developments highlight the need for more studies in afflicted regions to control this vector. This study analyzed outdoor *An. coustani* mosquitoes captured from high transmission settings in Nchelenge District, Zambia, and Kashobwe Health Zone and Kilwa Health Zone, the Democratic Republic of Congo using CDC light traps and pyrethroid spray catches. Species identifications were determined morphologically and confirmed by sequencing mitochondrial DNA cytochrome *c* oxidase subunit 1 (COI) and ribosomal internal transcribed spacer region 2 (ITS2). Furthermore, blood feeding host preference was determined by PCR and *Plasmodium falciparum* infectivity by ELISA. Maximum parsimony trees were constructed from the aligned sequences and genetic relationships showed three distinct clades. This study presents a more thorough understanding of *An. coustani* genetic diversity and bionomic characteristics, which will enable the development of more effective vector control strategies to limit indoor and outdoor transmission, and ultimately, prevent secondary vectors from becoming primary vectors.

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INVESTIGATING THE BIOLOGY AND BEHAVIOR OF *ANOPHELES SQUAMOSUS* AND ITS ROLE IN MALARIA TRANSMISSION IN SOUTHERN ZAMBIA

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In the last decade, malaria cases in Southern Zambia have declined by 90% due in part to national control efforts. Although some areas, such as the Nchelenge District in northern Zambia, still experience high transmission, Choma District in southern Zambia is approaching elimination. In 2011, higher than expected rates of anthropophily was observed among "zoophilic" species in the catchment area of Macha Hospital, including *Anopheles squamosus*, indicating the potential importance of secondary malaria vectors. The importance of *An. squamosus* as a secondary malaria vector was confirmed in 2016 when *Plasmodium falciparum* sporozoites were detected in the species within the pre-elimination zone of southern Zambia. *An. squamosus* have been shown thus far to be mainly exophilic and exophagic (feeding and resting outdoors). If they are feeding on humans outdoors, they may be feeding at different times and in different areas than the primary vectors. If this is the case, new control measures may be necessary for achieving and sustaining malaria elimination. Due to its previously presumed lack of importance to malaria transmission, little is known about the foraging behavior of *An. squamosus*. Mosquito collections were performed at three locations per household—inside a sleeping house, next to a cooking structure, and next to an animal pen—throughout a 24-hour period using primarily CDC light traps. Households were randomly selected among households enrolled in the ICEMR study with animal pens in the Macha area. After collection, mosquitoes were morphologically and molecularly identified, infectivity assessed by CSP ELISAs and blood meal origin determined by PCR. Ecological and human behavioral data were incorporated to evaluate associations between climatic and human population activity with *An. squamosus* population density and behaviors and to determine the level of exposure. Data from these collections will help identify the extent of the role *An. squamosus* has in malaria transmission in southern Zambia, evaluate the efficacy of current vector control methods, and provide data for alternative control strategies.

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AMERICAN ROBINS AND COMMON GRACKLES EXPERIMENTALLY INFECTED WITH WEST NILE VIRUS DIFFER IN THEIR INFECTIVENESS TO *CULEX PIPIENS* MOSQUITOES

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West Nile virus (WNV) is a mosquito-borne arbovirus of songbirds. In nature, songbirds are commonly infected with blood parasites (*e.g.*, microfilariae, haemosporidia, trypanosomes). In certain systems, arboviral infections in microfilaremic hosts have been shown to enhance the

infectivity of arboviruses to mosquitoes. Thus, the original aim of this study was to examine the reservoir competence of WNV-infected birds to mosquitoes, comparing birds with active blood parasitemias versus birds without blood parasitemias. To do this, American Robins and Common Grackles were live-trapped and tested for antibodies to WNV. Only WNV-seronegative birds were used in the study. Birds (six Robins, nine Grackles) were screened by microscopy for microfilariae and trypanosomes and by PCR for haemosporidia. Parasitized and non-parasitized birds were then inoculated with WNV. Groups of *Culex pipiens* mosquitoes were allowed to feed on the birds over the subsequent three nights. Viremia profiles were similar between Robins and Grackles, peaking at Days 1 and 2 after virus inoculation. Within a bird species, the presence or absence of blood parasites had little effect on the infectiousness of WNV-infected birds to mosquitoes. However, WNV-infected Robins and Grackles with comparable viremias differed significantly with respect to their infectivity to mosquitoes. Over a range of viremias, Robins were always more infectious than Grackles. These results suggest that the infection threshold of arboviruses may depend not only on the species combination of virus and vector, but also on host species. Estimates of reservoir competence based solely on the profile of host viremia following viral inoculation may lead to inaccuracies. Estimates of reservoir competence may be more accurately determined by feeding cohorts of vectors on experimentally-infected hosts over a range of viremias and determining the resultant vector infection rates.

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IDENTIFICATION OF PUTATIVE MOSQUITO VECTORS IN AN ARBOVIRAL ENDEMIC AREA OF COSTA RICA

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Costa Rica is an endemic country for several arboviruses. Ostional is a district located in the northwest where wet and dry seasons are clearly marked and with a landscape mainly constituted of tropical dry forest and cattle ranches. Recently, human and equine outbreaks of several arboviruses have occurred in Ostional and its surroundings. Our aim was to capture and identify potential vectors for West Nile Virus (WNV), Madariaga Virus (MADV), and Venezuelan Equine Encephalitis Virus (VEEV). We sampled 8 different estates during one year. Encephalitis Vector Survey (EVS) Traps, CDC Gravid Tramps, and ovitraps were placed in 4 different areas: intradomiciliary, peridomiciliary, pen, and forest. The traps were placed from 18:00 to 6:00 hours. The EVS traps were baited with 2 lbs of dry ice and the CDC Gravid Traps with hay infusion. Even though our sampling effort was limited, we were able to identify several species considered as vectors of WNV and VEEV among other viruses. We collected approximately 25 different species of mosquitoes. The EVS capture success was significantly lower during the dry season than in the wet season, whereas the CDC Gravid Trap success was greater during the dry season. Overall, the most common species captured with the EVS and CDC Gravid Traps during the wet season were *Culex quinquefasciatus* (36.3%), *Deinocerites pseudes* (22.9%), and *Anopheles albimanus* (12.2%); while during the dry season *De. pseudes* (60.0%) was the most common species found. Larvae of *Aedes aegypti* and *Aedes albopictus* were collected in the ovitraps during both seasons. Other species such as *Trichoprosopon digitatum*, a yellow fever vector, were captured only during the dry season. Despite an important drop in the number of mosquitoes and species captured during the dry season, the presence of known arboviral vectors such as *De. pseudes,* may indicate the maintenance of enzootic transmission cycles year-round though with sporadic arboviral spillovers. Further sampling and testing of mosquitoes for different arboviruses presence are required to correctly address arbovirus transmission dynamics in this endemic area.

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SEASONALITY OF MALARIA VECTOR ABUNDANCE ALONG IRRIGATION GRADIENT IN BWANJE VALLEY SCHEME IN MALAWI

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Irrigation schemes present a unique challenge when considering malaria control interventions. While many efforts have led to a decline in transmission intensity, the disease continues to be a major public health problem in Malawi. However, these efforts could prove to be inefficient as Malawi increases surface irrigation through the expansion of irrigation schemes. While this is an opportunity to address food insecurity and improve rural livelihoods; it also increases malaria risk through the proximity of vector breeding sites to human residences. Expanding surface water creates more Anopheles breeding habitats, thereby increasing local transmission. We conducted an entomological study to study the vector density and parasite transmission in Bwanje Valley Irrigation Scheme in Dedza, Malawi. Mosquitoes were collected in 14 villages around the Bwanje Valley at the end of the rainy season in April 2016 and 2017 involving 400 households per year. A total of 6700 mosquitoes were collected from 344 households in 2016 and 5300 mosquitoes from 283 households in 2017. These were sampled by indoor CDC light trap without dry ice and played in sleeping rooms. Mosquitoes were identified morphologically and by PCR methods to species. Of the malaria vectors, (in 2016 and 2017 respectively) Anopheles arabiensis (68%; 59%) was the dominant mosquito in collections and Anopheles funestus was second most common (10%; 19%), whilst Culex sp represented 18%; 21% and Mansonia sp represented 2% and 1% of total collections, respectively. An average of 19 mosquitoes per household were collected (5-56 range) per trap-night and six villages had counts above the average. All were close to the scheme. The malaria transmitting species were most abundant in six of the fourteen villages and in these villages. An. funestus was relatively more abundant in four villages only. Mosquito abundance in 2016, a drought year revealed that villages most proximal to the scheme had the highest Anopheles abundance compared to those further away. In 2017 under more abundant rainfall there were more breeding sites resulting in mosquito abundance independent of the irrigation gradient.

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APPLICATION OF A NOVEL HIGH-THROUGHPUT MOLECULAR APPROACH FOR VECTOR-BORNE DISEASE SURVEILLANCE TO MALIAN AND GUINEAN MOSQUITOES

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Efficient vector-borne disease surveillance is critical for reducing disease transmission and preventing outbreaks. Unfortunately, current surveillance approaches are resource-intensive: the collection of samples is time consuming and requires trained personnel, the vector species identification is laborious, and the detection of pathogens is expensive. As a consequence, efforts typically focus on monitoring only a few specific pathogens associated with the most current threats, which hamper early detection of emerging pathogens. Recently, we developed a PCR-based assay which, combined with high-throughput sequencing, allows the detection and characterization of a large variety of eukaryotic parasites in a single sample. Here, we apply this assay to analyze more than 600 *Anopheles* mosquitoes collected from several locations in Guinea and Mali. We screened each individual mosquito for DNA from *Microsporidia,*

Plasmodium, Spirurida, Trichocephalida, Apicomplexa, Kinetoplastida, Parabaslia, Platyhelminthes and RNA from Arboviruses. In addition, we simultaneously characterized the species of each mosquito, preliminarily assessed the mosquitoes' genetic diversity at the ND5 locus, genotyped insecticide resistance alleles and determined the composition of each blood meal. In this comprehensive approach, we are able to gain insight about the distribution and diversity of both the pathogens and vectors in a high-throughput and cost-efficient manner. This study offers an examination of this assay as a preliminary surveillance tool that can provide a framework for designing targeted methodology to monitor pathogens and vector species.

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DIVERSITY OF *ANOPHELES* MOSQUITOES FROM ANTHROPIC LANDSCAPES OF AN ENDEMIC MALARIA REGION OF COLOMBIA

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The landscape structure influences the distribution and abundance of *Anopheles* mosquitoes. This work aimed to determine the relationship between *Anopheles* diversity with patch number and with landscape diversity in five localities of a malaria endemic region in northwest Colombia. Mosquitoes were collected at six sites per locality; land covers at 1.5 Km radius were characterized using orthorectified aerial photographs and landscape metrics were calculated. The diversity of the *Anopheles* community in each locality was estimated, and a linear regression model was applied to evaluate the relationship between *Anopheles* diversity with the number of patches and the landscape diversity. In general, landscapes with land covers associated with mining and livestock activities were mainly observed. A total of 2,458 mosquitoes were collected and corresponded to 10 species. The highest species richness was registered in Puerto Triana locality, with eight species, and the lowest, in La Lucha and Villa Grande, with five species each. The highest species diversity was registered in Cuturú and Puerto Triana (Simpson 1-D= 0.714 and 0.701 respectively) and the lowest in La Lucha (1-D= 0.239). The linear regression model showed a negative relationship between patch number with *Anopheles* richness (r^2= 0.84; r = -0.92; p<0.05), and with *Anopheles* diversity (r^2= 0.83; r =-0.91; p<0.05). No significant relationship was found between landscape diversity and *Anopheles* diversity (r^2= 0.59; r= 0.77; p>0.05). The results indicate that landscape fragmentation, expressed as the number of patches, influences *Anopheles* diversity. Additionally, they suggest that changes in forest cover associated with human activities exert a selection pressure on the species, favoring the presence of malaria vectors. This information contributes to the planning of vector control interventions.

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IMMUNOMODULATORY ROLE OF ARYL-HYDROCARBON RECEPTOR IN INSECTS

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Insect have developed an efficient immune system during the evolutionary interactions with microbes, to defend against various microbial pathogens. The immune homeostasis is well maintained by different negative feedback mechanisms. The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor. Upon activation, AhR is translocated into the nucleus and transcribe genes for various functions. In this study, we examined the role of insect AhR signaling in immune regulation in mosquito *Anopheles gambiae* and fruit fly *Drosophila melanogaster*. In the mosquito model, we used different chemical AhR modulators to control AhR activation, and RNAi mediated AhR knockdown. When AhR was inhibited by feeding chemical inhibitors, CH223191, StemRegenin1 and 680C91; mosquitoes were more resistant to systemic infection with a bacterium *Serratia* sp. S1. The survival rate increased from 58% (in naïve control) to 75-85% in treated mosquitoes (P<0.01). Similar trends were observed after AhR gene silencing by RNA interference, which resulted in higher survival. On the opposite, when mosquitoes were treated with agonist kynurenine, survival rate was reduced from 58% to 38.7% (P<0.01). In *Drosophila*, the AhR is encoded by gene *spineless, Ss.* There are *Ss* loss of function mutant lines. Bacterium *Providencia* is pathogenic and causes mortality in flies when ingested. The *Drosophila* wild type flies were more sensitive to the oral infection, showing a higher mortality than the two mutant lines, Ss^1 and Ss^a (P<0.01). When wild type flies were treated with an AhR antagonist CH233191, the survival was improved (P<0.05). Furthermore, the interrogation of transcriptome of before and after infections in AhR manipulated mosquitoes revealed various genes that may be involved in the AhR mediated immune regulation. The data indicate that insect AhR plays a role in negative modulation of immunity. In the context of host-microbiota symbiosis, AhR may recognize microbe-derived metabolites and mediate immune tolerance to maintain symbiotic homeostasis.

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QUANTIFYING SOCIODEMOGRAPHIC HETEROGENEITIES IN THE DISTRIBUTION OF *AEDES AEGYPTI* AMONG CALIFORNIA HOUSEHOLDS

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The arrival of travelers infected with Zika virus in California and other regions of the U.S. has created an urgent need to understand the genesis of potential local chains of transmission and whether variation in risk can be attributed to socio-demographic factors. Since the first detection of *Aedes aegypti* in Los Angeles County in 2014, the mosquitoes have continued to spread to new cities and have now been detected in 62 cities within the county. Understanding heterogeneities in the distribution of household *Ae. aegypti* abundance is important in estimating the risk of local Zika transmission. We conducted a cross-sectional study in Los Angeles County during summer 2017 to identify heterogeneities in relative household abundance of *Ae. aegypti*. 163 houses were surveyed, representing a wide range of incomes. Surveys consisted of systematic mosquito collections, inspection of the household and property, and administration of a questionnaire in English or Spanish. Households were surveyed on lifestyle and household characteristics, including those that may affect *Ae. aegypti* biting exposure and breeding. We obtained sociodemographic variables, including median income, population density, and property size from the U.S. Census Bureau. Generalized linear models were used to determine whether lifestyle, household, and socioeconomic risk factors were associated with increased *Ae. aegypti* abundance. Overall, 72% of households had *Ae. aegypti* present, 12% of households had *Ae. aegypti* present indoors, and an average of 3.33 *Ae. aegypti* were collected at each household. We found that higher median household income within each household's census tract was associated with lower outdoor *Ae. aegypti* abundance, and was not associated with indoor abundance. We also found that the density of containers with standing water present on properties (number of containers per 1,000 square feet) was the best predictor for *Ae. aegypti* abundance both indoors and outdoors. This study will provide tools for vector control and public health agencies to develop appropriate management strategies for communities at-risk for *Aedes*-borne virus transmission.

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IMPACTS OF LARVAL NUTRIENT ENVIRONMENT ON GUT MICROBIOME FORMATION IN *AEDES AEGYPTI*

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The midgut microbiome is critical for *Aedes aegypti* larval development and can have important implications on vector competence for multiple human pathogens including dengue virus. Given its multifaceted role in vectorial capacity, the microbiome is a prime target for vector control efforts. However, the factors that determine its formation and size are still not well understood. The environment has been shown to play a substantial role in determining microbiome composition. In the current work, we investigate the impact of nutritional availability during larval development on midgut microbiome composition and microbiome size during multiple stages of development. We reared *Aedes aegypti* larvae under four larval food regimes: 0.25mg/larva/day ("low"), 0.75mg/larva/day ("medium"), 1mg/larva/day ("standard"), 2mg/larva/day ("high"). We determined the impact of nutrient availability on body size by measuring wing length in adults from all treatments. We collected DNA samples from larval water, whole 4th instar larvae, sugar fed adult midguts, and blood fed adult midguts 24 hours post blood meal. We performed 16S qPCR to determine the relative level of bacteria in each sample type as well as high-throughput 16S amplicon sequencing to determine composition of the microbiome in each sample type. Adult mosquitoes reared in the low nutrient environment were smaller than those reared in standard nutrient conditions. Preliminary 16S quantification suggests that larvae reared in the low nutrient environment harbor fewer bacteria in their midguts relative to those reared under standard conditions. This persists through adulthood and even after blood feeding, with lower levels of bacteria detected in low treatment adult midguts relative to adults reared in standard conditions. Investigation into differences in microbiome composition are ongoing.

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IMPACTS OF TEMPERATURE ON ZIKA VIRUS TRANSMISSION POTENTIAL

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Diseases like Zika, dengue, and chikungunya, which were once considered tropical and sub-tropical diseases, are now threatening temperate regions of the world due to climate change and increasing urbanization. Temperature is a strong driver of vector-borne disease transmission, and characterizing the thermal range and optimum for transmission is crucial for accurately predicting arbovirus emergence and spread. To address the lack of data on the relationship between temperature and key pathogen traits for emerging arboviruses, we conducted a series of experiments to estimate the thermal performance of Zika virus (ZIKV) in field-derived *Aedes aegypti* across eight constant temperatures. We observed strong, unimodal effects of temperature on vector competence, extrinsic incubation period, and mosquito survival. We used thermal responses of these traits to update an existing temperature-dependent R_0 (the basic reproductive number) model, to infer how temperature impacts ZIKV transmission. We demonstrated that ZIKV transmission is optimized at a mean temperature of approximately 29°C, and has a thermal range of 22.7°C to 34.7°C. The predicted thermal minimum for Zika transmission is 5°C warmer than for dengue virus which suggests that current estimates on the global environmental suitability for Zika transmission are over-predicting its possible range. Accurately characterizing the unimodal effect of temperature on emerging arboviruses, like ZIKV, is critical for estimating the potential geographic and seasonal range for transmission, and accurately predicting where future climate change will increase, decrease, or have minimal impact on transmission.

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INFECTION DYNAMICS AND DISEASE IN CHIKUNGUNYA VIRUS FIDELITY VARIANT INFECTED MICE

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Replication fidelity of RNA viruses has evolved to maximize the trade-off between adaptability through genetic diversity and deleterious mutations from error-prone replication. Mutations that modulate replication fidelity have been derived *in vitro* under mutagen treatment for several RNA viruses, including the re-emergent global health threat, chikungunya virus (*Alphavirus*, *Togaviridae*; CHIKV). For CHIKV, mutations in viral non-structural proteins 2 and 4 produce high and low fidelity variants (HiFi and LoFi) with *in vitro* mutation frequencies ranging from 60% to 140% of wild type CHIKV. These fidelity variants can be used to address the gap in knowledge about the effects of replication fidelity on CHIKV fitness and disease. Additionally, high fidelity mutations have been proposed as modifiers of live-attenuated vaccines (LAV) that could improve vaccine safety by reducing the risk of reversion to virulent genotypes. We hypothesized that any deviation from wild type replication fidelity would result in attenuated viral replication and disease, and lower neutralizing antibody titers relative to wild type CHIKV. To test our hypothesis *in vivo*, we inoculated cohorts of 6-week old female C57BL6 mice bilaterally in the rear footpad with CHIKV LoFi, HiFi, double mutant (DM) HiFi, or wild type. We tracked foot swelling and viral titers in tissues and collected serum 30 days post-inoculation. Surprisingly, we found that both HiFi and DM HiFi CHIKV produced more severe foot swelling, but similar viral titers in tissues compared to wild type. Conversely, LoFi CHIKV generated milder footpad swelling than wild type, and lower viral titers. We observed a small reduction in neutralization of WT CHIKV by anti-fidelity variant sera relative to wildtype sera, but no differences in neutralization of fidelity variant CHIKV. These results indicate that fidelity mutations do not necessarily confer an attenuated phenotype for CHIKV in immunocompetent vertebrate hosts.

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QUALITY OF LIFE TEN YEARS AFTER A CHIKUNGUNYA OUTBREAK : THE QOL-CHIK POPULATION-BASED STUDY ON RÉUNION ISLAND

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Chikungunya virus (CHIKV) infection is an arboviral disease, which long term manifestations have been shown to impair durably the health-related quality of life (QoL). Most longitudinal studies of patients affected by chikungunya chronic illness are hospital-based or based on outpatient populations skewed towards selection bias given initial severity at enrolment. In 2015-2016, we assessed the QoL of the general population using a cohort of subjects exposed to CHIKV in 2005-2006 on the island of La Réunion. To achieve this goal, we conducted a telephone follow-up study on a sample of 580 subjects from a population-based

serosurvey (2,442 subjects at inclusion). The primary endpoint was the physical component of the SF-36v2 (PCS). Secondary endpoints were the mental component (MCS) of the SF-36v, as well as SF-36v2 subscales. Painful phenotypes (e.g., musculoskeletal pain, chronic fatigue, or depression), incident morbidities and health care were also measured. Of the 386 responders, the 193 CHIKV-infected individuals had no more musculoskeletal pain, fatigue, or depression than the 193 uninfected individuals 10 years post exposure. The mean PCS score was 66.6 (± 21.6) in the infected and 71.0 (± 19.7) in the uninfected (*P*=0.06) groups. Of the 8 dimensions of the SF-36v2, only bodily pain was impaired in infected subjects compared to uninfected peers (66.7 versus 61.6, *P*=0.04). Infected subjects with pain two years post onset of infection (57%) reported complaints in 88% ten years after: fatigue (57%), mood disorders (46%) and musculoskeletal pain (45%). These symptoms were associated with impairment of all dimensions of SF36v2 QoL for 36% of infected subjects. Indeed, these individuals required rheumatologic care (OR 4.6) or evolved more into osteoarthritis (RR 2.5). Infected subjects did not develop more type 2 diabetes mellitus, cancer, or other known illnesses, than uninfected peers. Ten years after a chikungunya outbreak, infected people have a comparable quality of life than uninfected peers. However, these results are falsely reassuring because more than a third of infected people are sicker and report impaired quality of life.

1597

EXPERIMENTAL EVOLUTION OF CHIKUNGUNYA VIRUS TO STUDY EMERGING VARIANTS AND THE IMPACT OF DEFECTIVE GENOMES ON EVOLUTION

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Chikungunya virus has recently extended to previously disease-free areas. Its high mutation and fast replication rates lead to the emergence of new strains potentially able to disseminate in these new territories, through the acquirement of novel characteristics. Favored by a high recombination rate this virus can also produce defective genomes that may interfere with the parental virus and hence influence its evolution. These defective genomes are also known to promote persistence in invertebrate vectors of arboviruses. The La Reunion Island and the Caribbean chikungunya virus strains were passaged several times *in vivo* in their mosquito vector (bypassing the mammalian host) to force invertebrate adaptation. RNA deep sequencing permitted us to select consensus changes or minority variants reflecting invertebrate adaptation. Direct mutagenesis was performed to reproduce these mutations in the virus clone in order to test for attenuation in a vertebrate environment. Interestingly, these repeated *in vivo* mosquito passages give rise to a heterogeneous cloud of defective genomes. Some of them show an interesting shuffled pattern, multiple deletions or duplicated sequences. Contrary to defective genomes produced in mammalian cells that decrease drastically the number of infected mosquitoes after a chikungunya blood meal, these mosquito-derived defective genomes don't seem to decrease viral infection or titer in mosquitoes. Mutations selected by NGS are tested for their impact on the generation and characteristics of these defective genomes. Generation of cloned defective genomes will permit us to compare the impact of mammalian or invertebrate defective genomes on the replication and the evolution of chikungunya virus.

1598

IMMUNOLOGICAL INSIGHTS BASED ON ANTIBODY BINDING EPITOPES ON THE CHIKUNGUNYA VIRUS ENVELOPE

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Chikungunya virus (CHIKV) infects millions of people, causing a debilitating arthritic disease with no specific treatment. To identify the CHIKV structures that elicit a protective humoral immune response, we have epitope mapped the binding sites of over 70 monoclonal antibodies (MAbs) against the CHIKV envelope glycoprotein E2/E1 using a comprehensive shotgun mutagenesis library of 910 E2/E1 alanine-scan mutants. Published studies have used these epitopes to characterize broadly cross-reactive and ultrapotent neutralizing MAbs that blocked post-attachment steps, and whose binding mapped to functionally-important domains A or B. MAb binding sites suggest a mechanism of action where MAbs inhibit virus-host membrane fusion by preventing exposure of the fusion loop on E1. Other studies characterized MAbs that induce structural changes on Domains and B, and viral receptor binding residues involved in virus fusion. In addition, we have isolated seven human MAbs against CHIKV E2/E1 derived from infected human patients. The most potent MAb, IM-CKV063, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), showed high-affinity binding (320 pM), and was capable of therapeutic and prophylactic protection in multiple animal models up to 24 h post-exposure. Epitope mapping identified an inter-subunit conformational epitope on domain A of E2. Subsequent analyses confirmed this by cryo-EM, and demonstrated that IM-CKV063 blocks both virus entry and virus release steps. The isolated MAbs included previously unreported MAbs against the highly conserved fusion loop. These were broadly cross-reactive against alphaviruses but were non-neutralizing, suggesting that the fusion loop is hidden in mature infectious virions. Analysis of MAbs that bind the E2/E1 spike suggest that the neutralizing epitopes are confined to the exposed topmost and outer surfaces of the E2/E1 trimer, providing a rationale for using the intact E2/E1 trimer for vaccine design and therapeutic MAb development.

1599

UNDERSTANDING THE ROLE OF RECOMBINATION IN ARBOVIRUS EVOLUTION

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Recombination in RNA viruses is a genetic exchange phenomenon that leads to the generation of hybrid RNA molecules from different parental RNA strands. This event, carried out by the viral polymerase as it synthesizes viral RNA, can provide an evolutionary advantage because it can lead to the generation of a new population of viruses with a new genotype and phenotype. These new characteristics may enhance virus replication or enable better adaptation to the host. Importantly, a by-product of recombination is the generation of defective genomes with internal deletions that render them non-replicative *per se*. This poster will focus on the identification of aminoacid residues that regulate recombination in arboviruses, using Sindbis virus as a model. The identified residues were tested for their role in recombination using replicon assays. These results will enable us to generate viruses with lower or higher ability to recombine, which could serve as an invaluable tool to understand the role that genetic recombination may play in arbovirus evolution, transmission or dissemination within the host or arthropod.

1600

PRECLINICAL DEVELOPMENT OF A COMBINATION ZIKA AND CHIKUNGUNYA VIRUS-LIKE PARTICLE VACCINE

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Zika virus (ZIKV) and Chikungunya virus (CHIKV) are pathogenic, arthropod-borne viruses that pose serious public health threats to humans. Recently, both viruses have emerged throughout much of the western hemisphere and now circulate globally in many of the same tropical and subtropical geographical regions where the *Aedes aegypti* mosquito vector resides. The unmet medical need and the co-circulation of the viruses in the same geographic regions suggest that a combination vaccine could have public health, commercial, and economic advantages. We have sought to develop a ZIKV and CHIKV combination vaccine based on virus-like-particles (VLPs) generated separately in HEK293 mammalian cells by transient transfection of expression plasmids encoding for ZIKV prME or CHIKV C-E3-E2-6K-E1 structural proteins. VLPs were purified by tangential flow filtration and anion exchange column chromatography. To assess the immunogenicity of the single and combination vaccines, a study was conducted in C57Bl/6 x Balb/c F1 hybrid mice using a dose titration of either ZIKV VLP, CHIKV VLP, or both VLPs formulated with aluminum hydroxide adjuvant. Serum samples from VLP-immunized mice were tested in neutralization assays using reporter virus particles expressing luciferase. The combination VLP vaccine elicited high neutralization titers specific for ZIKV and CHIKV, comparable to antibody titers achieved with either VLP vaccine alone. These results indicate that purified ZIKV and CHIKV VLP can be combined into one vaccine, without inhibitory effects, that has the potential for use in endemic areas. Given the sporadic and unpredictable characteristics of the epidemics caused by these viruses, the demonstration that neutralizing antibody represents a surrogate or correlate of protection for both of these viruses could enable an accelerated approval path to initial licensure.

1601

DEVELOPMENT OF A HIGH THROUGHPUT LUCIFERASE-BASED CHIKUNGUNYA NEUTRALIZATION ASSAY

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PaxVax, Inc. is developing a virus-like particle (VLP) vaccine candidate intended for active immunization against disease caused by the chikungunya virus (CHIKV). The VLP vaccine has been in-licensed from the National Institutes of Health/Vaccine Research Center (NIH/VRC) and is being further developed with the goal of licensure. To facilitate evaluation of vaccine immunogenicity, PaxVax has developed a high-throughput Luciferase (Luc)-based assay for assessing neutralizing antibody titers against CHIKV (CHIKV-Luc). To aid in the development of the CHIKV-Luc assay, serum samples from the Phase 1 VRC311 study were provided to PaxVax by the NIH/VRC. Evaluation of these samples using the PaxVax method is part of the assay qualification to be completed prior to the company's Phase 2 dose-selection study. The virus used in the assay is the 181/25 vaccine strain of CHIKV that has been engineered to express a Luc transgene. Antibody neutralization titers can be determined via characterization of reductions of Luc activity in the presence of immune serum. We next compared our CHIKV-Luc assay results with those obtained using a flow cytometry-based assay employing a chimeric Semliki Forest-CHIKV OPY1 virus that expresses the green fluorescent protein (GFP). This assay was used previously to characterize the immunogenicity and neutralization breadth of the VLP vaccine in support of the VRC311 Phase 1 study. Finally, both high-throughput assays were compared to the gold-standard plaque reduction neutralization (PRNT) assay using the same immune sera samples from the VRC311 clinical trial. The PRNT assay was evaluated because it was used to test samples in the AFRIMS Philippine field study that established a link between antibody titers and protection: pre-existing PRNT80 titers greater than or equal to 10 protected against symptomatic CHIKV infection (Yoon et al, 2015). Strong and statistically significant correlations were demonstrated between the measurable results for all three assays indicating the feasibility of using the CHIKV-Luc assay for clinical testing of serum samples for CHIKV-specific antibody neutralization activity.

1602

FIRST REPORT OF MADARIAGA VIRUS FROM A CLAY-COLORED TRUSH (*TURDUS GRAYI)* IN GUANACASTE, COSTA RICA

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Madariaga virus (MADV) is the new designation for the South American strains of Eastern Equine Encephalitis virus (EEEV). The first outbreak was documented in 2010 in Darien, Panamá in an area that is endemic for the Venezuelan Equine Encephalitis virus (VEEV). VEEV and other arboviruses are endemic in equines in Costa Rica. Several cases of equine encephalomyelitis are reported annually in Ostional, Guanacaste at the northwest part of the country. This region is composed by dry forest and multiple cattle ranches. Based on previous reports of the disease in this area, eight different locations were selected for field sample collection during two different tropical weather seasons (rainy and dry season). Birds were sampled in order to identify their potential role as arboviral reservoirs. Mist netting was performed at peridomiciliary, forest, and stockyard areas at dusk and dawn. Birds were euthanized, organs were collected (heart, lung, liver, spleen, kidney, central nervous system, reproductive system, eye, proventriculus, intestine, stifle joint), and a complete post-mortem analysis was done for histopathological and molecular analysis. A RT-PCR for flavivirus and alphavirus was performed on individual and pooled organs. One *Turdus grayi* from ranch "D" was positive for alphavirus (NSP4 region, 210 pb product). Phylogenetic analysis showed a 97% identity with the MADV isolated in Panamá in 2010. Histopathological analysis revealed multifocal inflammatory aggregates composed predominantly by lymphocytes, few histiocytes, plasma cells, and scattered heterophils in myocardium, liver, kidney and lung. Additionally, lymphoid hyperplasia was observed. This is the first report of MADV in Costa Rica. Further sampling will be conducted in order to characterize a putative zoonotic/epizootic virus cycle.

1603

THE CHIKUNGUNYA VIRUS OUTBREAK IN GRENADA 2013 - EVOLUTION, EPIDEMIC ACTIVITY, AND PHYLODYNAMICS

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Chikungunya virus (CHIKV) is a rapidly re-emerging global pathogen causing both acute and chronic disabling illness. There is currently no vaccine. An RNA virus within the family *Togaviridae*, CHIKV is transmitted to humans by the mosquito vector *Aedes aegypti* and related species. *A. aegypti* is the same species that transmits Dengue and Zika viruses. CHIKV exists in three phylogenetically distinct groups: West Africa and East, Central, and Southern Africa (ECSA), and Asian genotypes. The rapid spread of CHIKV has been driven by the expanding mosquito vector populations as a result of urbanization and climate change as well as increased global travel. CHIKV was introduced into the Caribbean in 2013, which led to an explosive outbreak in Grenada. During this outbreak a total of 143 CHIKV samples from acute case symptomatic patients were collected and confirmed positive with PCR. At the California Academy of Sciences, RNA was extracted from CHIKV positive sera and Illumina Miseq sequencing was performed yielding 17 complete genomes. Despite CHIKV positive sera, a handful of samples illuminated an underlying-and undetected-dengue virus (DENV) co-infection. These DENV co-infections yielded near complete genomes of DENV serotypes 1-3, indicating co-circulation of both CHIKV and DENV in a single epidemic season. Phylogenetic analyses were performed using maximum likelihood methods. All of the CHIKV Grenada samples form a monophyletic group, suggesting a single main introduction into Grenada. By employing phylogenetic and whole-genome analyses of CHIKV we reveal a deeper understanding of evolutionary patterns, phylodynamics and epidemic trends, as well as public health challenges.

1604

CHARACTERIZATION OF PROTECTIVE MECHANISMS BY ANTIBODIES AGAINST DENGUE VIRUS NS1

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Plasma leakage, which can lead to hypovolemic shock and potentially fatal complications, is a pathogenic hallmark of severe dengue disease. Previously, we demonstrated that dengue virus (DENV) non-structural protein 1 (NS1) directly triggers endothelial permeability and vascular leak. We also showed that NS1-immune serum and anti-NS1 monoclonal antibodies (mAbs) block NS1-induced endothelial dysfunction of human endothelial cells *in vitro* and prevent NS1-mediated lethality *in vivo*. Recent studies identified NS1 immunodominant regions and established that antibodies against the NS1 wing domain can inhibit DENV infection and prevent DENV-induced mortality in mice. However, the molecular mechanisms by which antibodies prevent NS1 pathogenesis are not fully understood. Here, we studied the relative contribution of DENV NS1 domains to NS1-induced pathogenesis *in vitro* and whether antibodies against specific NS1 regions are associated with protective responses *in vivo*. We found that the DENV NS1 hexamer, but not individual domains, increase permeability of human pulmonary microvascular endothelial cells (HPMEC). Further, only full-length DENV NS1 was able to bind to endothelial cells and trigger disruption of the endothelial glycocalyx. We also found that sera against the wing domain partially block NS1-induced endothelial hyperpermeability, whereas sera against the β-ladder and tip domains did not. In ongoing experiments, we are assessing a panel of anti-NS1 mAbs for their ability to block NS1 binding to endothelial cells and to prevent endothelial dysfunction *in vitro*. Initial results suggest that only parts of each domain are critical for NS1 blockade, as a number of mAbs with the same NS1 domain specificity differ in their capacity to abrogate NS1 binding and/or endothelial permeability. Finally, blocking antibodies are being evaluated for their capacity to prevent vascular leak in a mouse model. Together, these studies provide a comprehensive assessment of the specificity, mechanism of action and efficacy of anti-NS1 humoral responses and identify critical regions that can be targeted to block NS1-mediated pathogenesis.

1605

UNRECOGNIZED DENGUE AND CHIKUNGUNYA HUMAN TRANSMISSION IN WESTERN AND COASTAL KENYA

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Dengue (DENV) and chikungunya (CHIKV) viruses cause human disease in Kenya, but remain poorly controlled and under-recognized. To understand the true burden of exposure and resultant disease from these important arboviruses, our objective was to determine the distribution of CHIKV and DENV cases among children in Kenya. Two cohorts of children (Jan 2014-present) were enrolled at four Kenyan study sites (rural west, rural coast, urban west, urban coast): a healthy child cohort followed every six months to document asymptomatic CHIKV and/or DENV infections via IgG ELISA testing, and an acutely febrile child cohort followed to convalescence to document symptomatic disease via RT-PCR and IgG ELISA. Questionnaire data were collected to describe demography, socioeconomic status (SES), and household environment. 3% (121/4263) of febrile disease was attributable to CHIKV and 4% (173/4592) to DENV. Approximately 6% (393/6980) of febrile illness was attributable to either virus. We detected unrecognized outbreaks of both CHIKV and DENV and alerted Ministry of Health officials to promote control policy. The majority of children with single infections presented with mild syndromes and fully recovered by one month; however, those with malaria co-infections (158/4478) were sicker and recovered more slowly. Among healthy cohorts (500 children per site), asymptomatic infections were rare: only 8 asymptomatic CHIKV seroconversions (0.3%) and 9 DENV seroconversions were noted (0.3%) over four years of active surveillance. Exposure to CHIKV or DENV was associated with age, SES, mosquito exposure and avoidance behaviors, and hygiene and wealth indices. Continued misdiagnosis as malaria and the slow uptake of policies to prevent arboviral disease affect child health in Kenya. Better recognition of CHIKV and DENV in the health care setting, coupled with targeted vector control interventions at the local level, could dramatically decrease the impact of these enduring disease threats.

1606

COULD VIRAL CO-INFECTION OF MOSQUITOES IMPACT VECTOR CONTROL? A NEW DEVELOPMENT FOR BIOLOGICAL VECTOR CONTROL

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Could Viral Co-infection of Mosquitoes Impact Vector Control? A New Development for Biological Vector Control Mosquito-borne diseases are responsible for several million deaths and hundreds of millions of cases of human illness yearly. Mosquitoes can be vectors of many different pathogens that may cause a variety of human and veterinary illnesses (e.g. dengue, Zika, malaria and chikungunya). Many of these arboviruses may be found circulating in the same region. Dengue virus, one of the most important arboviruses in terms of public health, circulates in in Africa, Asia, the Americas, and the Pacific and is primarily found in the tropics and the sub-tropics. This is the same footprint of many other viruses, such as Zika and chikungunya. In addition to having the same

spatial distributions, the viruses are also carried by the same vector: *Aedes aegypti* and *Aedes albopictus*. This overlap in regions and vectors could possibly lead to co-infection in a human subject. In addition to being vectors to arthropod-borne viruses that can cause disease, mosquitoes are also hosts to viruses that are not transmitted to vertebrates, that do not cause human disease. In Espirito Santo, Brazil, a biological sample containing DENV-2 was analyzed, leading to the discovery of a new virus aptly named, Espirito Santo Virus (ESV). Preliminary studies on this virus showed the virus replicates in C6/36 (Ae. albopictus) insect cells but not Vero (African green monkey kidney; mammalian cells). In addition, to these studies ESV has initially been shown reduce titer values of DENV-2, when co-infected simultaneously. Current preliminary studies have also shown a reduction in infection rates of DENV-2 of ESV infected mosquitoes. Ongoing research into the relationship of co-infection of ESV-DENV could have dramatic impacts of vector control. The manipulation of mosquitoes to reduce their vector competence, could open new avenues of vector control. Our research aims to build on these preliminary results and study the relationship of how these two viruses affect each other during co-infection.

1607

CONTRIBUTION OF TYPE-SPECIFIC VERSUS CROSS-REACTIVE ANTIBODIES TO NEUTRALIZATION FOLLOWING REPEAT DENGUE VIRUS INFECTIONS

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The four dengue virus (DENV) serotypes can cause repeat infections, each time with a new serotype. While primary infection generates life-long neutralizing antibodies to the infecting serotype, secondary DENV infections elicit cross-neutralizing antibodies to two or more serotypes. As recent vaccine trial results and natural DENV infection studies highlight, the quality of the neutralizing response is a complex matter that needs further investigation. One important question to be addressed is whether neutralizing antibodies after secondary infection target cross-reactive epitopes conserved across serotypes or epitopes specific to each serotype previously encountered. Antibody depletion methods developed previously provide a tool to evaluate the sequence of DENV infections and the contribution of type-specific (TS) versus cross-reactive antibodies to neutralization by polyclonal sera. In this study, we analyzed sera from subjects enrolled in a long-term prospective pediatric cohort study in Nicaragua, which allowed us to evaluate repeat infections in individuals with a well-characterized history of first, second and in some cases third DENV infections. Using depletion of antibody subpopulations, we found that after a first infection, a substantial proportion of TS antibodies contribute to the neutralizing response, and after a second infection, the cross-reactive response dominates. In some individuals, TS antibodies to both infecting serotypes can be detected. Moreover, we simultaneously interrogated B cell serotype-specificity and cross-reactivity in the same individuals via the Quad-Color FluoroSpot (QFC) assay, which enables analysis of the memory B cell response at a single-cell level. This unique set of samples from individuals with repeat infections in a dengue-endemic area allows dissection of both antibody and B cell responses at the individual level and in an epidemiological context. Altogether, this study advances our understanding of natural DENV infections and has direct implications for vaccine design.

1608

COMPREHENSIVE MUTAGENESIS OF DENGUE VIRUS ENVELOPE PROTEINS TO MAP ANTIBODY EPITOPES AND IDENTIFY RESIDES ESSENTIAL FOR FUNCTION

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To characterize the immune response to dengue virus (DENV) infection, we have epitope mapped 200 anti-DENV monoclonal antibodies (MAbs), using high-throughput, rapid screens of MAb binding to DENV prM/E comprehensive mutation libraries for all four DENV serotypes, 3,380 mutations in total. Each library of individual mutant expression plasmids was transfected into human cells to achieve native protein expression and folding, and immunoreactivity of MAbs to each individual prM/E variant was quantified by high-throughput flow cytometry. The epitopes obtained were both conformational (including quaternary) and non-conformational, were spread across prM and E domains I-III, and many have been correlated with their abilities to protect against DENV infection. A number of anti-DENV MAbs cross-reacted with ZIKV prM/E, predominantly within the fusion loop but also within Domain II of the E protein, identifying critical immunogenic residues shared by DENV and ZIKV. We have also produced DENV virions from all four DENV mutation libraries using a previously developed DENV reporter virus particle (RVP) system, allowing us to screen each individual DENV Env variant protein for DENV particle budding and infectivity. For DENV3, we identified residues whose mutation eliminated virus infectivity but did not impact E protein expression, antigenicity, virion assembly, or particle budding. We identified variants that showed increased DENV virion budding, up to 5-fold above wild-type, indicating the ability to engineer highly expressed, non-infectious DENV variants for use in vaccine design. To identify uncharacterized DENV cellular receptors we assayed wild-type DENV RVP infectivity in non-permissive cells expressing our membrane proteome array (MPA) of 5,300 unique human membrane proteins. This has identified candidate membrane proteins that enable DENV infectivity. We have identified neutralizing epitopes in DENV prM/E and specific sites that are critical for DENV infectivity, providing new targets and opportunities for vaccine development.

1609

QUALITY OF THE ANTIBODY RESPONSE INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE IN NAIVE AND DENGUE EXPOSED INDIVIDUALS

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Dengue is the most prevalent arthropod-borne viral disease in humans. Cocirculation of 4 dengue virus (DENV) serotypes and increased risk of severe disease after a secondary DENV infection have made the development of a dengue vaccine especially challenging. Reports from clinical trials of a leading tetravalent vaccine candidate suggest that qualitative as well as quantitative aspects of antibodies, may play an important role in determining protection. Humoral immunity to DENV infection in humans is characterized by both serotype-specific (TS) and cross-reactive (CR) neutralizing antibodies (NAbs). While TS NAbs have been linked to long protective immunity after primary infection with one serotype, CR NAbs seem to provide broad protection to serotypes not previously encountered, after secondary infections. The relative role of TS and CR NAbs induced after simultaneous administration of 4 DENV serotypes in a tetravalent vaccine is not well understood and may vary depending on the history of previous DENV infections. Takeda Vaccines Inc. has developed a tetravalent live-attenuated dengue vaccine (TDV)

currently in phase 3 clinical trials. To determine the levels of TS and CR NAbs to each serotype induced by TDV, we depleted specific populations of Abs from TDV-immune sera, either reactive to the homologous or the heterologous serotype, and measured the impact on neutralization. We found that the proportion of TS and CR NAbs varied among vaccine recipients and among serotypes. We also used competition assays with human monoclonal Abs and neutralization assays with recombinant chimeric DENVs to map the epitopes targeted by TDV-induced TS NAbs. We will discuss the implications of our results for evaluating vaccine performance and identifying correlates of protection.

1610

PRE-EXISTING T CELL SUBSETS AND THEIR ASSOCIATION WITH SUBSEQUENT SUBCLINICAL VERSUS SYMPTOMATIC DENGUE INFECTION

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Dengue is an ongoing global epidemic, with increasing co-circulation of the four virus types (DENV-1-4). As more severe dengue disease is associated with secondary heterologous infection, pre-existing immunity to at least one DENV type is thought to influence the outcome of subsequent infection. Through a school-based cohort study of Thai children in grades 1-6, we defined symptomatic and subclinical DENV infections based on seroconversions that were or were not associated with acute febrile illnesses, respectively. Previous data from this cohort suggested that higher levels of pre-existing IFNγ-producing T cells positively correlated with subsequent subclinical infection. A separate study of patients from Vietnam suggested that a higher ratio of T regulatory to T effector cells correlated with milder disease. These data support the notion that the balance of different T cell subsets influences the clinical outcome of DENV infection. To investigate the contribution of different T cell subsets to the outcome of subsequent DENV infection, we used a 13-color flow cytometry panel to analyze the production of IFNγ, TNFα, IL-4, IL-10, IL-17A, IL-17F, IL-21, and IL-22 after overnight stimulation of PBMC collected prior to a documented DENV infection. We studied samples from 20 subjects with subsequent subclinical DENV infections and 20 subjects with subsequent symptomatic DENV infections. We stratified the data based on clinical outcome and compared the frequencies of various CD4+ and CD8+ T cell subsets. The data suggest that greater diversity in cytokine production is associated with subsequent subclinical outcome. These findings extend prior observations on T cells in dengue to additional T cell subsets, and lend further support to a role for T cells in shaping the outcome of DENV infection.

1611

DTK-DENGUE: A NEW AGENT-BASED MODEL OF DENGUE VIRUS TRANSMISSION DYNAMICS

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Dengue is a mosquito-borne viral disease that exerts a growing impact on global health and presents a formidable challenge for public health officials, in part because many factors related to its transmission are difficult to observe and measure (e.g., vector population dynamics and number of inapparent infections). Agent-based models (ABMs) can explicitly represent these factors and can be calibrated to empirical data to provide insight about the dynamics of both observed and unobserved variables. Disease Transmission Kernel-Dengue (DTK-Dengue) is an ABM for dengue virus (DENV) transmission that extends the Institute for Disease Modeling's Epidemiological Modeling Disease Transmission Kernel (EMOD-DTK). DTK-Dengue includes three major modifications to EMOD-DTK: 1) it incorporates the influence of climatic variables on *Aedes aegypti* mosquito population dynamics, 2) it modifies adult vector activity to reflect *Ae. aegypti* behavior, and 3) it explicitly includes all four DENV serotypes, including their effects on human immunity and rates of symptomatic disease. Although ABMs such as DTK-Dengue are capable of representing a variety of observed and unobserved variables, little is known about the relative contributions of different data types from routine surveillance systems for calibrating such models. We calibrated DTK-Dengue with three complementary datasets from San Juan, Puerto Rico in 2007: the monthly incidence, the monthly number of cases by serotype, and the age distribution of cases. We ran seven calibrations, each incorporating one possible combination of datasets into the model likelihood. We evaluated the fit of the model to all data types, regardless of whether they were used in the calibration, and compared simulated output of mosquito dynamics to trap data, which is not routinely collected by surveillance systems and was thus withheld from the calibration process. These results provide insight into the relative importance of different types of commonly collected data for calibrating ABMs of DENV transmission and how the inclusion of these types influences predictions of observed and unobserved variables.

1612

SURVEILLANCE OF DENGUE VIRUS INFECTION IN NEPAL IN 2015

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Dengue virus (DENV) is estimated to infect 390 million people each year. Of that, Asian countries account for 70% of cases. The first case of DENV in Nepal was documented in 2004; since then all four serotypes (DENV 1-4) have been documented in most of the southern tropical zone and as far north as the temperate Kathmandu Valley. We set out to study circulating DENV in Nepal in 2015. Patients were recruited from local hospitals who presented with dengue-like signs and symptoms. DENV IgM and IgG ELISA, DENV NS1 ELISA and DENV RT-PCR were performed on each patient and they were classified by likely primary infection, likely secondary infection, or negative. Select samples were tested by neutralization assay to assess any existing cross-protective antibody levels. Of 91 patients tested, 93% presented with fever and 86% with myalgia. Surprisingly, no patients were noted to have rash. Eight percent of patients had thrombocytopenia less than 50 x10^3/μL. Nineteen patients were found to have likely primary DENV and 5 were found to have likely secondary disease. No convalescent serum was available for comparison due to the single time point blood collection. Six patients were found to have positive RT-PCR results. Two of these patients were found to have DENV-2 serotype and both serum samples were cultured on mosquito C6/36 cell lines. Full-length genome sequencing was performed directly from sera and on the viral isolates after amplification in C6/36 cells. This is the first known full-length sequence of DENV circulating in Nepal to date. Nepal, with its

90 ethnic groups, is a unique ecologic niche where most adults have no pre-existing immunity to DENV and it circulates in a population that has been immunized against the related flavivirus, Japanese Encephalitis virus. It is a unique ecologic niche also in that it rises in elevation from 50 m to the highest point on Earth (8,848 m) within the span of 200 km. This could serve as a model to study transmission dynamics from subtropical to temperate regions. Data from this population could significantly contribute to our knowledge about DENV-host interactions and inform ongoing therapeutic and vaccine development efforts.

1613

CHARACTERIZATION OF SERA FROM DENGUE INACTIVATED VACCINE PRIMED VOLUNTEERS THAT HAD IMPROVED NEUTRALIZING ANTIBODY RESPONSES AFTER LIVE ATTENUATED DENGUE VACCINE BOOSTING

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A previous clinical study evaluated heterologous vaccine prime-boost regimens that consisted of a formalin inactivated dengue virus (DENV) vaccine (DPIV) prime on day 0 followed by a live attenuated DENV vaccine (TDENV) boost at 1 month (0,1 group) or 6 months (0,6 group). The 0,6 group had significantly higher neutralizing antibody (NAb) titers than the 0,1 group, and both groups elicited significantly higher NAb titers compared to unprimed volunteers given TDENV. To better understand the DPIV-primed immune profile that mediated these improved responses, we characterized sera collected on the day of TDENV booster vaccination. The NAb titers to DENV 1-4 were very low or undetectable for volunteers in both groups. The levels of DENV 1, 3, and 4 virion-reactive IgG were relatively high for the 0,1 group (mean titers >1,000), but had waned significantly for the 0,6 group. Surprisingly, volunteers in both groups had very low levels of DENV-2 reactive IgG. The DENV-2 vaccine virus was the dominant replicating component of TDENV so we also measured DENV-2 antibody dependent enhancement (ADE) *in vitro*. All subjects in the 0,1 group had detectable DENV-2 ADE curves, whereas most of the ADE curves for the 0,6 group had waned below the assay limit of detection (<1:40 dilution of serum). The magnitude of observed viremias in the 0,1 and 0,6 groups were not significantly different from unprimed volunteers that received TDENV. However, we observed that a higher proportion of subjects in the 0,6 group had detectable DENV-2 viremia (46%) compared to those in the 0,1 and unprimed groups (17% and 23%, respectively). Therefore, it is possible that waning levels of DPIV-elicited, DENV-reactive antibody improved immunogenicity of the TDENV booster vaccination in the 0,6 group by increasing vaccine virus infectivity through an ADE mechanism. However, this does not appear to explain the improved immunogenicity of the 0,1 group relative to unprimed volunteers and indicates that other immunological mechanisms might be involved.

1614

SAFETY, EFFICACY AND IMMUNOGENICITY OF DENGUE VACCINES: SYSTEMATIC REVIEW AND NETWORK META ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Dengue virus is a global health problem. Tetravalent Dengue Vaccine (CYD-TDV) was the first vaccine to gain regulatory approval to try and address this problem. There is an urgent need for an effective and safe dengue vaccine to reduce morbidity and mortality in this high-risk population given the lack of specific treatment at present. A safe vaccine that protects against Dengue virus diseases is a global health priority. This review and network meta-analysis aimed to summarize all available evidence on the efficacy, safety, and immunogenicity of four serotypes of all available types of dengue vaccines. The data is gathered from randomized controlled clinical trial from 13 databases. The Protocol was registered at PROSPERO with the registration number CRD42016052578. Studies that reported the efficacy and immunogenicity of vaccines in the prevention of dengue virus infection and effects on the severity of the disease were included in this review with no restriction to language. Data was extracted by three independent authors. Data were analyzed by R software. Out of 518 studies identified by the electronic and manual search, 44 studies were included in our systematic review and network meta-analysis. Vaccines used were CYD-TDV, TDV, AvP TDV, TDENV PIV, TDEN F17, TDEN F19, TV003-TV005, WRAIR& GSK vaccine DEN1, DEN2 and DEN4 versus controls which were placebo or other vaccines such as meningococcal polysaccharide A+C, typhoid VI polysaccharides, varicella etc. Regarding risk ratio (RR) of injection site swelling, arthralgia, and fatigue in vaccine groups; results were insignificant in network meta-analysis in contrast to control group. However, in CYD-TDV and AvP TDV groups, the headache was significantly in contrast to controls RR=1.23 95%CI (1.06-1.43) and 18.45 95%CI (1.21 - 281.98) respectively. Seropositive conversion against serotype 1 was significantly higher in most of the vaccine groups in contrast to control. *We expect to find safety, efficacy, and immunogenicity of vaccines in the prevention of dengue virus infection, and effects on the severity of the disease as we complete the analysis of the study.*

1615

COST-EFFECTIVENESS OF DENGVAXIA VACCINATION OF PEOPLE WITH PRIOR DENGUE VIRUS EXPOSURE IN TEN LATIN AMERICAN AND ASIAN COUNTRIES

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As of March 2018, the Dengvaxia vaccine for dengue from Sanofi Pasteur has been registered in 20 countries in Latin America and Asia. In light of recently announced safety concerns for people with no prior dengue virus exposure, the World Health Organization revised its position on this vaccine to recommend that it only be used in people with prior dengue virus exposure. To date, no studies have estimated the public health impact and cost-effectiveness of Dengvaxia when applied only to people with prior dengue exposure. To provide such an assessment, we used a computational model of dengue virus transmission to simulate the impact of routine vaccination programs under various ranges of transmission intensity and with screening tests of varied sensitivity and specificity. We also simulated different combinations of routine vaccination at different ages and catch-up campaigns with different age ranges and coverages. This study analyzed and compared the cost-effectiveness of screening-based vaccination targeted at nine-year-old children and of an intervention with various catch-up cohorts based in ten countries across Asia and Latin America. We applied the simulated infections derived from the model to the cost-effectiveness analysis framework provided by WHO. The work also relied on literature for local vaccine cost and cost per DALY. An intervention would be evaluated as cost-effective if the threshold cost of the intervention was predicted positive. Overall, our results suggest

that screening-based vaccination could be cost-effective in six countries: Colombia, Brazil, Mexico, Malaysia, Thailand, and Puerto Rico, which have a GDP per capita ranging from $5,742 to $29,360. We have also found that screening-based vaccination would be cost-effective with a threshold cost ranging from $5 to $50 in the first five countries when the transmission intensity is high (SP9>0.5) with a highly sensitive and specific diagnostic; for Puerto Rico, the intervention could be cost-effective even when the transmission intensity is low (SP9≤ 0.3) and the threshold cost could reach $100 under stronger transmission.

1616

POST-VACCINATION ANTIBODY RESPONSE OF DENGUE SEROTYPE 3 BREAKTHROUGH INFECTIONS

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The four serotypes of Dengue virus (DENV1-4) are estimated to annually infect over 200 million people worldwide. In an effort to prevent DENV infection and disease, Sanofi Pasteur has developed a tetravalent live attenuated chimeric DENV vaccine (Dengvaxia) that aimed to elicit a protective response against DENV1-4. While Dengvaxia was deemed effective in two separate clinical trials in Asia and Latin America, vaccine efficacy varied depending on DENV serotype and pre-vaccination DENV immune status. Here we present analysis of vaccine elicited immune response of naïve and pre-immune subjects that experienced a DENV3 breakthrough infection. To understand the antibody response, we depleted specific populations of DENV-cross reactive antibodies from immune sera taken one month after final vaccination dose to estimate the contribution of serotype-cross reactive and type-specific antibodies to neutralization of DENV3. The majority of DENV naïve subjects who received the vaccine and then experienced a DENV3 breakthrough infection had no serotype specific response to DENV3 while developing other DENV serotype specific responses. For individuals who had neutralizing antibody response that was serotype-specific to DENV3 and still experienced a breakthrough infection, we mapped the DENV3 antibody response to specific epitopes using chimeric recombinant viruses. We discuss the implications of our results for understanding immune correlates and mechanisms of protective immunity against DENV3 following tetravalent DENV vaccination.

1617

EMERGENCE OF A DENGUE VIRUS SEROTYPE 2 CAUSING A LARGE DENGUE EPIDEMIC IN SRI LANKA

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Severity of disease associated with dengue virus(DENV) infections in Sri Lanka has been increasing since 1989. It has been a reportable disease in Sri Lanka since 1996 and a severe epidemic occurred in 2009, involving the DENV1 serotype. Although many DENV serotypes, have been co-circulating in Sri Lanka, DENV1 was the predominant serotype from year 2008 to 2015, responsible for over 92% of infections, whereas DENV-2 and DENV-3 were not detected. There were 28,000 to 47,000 dengue cases per year reported between 2008 to 2015. Starting from mid-2016 there was a rapid increase in cases and the year 2017 had a total of 186,101 reported dengue cases with over 320 dengue related deaths. There was an increase in hospitalizations and severe disease. During our investigations it was discovered that a DENV2 strain was largely responsible for this outbreak and displaced the existing circulating DENV1. This study focuses on this dominant DENV2 strain, responsible for the 2016/2017 outbreak. For this study, the DENV2 strain was fully sequenced and phylogenetic trees (one using the full length and the second using the envelope region) were generated using the Neighbor joining method, based on Tamura-Nei model, with a bootstrap of 1000 replications. Phylogenetic analysis showed these DEN2 isolates belong to the cosmopolitan strains and are closely related to the 2015-2016 Singaporean strains. With daily frequent flights it is possible this strain spread from Singapore to Sri Lanka.

1618

MAGNITUDE AND FUNCTIONALITY OF NS1-SPECIFIC ANTIBODY RESPONSE ELICITED BY TAKEDA'S TETRAVALENT DENGUE VACCINE CANDIDATE

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Dengue virus (DENV) is the most medically important arbovirus, affecting 40% of people worldwide and infecting ~390 million individuals annually. Cases range from dengue fever to life-threatening dengue hemorrhagic fever/dengue shock syndrome, characterized by endothelial dysfunction and vascular leakage. Previously, we demonstrated that DENV nonstructural protein 1 (NS1) can induce endothelial hyperpermeability in both human cell culture and mouse models and that vaccination with NS1 confers antibody-mediated protective immunity. Takeda's live attenuated tetravalent dengue vaccine (TDV) candidate has structural proteins from each serotype on the DENV2 genomic backbone. Here, we evaluated the magnitude and functionality of the TDV NS1-specific antibody response. An indirect ELISA was used to measure DENV NS1-specific IgG in serum from 15 dengue-naïve and 15 pre-immune subjects, pre- (day 0) and 4 months post-vaccination (day 120). Naïve day 0 samples showed background IgG levels, whereas pre-immune day 0 and all post-vaccination samples displayed varying levels of IgG titers against DENV NS1. Vaccination with TDV increased DENV2 NS1-specific IgG levels in serum from all naïve and a few pre-immune subjects and was cross-reactive with NS1 from DENV1, 3, and 4. Using an *in vitro* model, we found that DENV2 NS1 induced endothelial hyperpermeability in human endothelial cell monolayers as measured by trans-endothelial electrical resistance, and hyperpermeability was not inhibited by pre-vaccination day 0 serum from naïve subjects. Pre-existing NS1-specific antibodies in day 0 serum from pre-immune subjects partially inhibited NS1-induced hyperpermeability. All post-TDV vaccination samples from both naïve and pre-immune vaccine recipients completely abrogated NS1-induced hyperpermeability. These effects correlated with anti-NS1 titers as measured by ELISA (r=0.6303, p=0.0089). Post-TDV vaccination serum also inhibited hyperpermeability induced by DENV1, 3, and 4 NS1 and reflected IgG titers. This is the first indication of functionality of NS1-specific antibody responses elicited by a candidate dengue vaccine.

1619

CO-DEVELOPING CLIMATE SERVICES FOR PUBLIC HEALTH: STAKEHOLDER NEEDS AND PERCEPTIONS FOR THE PREVENTION AND CONTROL OF *AEDES*-TRANSMITTED DISEASES IN THE CARIBBEAN

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Diseases transmitted by *Aedes aegypti* (dengue, chikungunya, and Zika) are among the top public health concerns in the Caribbean. The 2017 Atlantic Hurricane Season was one of the most active on record; certain territories are now at greater risk of *Ae. aegypti* transmitted diseases due to damages to housing, infrastructure, and health systems, and increased mosquito larval habitats. Given the effects of local climate conditions (e.g., temperature and rainfall extremes) on the mosquito vector and disease transmission, the health and climate sectors have partnered to co-develop climate services to improve the management of these diseases, for example, through the development of climate-driven early warning systems. Here we present the results of a qualitative analysis of key stakeholder needs and perceptions in the region, with a focus on Barbados and Dominica. Stakeholders included public decision makers and practitioners from regional and national climate and health sectors. From April-June 2017, we conducted interviews (n=36), surveys (n=32), and national consultations with informants. Survey results were tabulated, and audio recordings were transcribed and analyzed by qualitative coding to identify themes. In surveys, 78% of health practitioners indicated that their jurisdiction is currently experiencing an increased risk of diseases transmitted by *Ae. aegypti* due to climate variability, and 81% of respondents anticipated that this risk will increase in the future. Interviewees identified strategies to strengthen the partnership between the climate and health sectors, and emphasized the need for local scientific evidence linking climate to health outcomes. Interviewees identified mechanisms for mainstreaming climate services for health operations to control *Ae. aegypti* transmitted diseases, such as an online GIS platform that would allow for data sharing and the generation of seasonal epidemic forecasts. These findings support the creation of transdisciplinary and intersectoral 'communities of practices' and the co-design of climate services for *Ae. aegypti* transmitted diseases in the Caribbean.

1620

CLINICAL AND ANALYTICAL PERFORMANCE OF THE TRIOPLEX REAL TIME RT-PCR ASSAY DURING THE 2016 ZIKA EPIDEMIC IN PUERTO RICO

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The emergence of Zika virus in 2015 presented a challenge to diagnostics in areas where transmission of dengue and chikungunya viruses has been detected. In response to this public health emergency, the Centers for Disease Control and Prevention developed the Trioplex Real Time RT-PCR Assay designed for the simultaneous detection of dengue, chikungunya and Zika virus RNA in human clinical specimen. We determined the analytical and clinical performance characteristics of the Trioplex in detecting each target virus serum, urine and whole blood-EDTA specimens. The assay was adapted to RNA extraction, PCR equipment and procedures commonly found in US and international public health laboratories. To determine the limit of detection (LoD) of the Trioplex for each target virus on each these modalities, inactivated dengue, chikungunya and Zika viruses were suspended in human serum, urine or whole blood and diluted to undetectable levels. The overall LoD for the assay was determined to approximate 10^3 genome copy equivalents per milliliter of specimen (GCE/mL) for every target virus. The assay modality that achieved the highest sensitivity included RNA extraction from 1 mL of specimen. Independent studies confirmed that the sensitivity of Trioplex is similar to other CDC and non-CDC tests; and that the Trioplex modality with the highest sensitivity compares favorably to the most sensitive commercial tests. In order to determine the clinical sensitivity of Trioplex during the first 6 days of illness, 373 concurrently collected serum, urine and whole blood samples from patients with positive Zika IgM after 7 days were tested. In 373 confirmed cases in Puerto Rico, the Trioplex detected 85% (317/373) in serum, 83% (311/373) in urine and 82% (285/347) in whole blood. Testing simultaneously collected serum and urine provides an additional 3% sensitivity over serum alone; whereas the value of testing serum-whole blood provides an additional 5% over serum alone. The high sensitivity of the Trioplex demonstrates the utility of the assay resolving Zika cases in endemic areas. More than 39 thousand Zika cases in Puerto Rico have been confirmed with the Trioplex.

1621

IMPROVEMENT OF THE DENGUE NON-HUMAN PRIMATE MODEL *VIA* A REVERSE TRANSLATIONAL APPROACH BASED ON DENGUE VACCINE CLINICAL EFFICACY DATA

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The non-human primate (NHP) model is the most widely recognized tool for assessing the protective activity of dengue vaccine candidates, based on the prevention of post-infection Dengue virus (DENV) viremia. However, the use of this model has been questioned after the recent Phase III trials with the tetravalent dengue vaccine (CYD-TDV) in which moderate protective efficacy against DENV-2 was reported, despite full protection against DENV-2 viremia previously being demonstrated in monkeys vaccinated with CYD-TDV. The aim of the present work was to re-assess the protection conferred by CYD-TDV in macaques, based on a reverse translational post-Phase III approach under more stringent conditions of DENV infection than previously employed. This strategy was based on the assumption that using viremia levels approximating those observed after natural dengue infection in humans, the new NHP model would have better ability than previously to predict the clinical efficacy of CYD-TDV against serotype 2. We show that the NHP model can be improved to achieve DENV-2 protection levels which show better agreement with clinical efficacy. We also demonstrate with this new model that injection of the CYD-2 component of the vaccine, either in a monovalent or a tetravalent formulation, is able to reduce DENV-2 viremia in all immunized animals. We also provide clear statistical evidence that DENV-2 neutralizing antibodies are able to reduce viremia in a dose-dependent manner. Collectively, these results reinforce the value of the NHP model in assessing the efficacy of dengue vaccines, despite inherent limitations.

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IDENTIFICATION OF NOVEL TYPE-SPECIFIC NEUTRALIZING ANTIBODY EPITOPES IN THE DENGUE VIRUS TYPE 3 ENVELOPE GLYCOPROTEIN

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Four serotypes (1-4) of dengue virus (DENV) circulate in human populations, and immunity to one serotype does not confer long-lasting immunity to the others. Rather, pre-existing DENV immunity may actually increase the risk of severe dengue after exposure to a second serotype. The possibility of antibody-mediated enhancement has complicated vaccine development because of the need to induce robust immunity to all 4 serotypes simultaneously. After a primary infection, type-specific (TS) antibodies to the individual serotypes of DENV is thought to be associated with robust, life-long homotypic protection, but the full repertoire of primary neutralizing antibody epitopes in each DENV serotype remains incomplete. Currently, the only DENV3 TS neutralizing human monoclonal antibody (mAb) is 5J7, which recognizes a complex quaternary epitope that spans 3 monomers of the envelope (E) glycoprotein. Importantly, several studies have suggested that 5J7 may not be the only neutralization epitope in DENV3. Rather, studies in natural DENV-infected cohorts and in vaccinees receiving tetravalent DENV vaccine suggest only a fraction of

the polyclonal response targets the 5J7 epitope and there are additional neutralizing epitopes. To test this hypothesis, we immortalized memory B cells from DENV3infected individuals from a cohort in Nicaragua. Several additional DENV3 TS neutralizing mAbs were identified that do not compete with 5J7 in competition assays. One mAb, 66, neutralizes genotype III but not genotype II variants of DENV3. A second neutralizing human mAb, 144, binds to E domain 1 (EDI) as determined by lack of neutralization of chimeric viruses. A third DENV3- specific neutralizing human mAb, 115, binds to a novel epitope located outside the EDI/II hinge and EDI regions. Refined epitope mapping and epitope exchange studies are in progress to definitively map the epitopes of these new DENV3 neutralizing mAbs. These findings provide new insights into the mechanism of DENV3 neutralization and will lead to assays for defining the primary neutralizing epitopes associated with DENV3 protective immunity following natural infection or vaccination.

1623

POTENCY AND BREADTH OF HUMAN IMMUNE SERA FOLLOWING PRIMARY DENGUE VIRUS INFECTION

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Characterization of the antibody response following infection with one of the four dengue virus (DENV1-4) serotypes is often incompletely defined, in part because of antigenic diversity between DENV within each serotype. Historically DENV immune sera are characterized using either locally circulating or reference DENV isolates, often with only one virus per serotype. However, the DENV field has increasingly recognized the potential for within serotype antigenic variation to contribute to breakthrough infection following DENV vaccination and, potentially, natural DENV infection. Consequently, approaches to characterize immune sera that capture and compare the interplay between antigenic diversity and antibody neutralization are called for. The objective of this study is to characterize and compare the potency and breadth of neutralization of 1° DENV immune sera by applying approaches developed in the HIV field to characterize breadth and potency of HIV neutralizing antibodies. In this context, breadth refers to the overall antigenic diversity contained within a panel of unique viruses and potency refers to the each serum neutralization titer (NT) against each unique virus. To execute this study, immune sera from individuals with diverse geographic (South and Central America, South Asia, Southeast Asia and Oceana) and temporal (0.7 to 36 years prior) 1° DENV1 infection histories were characterized against a panel of 10 genetically distinct DENV1 virus isolates. For each serum and virus set, proportion of viruses neutralized are plotted against serum dilution, generating a unique curve for each serum that characterized the potency and breadth of serum neutralization. We then describe and compare the curves using both traditional and novel statistical methods, providing a robust means of comparing individual serum neutralization properties in a manner that captures the full activity of each serum against a diverse panel of virus isolates. This approach is generalizable to all four DENV serotypes and should provide a more rigorous approach to characterizing DENV immune sera following both vaccination and natural infection.

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SEROLOGICAL DISCRIMINATION OF DENGUE AND ZIKA INFECTIONS

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The arrival of Zika virus (ZIKV) to dengue DENV endemic areas came along with diagnostic challenges in serology. The current ELISA assays cannot distinguish these viruses. Furthermore, cross-reactive antibodies produced in DENV and ZIKV infections make the PRNT unreliable for diagnostic purposes as most dengue endemic countries experienced ZIKV mainly as a secondary flavivirus infection. The goal of this study was to develop a MAC-ELISA that could simultaneously discriminate between DENV and ZIKV infections during the convalescent phase during which molecular diagnosis is no longer reliable. A ZIKV/DENV MAC-ELISA was developed to detect the presence of ZIKV or DENV IgM simultaneously. We analyzed PCR-confirmed acute and convalescent cases of ZIKV, DENV, and non-flavivirus febrile illness from our established Sentinel Enhanced Dengue Surveillance System (SEDSS) in Ponce, Puerto Rico. Infections were identified with a diagnostic algorithm that utilized the ratio of the average OD450 reacting to ZIKV antigen/average OD450 reacting to DENV antigen to determine ZIKV positive cases. This was followed by calculating the average OD450 to DENV/the average OD450 to the normal antigen to determine DENV positive cases. Specimens that did not react above the cutoff value for DENV were considered negative for both viruses. The ZIKV/DENV duo MAC-ELISA was able to detect 103/103 (100%) ZIKV+ specimens and discriminated 103/103 (100%) of the specimens correctly. For DENV+ specimens, the ZIKV/DENV duo MAC-ELISA detected 133/134 (99.25%) and correctly discriminated 133/133 (100%). The sole specimen not detected or discriminated was equivocal in the assay. No false positives were detected from 143 negative specimens tested. A novel approach to differentiate DENV and ZIKV infections serologically was developed. Our ZIKV/DENV duo MAC-ELISA displayed sensitivity that was equivalent to both ZIKV and DENV stand-alone assays. The assay specificity was high enough that it can potentially replace the highly laborious PRNT for confirmation of ZIKV or DENV IgM detection.

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CHARACTERIZATION OF A MURINE MODEL OF NON-LETHAL, SYMPTOMATIC DENGUE VIRUS INFECTION

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The mosquito-borne disease dengue is caused by four serologically- and genetically- related viruses, termed DENV-1 to DENV-4. Clinical dengue infections range in severity from self-limiting, debilitating disease to life-threatening illness, such as dengue hemorrhagic fever and dengue shock syndrome. A major obstacle in dengue research had been the lack of appropriate animal models that mimic human disease. Recent advances using the immunocompromised AG129 mice (deficient in IFN-α/β/γ receptors) resulted in development of lethal murine infection models for DENV-1-4 that reproduce key features of severe dengue. In this study, we compared a non-lethal, disseminated model of DENV-3 infection using strain D83-144 to that of the lethal outcome following infection by strain DENV-3 C0360/94. Intraperitoneal inoculation of AG129 mice with strain D83-144 led to a neutralizing antibody response and to clinical signs of dengue infection, such as serum cytokine induction, thrombocytopenia, leukopenia, organ pathology, and disseminated viral loads. However, C0360/94 infection led to increased clinical disease and to additional features of severe human dengue, such as coagulopathy and lethal outcome. Genome sequencing revealed that both DENV-3 strains belong to the same phylogenetic group, genotype II, and differ by only 13 amino acids. The results suggest that the AG129 mouse model has applications to investigate differences between mild and severe disease. This study is the first to investigate a low passage, non-mouse lethal strain in AG129 mice that causes a productive, mild infection with features of human dengue.

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ENDEMICITY AND EMERGENCE OF ARBOVIRUSES IN PIEDECUESTA, COLOMBIA

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For diseases like dengue (DENV) or Zika (ZIKV), where a large proportion of infections are asymptomatic, population-based serological studies remain the gold-standard to quantify transmission. Here, we present preliminary findings from our population based studies conducted in Piedecuesta, Colombia since 2014. These studies include a baseline household-based cross-sectional sero-survey conducted among 1200 individuals in 2014, as well as an ongoing longitudinal cohort that enrolled 2400 participants in 2015. Follow-up within the cohort study involves active fever surveillance (via a call center) as well as yearly visits in which a blood sample is collected. While these studies were originally designed to characterize dengue transmission, chikungunya (CHIKV) and ZIKV were introduced into the study area in 2014 and 2016, respectively. Thus, they provide a unique opportunity to quantify transmission of the three viruses and identify risk factors for infection and disease. At baseline, 67% (1636/2453) of participants tested positive for IgG against DENV and the age-specific seroprevalence pattern was consistent with endemic circulation of DENV in this population. Up to now, during over 5000 person-years of follow-up, there have been 813 incident cases of febrile illness, including 14 cases of DENV, 14 cases of CHIKV and 73 cases of ZIKV confirmed by PCR and/or serology. We are currently testing the samples from the yearly visits to ascertain seroconversion. Serological testing is being performed using a multiplex recombinant antigen-based microsphere immunoassay that simultaneously quantifies IgG against multiple arboviruses. This assay was validated using over 400 well characterized samples from the cohort study and shown to have good sensitivity and specificity for CHIKV, DENV and ZIKV. Once testing is complete, we will estimate the attack rates of the three viruses, the symptomatic to asymptomatic ratios, and risk factors associated with infection and disease. In particular, we will explore whether prior exposure to DENV modified the risk and/or outcome of ZIKV infection during the 2016 outbreak.

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AVIAN IGY ANTIBODIES RECOGNIZE NOVEL DENGUE NS1 EPITOPES WITH THE ABILITY TO NEUTRALIZE INFECTION AND REDUCE VASCULAR LEAKAGE WITHOUT INDUCING ANTIBODY DEPENDENT ENHANCEMENT

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Dengue virus (DENV), the most prevalent arbovirus, causes 96 million infections worldwide resulting in 500,000 cases of Dengue Hemorrhagic Fever (DHF) cases and 22,000 deaths. Symptoms of DENV infection range from a mild fever to extensive vasculature permeability resulting in hemorrhagic fever, shock, and death. The severity of DENV infection is greatly enhanced by antibody dependent enhancement (ADE); increased viral load due to increased monocyte opsonization by non-neutralizing low avidity Abs from a different DENV serotype or secondary flavivirus infection. ADE has made both passive and active vaccines difficult to develop. We have previously demonstrated that polyclonal avian anti-DENV-2 IgY ameliorates DENV infection in mice without inducing ADE. We had hypothesized that IgY would not induce ADE as mammalian Fc receptors are unable to bind the Fc portion of IgY, and thus IgY would have the potential to provide a viable treatment for DENV infections. Epitope-mapping of anti-DENV-IgY identified numerous epitopes of the envelope, membrane, and non-structural (NS) proteins that were recognized. Here we demonstrate that two purified pools of anti-DENV IgY with specificity for two different regions of NS1 had a neutralization capacity equivalent to the whole polyclonal DENV IgY preparation *in vitro*. We assayed each pool of NS1-specific IgY for the potential to induce ADE and demonstrated no ADE when cells were treated with IgY and challenged with a secondary flavivirus. We then utilized a trans-endothelial electrical resistance (TEER) assay and determined that treatment with DENV NS1-specific IgY reduced vascular leakage in our model system. We propose IgY may provide a novel oligoclonal therapeutic for human DENV infection without inducing ADE.

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TOWARDS THE DEVELOPMENT OF A FEASIBLE DENGUE FORECASTING SYSTEM IN AN ENDEMIC COUNTRY

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Disease forecasting systems vary in complexity and are largely based on the analysis of routine data such as disease surveillance, socio-economic, meteorological, and land use data. In countries with limited resources, a user-friendly forecasting system based on strategically chosen input data sources may be necessary. The objective of this research was to develop and validate such a forecasting model for short-term (1 and 2 month-ahead) and long-term (year-ahead) predictions of dengue incidence based on monthly municipality-level data for Colombia from 2014 to 2016. We used a machine learning approach and specifically, a random forest algorithm, as it is known to have good prediction accuracy in a variety of contexts and can rank predictors/inputs in terms of their relative importance to prediction. We considered the following inputs: socio-economic, meteorological, land cover, and historical arbovirus disease surveillance notifications (i.e., cases of Zika, chikungunya, dengue). In our preliminary results of year-ahead forecasts of dengue incidence in 2016, based on 2014-15 predictors, the best model included land cover, SES, and historical arbovirus disease notifications with a good prediction accuracy ($R^2 = 0.66$). Historical dengue incidence was the strongest predictor followed by evergreen/deciduous needleleaf tree landcover and the average household index of unsatisfied basic needs. However, a simpler model using only historical chikungunya and dengue incidence provided forecasts with similar prediction accuracy. In short-term forecasts, the 1 month-ahead model with predictors, historical dengue and chikungunya, and rainfall had high prediction accuracy ($R^2 = 0.89$). The best 2 month-ahead model, allowing 1 month lead time, had the same predictors but had lower accuracy ($R^2=0.70$). Our next steps include exploring higher temporal resolution forecasts (e.g., weekly) at lower spatial resolutions (e.g., department level) for 2017. Our preliminary work suggests that investment in disease surveillance data may be more efficient than investing in the processing and management of climate/meteorological variables.

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VALIDATION OF CLINICAL ALGORITHMS FOR THE DIAGNOSIS OF DENGUE IN ENDEMIC AREAS OF COLOMBIA

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Due to the increase mortality of dengue, its prompt and correct diagnosis is a priority for clinical care in endemic countries. Clinical classifications and existing laboratory tests for diagnosis have a variable performance in terms of sensitivity and specificity. To develop and validate clinical algorithms for dengue diagnosis in endemic areas of Colombia. A Bayesian-adaptive quasi-experimental clinical trial has been conducted with a target of 2000 febrile subjects. In the first phase, diagnostic algorithms based on Bayesian methodologies are been developed and validated prospectively. Demographics, signs and symptoms of dengue, leukocytes and platelets are collected from all participants; clinical algorithms are compared to gold standard diagnostic dengue tests (combination of NS1-ELISA, IgM/IgG-ELISA, RT-PCR). In the second phase, the diagnostic accuracy of the best algorithm identified in the first phase will be validated in the routine clinical practice. Interim analyses will be performed when effective sample size is met. Bayesian estimates of sensitivity and specificity were made. To date, 8 clinical diagnostic algorithms have been developed. Four based on signs and symptoms and four that include leukocytes and platelet counts. The latter have reached up to 81,3% sensitivity and 88,2% specificity. In addition, in a prospective validation 536 febrile subjects were recruited and the clinical algorithm (headache, skin rash, hemorrhagic manifestations, warning signs, neurological alterations, absence of respiratory symptoms, leukopenia <4.200/mm³ and thrombocytopenia <165.000/mm³) had a sensitivity of 70,5% and a specificity of 65%. The inclusion of blood count parameters using Bayesian methods improves the sensitivity of dengue diagnostic algorithms based only on signs and symptoms. The development of highly sensitive and specific dengue diagnostic tests based on clinical criteria useful at point of care remains a challenge for dengue research.

1630

DIMER-DEPENDENT QUATERNARY EPITOPES ENHANCE QUALITATIVE IMMUNE RESPONSES IN FLAVIVIRUS E-SUBUNIT VACCINE CANDIDATES

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Flaviviruses are a group of arthropod-borne viruses that represent a major public-health threat all over the globe. Several hundred million people are infected by DENVs, of which many develop dengue fever or dengue hemorrhagic fever. The same populations are at risk to other closely related flaviviruses. Since early 2015, Zika virus (ZIKV) has spread from Brazil to most countries in Latin America, the Caribbean, and most recently, the USA. ZIKV infection can lead to neurological disorders and has been linked to severe birth defects. There is an urgent need for vaccines to protect individuals and to prevent the spread of these viruses. Results from flavivirus vaccine trials establish the importance of the "quality" of a neutralizing response for developing durable protective immunity. Neutralizing Ab quality refers to the capacity to recognize serotype-specific targets of complex structure on the surface of the virion. Recent studies have established that quaternary epitopes and larger antigenic sites displayed on flavivirus E protein homodimers, but not monomers, are major targets of type-specific and cross-protective neutralizing Abs. To explore the potential of E-protein based subunit vaccines, we have immobilized soluble E molecules on solid carriers, and induced the formation of stable homodimers These *in vitro* assembled homodimers were efficiently recognized by human Abs directed to quaternary structure epitopes. Most importantly, mice immunized with immobilized DENV2 and ZIKV rE-homodimers developed more rapid and higher neutralizing Ab responses compared to animals immunized with monomers. Our data indicates that targeting quaternary epitopes present on E-homodimers that are displayed on particles induced a higher quality immune response compared to E-monomers, therefore enhancing the quality of the immune responses. These findings represent promising tools to develop safe and efficacious flavivirus vaccine candidates.

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METABOLIC BIOSIGNATURES DEFINE THE PATHOGENIC STATE OF FLAVIVIRAL DISEASE

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Dengue viruses (DENVs) place over 2 billion people at risk of infection each year rendering them the most aggressive Arboviruses worldwide. There are four serotypes of DENVs. Infection with one serotype does not cross protect from infection with other serotypes. No treatment options exist due to complications in disease pathology mediated by the immune response. Using state of the art high resolution metabolomics analyses of serum derived from pediatric patients we have identified metabolite biosignatures that are strongly associated with dengue disease severity and pathogenesis. These metabolite biosignatures also very clearly distinguish the pathogenic states caused by Zika virus and chikungunya infections with a combined accuracy of 99-100%. Thus, they have a strong potential to play a critical role in better triaging and clinically managing these viral diseases at an early stage. Additionally, unbiased network and pathway analyses identified fatty acid metabolism as a critical pathway that differentiated the disease states associating observations made at a molecular level with those in humans. Given that lipids play an active role in immune cell development, maturation and immune-mediated pathology these metabolic changes may be critical for establishing productive infections primarily via detrimental effects on the immune response.

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FIRST ISOLATION OF DENGUE VIRUS SEROTYPE 3 IN A NORTH REGION OF PERU: MOLECULAR DIAGNOSIS AND CLINICAL CHARACTERISTICS

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Dengue virus (DENV) infection is the most rapidly spreading mosquito-borne viral disease. DENV-1 and DENV-2 have been associated with severe cases of DENV infection such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DENV-2 is the most commonly isolated serotype in Perú and DENV-3 has only been previously described once in Peru. This study aimed to describe an outbreak of DENV infection in Cajamarca during 2017 and to molecularly identify the involved serotypes. A total of 359 serum samples from patients with acute febrile illness (AFI)

were assessed for the presence of dengue virus via RT-PCR, ELISA NS1, IgM and IgG. DENV positive samples were classified by serotypes 1 to 4 depending on their amplified primers. Dengue virus was detected in 24,7% (n=89) of samples via RT-PCR. Serological analysis detected 35.7% (n=128) positive cases via ELISA NS1 antigen, 16.7% (n=60) via ELISA IgG and 9.7% (n=35) via ELISA IgM. DENV-2 serotype was isolated in 11.2% (n=10) and DENV-3 serotype in 77.5% (n=69). We could not identify the serotype of 11.2% (n=10) of DENV cases confirmed by RT-PCR. The most frequent symptoms found in patients positive for DENV (RT-PCR) overall were, headaches (88.8%), followed by arthralgias (71.9%) and myalgias (68.5%). DENV-3 infected patients reported headache (88.4%) followed by myalgias (73.9%) and arthralgias (66.7%), as their most common symptoms. No DHF, DSS or deaths were reported during this outbreak. In conclusion, in our study, we identified DENV in 24,7% of samples during a dengue outbreak in Cajamarca. The serotype characterization showed that 77,5% DENV infection cases were caused by DENV-3, which represents the first isolation of this serotype in Cajamarca and the second time DENV-3 is described in Peru. These findings demonstrate an increasing extension of serotype 3 in Peru and highlights the importance of molecular diagnosis and serotype characterization among the clinically-defined dengue cases to strengthen the Peruvian epidemiological surveillance and decrease under-reporting rates in the Americas.

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ANTIBODY PERSISTENCE AND IMMUNE MEMORY RESPONSE FOLLOWING VACCINATION WITH LIVE ATTENUATED SA 14-14-2 JAPANESE ENCEPHALITIS VACCINE [CD.JEVAX, CHENGDU INSTITUTE OF BIOLOGICAL PRODUCTS]

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Japanese encephalitis (JE) virus is the leading cause of viral encephalitis across temperate and tropical zones of Asia. The live attenuated SA 14-14-2 JE vaccine (CD-JEV) is one of several vaccines prequalified by the World Health Organization to prevent JE. Previous trials with CD-JEV have shown that more than 90% of vaccinees developed neutralizing antibody (nAb) titer at seroprotective levels of ≥1:10 one month after vaccination, and in two studies, more than 80% of participants maintained nAb titers ≥1:10 up to four years after vaccination. This Phase 4 open-label clinical study evaluated nAb titers in Bangladeshi children three and four years after primary CD-JEV vaccination in a previous study and 7 and 28 days following receipt of a CD-JEV booster dose in this study. Three years after initial vaccination, seroprotective nAb titers of ≥1:10 were measured in 328 of 561 children (58.5% [95% CI: 54.3-62.5]) using the plaque reduction neutralization test. No vaccine-associated neurologic adverse events or other serious adverse events or safety signals were noted in children who were given a booster CD-JEV dose. Neutralizing antibody titer results four years after primary vaccination and 7 and 28 days following the CD-JEV booster dose are expected in August 2018.

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ZIKA VIRUS FORECASTING AND PREDICTION STUDIES: A SYSTEMATIC REVIEW AND EVALUATION OF THEIR UTILITY DURING A GLOBAL HEALTH EMERGENCY

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Numerous quantitative predictions were published during the 2016-2017 Zika virus (ZIKV) pandemic yet it remains unknown how timely, reproducible and actionable they were. We therefore conducted a systematic review and evaluation of all ZIKV prediction studies published during the recent ZIKV pandemic. We adapted the PRISMA methodology. All studies which forecasted, predicted or simulated any ecological or epidemiological phenomenon about the Zika pandemic and published prior to March 01 2017 were identified via MEDLINE, EMBASE and grey literature review. Eligible studies underwent evaluation of methods, data sources, objectives, timeliness, reproducibility, accessibility and clarity. 2034 studies were identified, of which 73 met eligibility criteria. The majority of studies predicted spatial spread, Ro or epidemic dynamics. Few studies predicted Guillain-Barre Syndrome burden (4%), sexual transmission risk (4%) and intervention impact (4%). Most studies specifically examined populations in the Americas (52%), with very few African-specific studies (4%). Case count, vector and demographic data were the most common data sources. Few studies used real-time internet data (7%) and none used genomic data. Deterministic models were more commonly used. 22% of studies did not make any model data available, 62% did not provide model code, 39% did not present uncertainty in predictions and 46% did not provide complete methodological detail. 59% of predictions were published after the epidemic peak in the Americas. Pre-prints improved the accessibility of ZIKV predictions (median 132 days sooner than journal publication date), but were used in only 32% of studies. In conclusion, many ZIKV predictions were published during the 2016-2017 pandemic. The accessibility, reproducibility, timeliness and incorporation of uncertainty in these published predictions varied. Improvements in the sharing of model data and code, methodological reporting and use of pre-prints can improve future outbreak responses.

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EFFECT OF TEMPERATURE ON THE EXTRINSIC INCUBATION PERIOD OF ZIKA VIRUS IN *AEDES AEGYPTI*

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Since Zika virus (ZIKV) emerged as a global human health threat, numerous studies have pointed to *Aedes aegypti* as the primary vector due to its high competence and propensity to feed on humans. However, all published vector competence studies have been conducted between 26°C-28°C, and arboviral extrinsic incubation periods (EIPs), and therefore transmission efficiency, are known to be strongly affected by temperature. To better understand the relationship between ZIKV EIPs and temperature, we evaluated the effect of adult mosquito exposure temperature on ZIKV infection, dissemination, and transmission in *Ae. aegypti* at four temperatures: 18°C, 22°C, 26°C, and 30°C. Mosquitoes fed on mice infected with a 2015 Puerto Rican ZIKV strain and were sorted into the four temperatures with 80% RH and constant access to 10% sucrose. ZIKV infection, dissemination, and transmission rates were estimated via RT-qPCR from individual mosquito bodies, legs and wings, and saliva, respectively, at 3-5 time points per temperature from 3 to 31 days, based on data from other flavivirus EIPs. Twenty mosquitoes were tested at each time point. Of the 320 mosquitoes that fed and survived the duration of the study, 312 (97.5%) were infected with ZIKV, 264 (82.5%) disseminated, and 144 (45%) transmitted at the time point tested. The median time from ZIKV ingestion to transmission (median EIP, EIP50) at each temperature was estimated by fitting a generalized linear mixed model. EIP50 ranged from 5.1 days at 30°C to 25.0 days at 22°C. At 26°C, EIP50 was 9.4 days. At 18°C, only 15% transmitted by day 31 so EIP50 could not be projected. This is the first study to characterize the effects of temperature on ZIKV EIP in *Ae. aegypti*. These results deviate slightly

from EIPs of dengue virus in *Ae. aegypti* and West Nile virus in *Cx. tarsalis.* This information is critical for modeling ZIKV transmission dynamics to understand geographic and seasonal limits of ZIKV risk; it is especially relevant for determining risk in subtropical regions with established *Ae. aegypti* populations (e.g. California or Florida) as these regions typically experience cooler temperature ranges than tropical regions.

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CONTRASTING THE VALUE OF TARGETED VERSUS AREA-WIDE MOSQUITO CONTROL SCENARIOS TO LIMIT ZIKA VIRUS TRANSMISSION FOR DIFFERENT TROPICAL URBAN POPULATION CENTERS

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Transmission of vector-borne diseases is affected by various types of host movement. Here we consider the extent to which considering daily commuting patterns can help optimize vector control efforts to limit the size of Zika virus outbreaks. We examine three tropical urban centers (San Juan, Recife, and Jakarta) that have been exposed to Zika and/or dengue outbreaks in recent years and consider whether the distribution of human populations and resulting commuting flows has an impact on the optimal scale at which to implement control interventions. We developed a stochastic, spatial model adapted to these three urban areas and four control scenarios based on larval control. The scenarios differed in the spatial extent of their implementation and were: 1) a response at the level of an individual neighborhood; 2) a response targeted at a neighborhood with active transmission and one with which it was most strongly connected; 3) a limited area-wide response where all neighborhoods within a certain radius of the focal area were included; and 4) a collective response where all participating neighborhoods implemented control. The relative effectiveness of the scenarios varied only slightly between different settings, with the number of infected cases averted over the course of the epidemic increasing with the scale of implementation. This difference depended strongly on the efficacy of control at the neighborhood level. At low levels of efficacy, the scenarios mirrored each other in cases averted. At high levels of efficacy, impact increased with the scale of the intervention. This occurs because at high levels of efficacy, more focal responses lead to stronger metapopulation dynamics after the epidemic peak. Despite this, more focal responses still require a lower population coverage, and the choice between scenarios will largely be a function of the amount of effort decision-makers are willing to invest. Finally, we consider how the spatial configuration of control can improve the efficacy of control programs, pointing to ways in which improved knowledge of vector densities and human movement can optimize control efforts.

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THE EFFECT OF TIMELY INTENSIVE SPACE SPRAY ON DISEASE CONTROL IN TWO ZIKA VIRUS OUTBREAKS IN SOUTHERN THAILAND

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Main Zika virus (ZIKV) transmission is via mosquito vectors. Theoretically, rapid viremia occurs after onset in human case and short intrinsic incubation period in vector mosquito indicate the needs to lauch an intensive space spray within 3 days of onset of each case in order to prevent ZIKV transmission. However, whether this timeliness could be reached and whether it can actually stop the outbreak have never been epidemiologically examined. This study aimed to investigate the proportion of timely intervention and check whether it could reduce secondary infection rate. Data on two ZIKV outbreaks in 2015-2016 from two communities, A and B, were analyzed. Main variables included disease onset, house coordinates and date of spray around the cases' house. ZIKV was confirmed by RT-PCR. Potential secondary cases were patients with onset at 3 to 23 days after the index cases and lived nearest to them in the same community. Timely spray was achieved in 5 of 18 and 9 of 15 cases' households in Community A and B, respectively. In Community A, the mean (SD) of secondary case infection rates were 0.92 (1.12) and 0.20 (0.45) in the non-timely and timely groups, respectively. Consistent pattern was observed in Community B where mean (SD) of secondary case infection rate were 1.67 (1.63) and 0.33 (0.43) in the non-timely and timely groups, respectively. The timely spray could reduce secondary cases by (.92-.2)/.92 = 78.3% in Community A and (1.67-.33)/1.67 = 80.2% in Community B. Combining data from these two communities, rate of secondary infections in the timely group was significantly fewer than that in the non-timely group (ranksum test P value = 0.029). Thus, the timely space spray could reduce secondary case infection rate. However, delayed spray was still preponderant. Researches to increase timeliness for intensive spray are needed to improve future ZIKV outbreak control.

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ARBOVIRAL DISEASE IN PAKISTAN: 2015-2017

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Like most of the world, Pakistan has seen an increase in mosquito-transmitted diseases in recent years. The magnitude and distribution of these diseases are poorly understood as Pakistan does not have a nation-wide system for reporting disease. A cross-sectional study to determine which arboviruses were the cause of disease in Pakistan was instituted. Over the course of 2 years, 997 patients were enrolled presenting with clinical features suggestive of arboviral disease. Viral exposure was verified via detection of viral nucleic acids or virus-specific IgM with virus-specific neutralizing antibodies. In addition, clinical and pathological data were collected. It is not uncommon for Dengue viruses (DENV) to co-circulate West Nile virus (WNV), Japanese Encephalitis virus (JEV), and/or Chikungunya virus (CHIKV) which can lead to misdiagnosis. Overlapping clinical presentation and serological cross-reactivity complicate definitive diagnosis. Furthermore, secondary infections with these types of viruses can exacerbate clinical symptoms and complicate interpretation of diagnostic tests. Here we describe the active and persistent circulation of WNV, DENV, JEV, and CHIKV in Pakistan. We found that these viruses exhibited unique characteristics that could help local practitioners drive surveillance, diagnostics, and improve patient care. WNV infection occurred primarily in the early spring and fall months with many patients presenting with altered mental status (OR 4.25, CI 1.32-13.69) or seizures (OR 2.58, CI 0.66-10.14). Individuals with JEV infection were more likely to have gastro-intestinal disturbances (OR 7.16, CI 1.54-33.21) and a complete lack of neurological manifestations (OR 0.13, CI 0.03-0.62). We found that CHIKV has been circulating in Pakistan since at least 2015 and is a significant cause of neuroinvasive disease in nearly half of exposed patients (OR 3.5, CI 0.93-13.19). Moreover, the majority of CHIKV infections occurred in the winter months when *Aedes* mosquitos are typically dormant. DENV infections occurred primarily in the early summer months and all 4 serotypes co-circulated and co-infected patients.

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IDENTIFYING ENVIRONMENTAL RISK FACTORS AND MAPPING THE RISK OF HUMAN WEST NILE VIRUS

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Despite originally being a tropical disease, the highest incidence rates of human West Nile virus (WNV) within the United States are found in the cold and temperate Northern Great Plains, specifically in South Dakota. Disease transmission depends on complex interactions between the mosquito vector, the bird hosts, and humans, and is therefore highly variable in space and time. Understanding the spatial patterns of these interactions and being able to identify disease transmission hotspots are crucial for effective disease prevention and mosquito control. In this study, we used geospatial environmental data and machine learning techniques to assess the environmental drivers of human WNV cases in South Dakota and to map relative infection risk across the state. The binary dependent variable was derived from geocoded human cases from the years 2004 to 2016 and population weighted pseudo-absence points. We compared different environmental datasets to study their associations with the spatial pattern of cases. We used MODIS satellite data derived indices, such as the normalized differenced vegetation index (NDVI) and the normalized differenced water index (NDWI), as well as climatic data from the National Land Data Assimilation System (NLDAS). These remote sensing and climate datasets were both summarized over the 12 years of case data availability. We also used static environmental datasets, such as the National Land Cover Dataset (NLCD), National Wetland inventory (NWI), National Elevation Dataset (NED) and Soil Survey Geographic Database (SSURGO). We used a boosted regression tree model to identify the most important variables driving WNV risk. We generated a risk map by applying this model across the entire state. We found that inter-annual variation in humidity, temperature and surface water conditions were the strongest drivers of transmission risk. Land covers such as grasslands, low developed areas and wetlands were also found to be associated with human WNV risk. We suggest that combining measures of inter-annual environmental variability with land cover data can help to create improved disease transmission risk maps.

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SURVEILLANCE OF FLAVIVIRUSES IN MOSQUITOES, CARIBBEAN REGION OF COLOMBIA, 2017-2018

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The emergence of Zika virus (ZIKV) in the Americas, and its association with newborn microcephaly and Guillain-Barré syndrome have raised global concern. We aimed to detect ZIKV in mosquitoes of Coloso (Sucre), Colombia to determine if novel species of mosquitoes could carry ZIKV. We trapped peridomestic and sylvatic mosquitoes using *CDC-light*, *BG-sentinel*, and *resting traps* during the months of February, May, and September, 2017, and January, 2018. We extracted viral RNA from mosquito pools (organized by species, subsite and month), and screened all pools for flaviviruses by qRT-PCR with PF1S and PF2R-Bis primers, and tested flavivirus-positive pools for ZIKV by real-time RT-PCR. A total of 4,300 female mosquitoes were collected and grouped into 373 pools. The most abundant mosquito species collected were *Culex interrogator* (n=50 pools; 13%), *Cx. (Melanoconion) sp.* (n=41; 11%), and *Cx. declarator* (n=31; 8.3%). We found 68 or 18.2% of all pools flavivirus-positive. Positive pools were detected among *Haemagogus capricorni (*1/1*; 100%)*, *Ha. janthinomys (*1/1; *100%), Coquillettidia nigricans* (1/1; 100%), *Uranotaenia lowii* (10/10; 100%), *Psorophora ferox* (9/11; 81.8%), *Cx. (Mel.) ocosa* (12/16; 75%), *Aedes aegypti* (6/8; 75%), *Ha. equinus* (3/4; 75%), *Haemagogus sp.* (1/2; 50%), *Limatus durhamii* (3/8; 38%)*, Cx. (Mel.) sp.* (12/41; 29.3%), *Ps. columbiae* (4/15; 26.7%), *Cx. chidesteri* (3/11; 27%), *Mansonia pseudotitillans* (1/4; 25%), *Ps. (Janthinosoma) sp.* (3/12; 25%), *Ma. titillans* (1/8; 13%), *Psorophora sp*. (1/8; 13%), *Culex (Cx.) sp (3/36; 8.3%)*, *Cx. coronator* (1/19; 5%), and *Cx. declarator* (1/31; 3.2%). All the aforementioned pools were negative for ZIKV virus infection. Flavivirus-infecting mosquitoes, predominantly of the genus *Culex, Psorophora* and *Aedes*, were found to circulate in Coloso, Sucre. Although we did not find ZIKV infection in these insects, other flaviviruses were common, and will be characterized to determine public health importance. Non-human primates inhabit this area and should be evaluated as reservoirs for these viruses.

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ZIKA VIRUS PERSISTENCE AND VIRAL LOAD IN BODY FLUIDS OBTAINED FROM INFECTED PATIENTS

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Since the recognition that some Zika infections can be detected in fluids other than blood (sometimes for extended periods), several studies have been performed to try to understand the prevalence and impact of this upon disease transmission. Given our access to Zika patients in Peru since 2016, we initiated an enhanced surveillance program to detect cases, and follow them over time in an effort to characterize such events. Beginning in late 2017, this program detected numerous Zika cases in Yurimaguas, Loreto, Peru. To estimate the frequency, duration and viral load shedding we implemented a prospective study whereby newly confirmed Zika cases (positive by EUA CDC Trioplex real-time RT-PCR) were enrolled for follow up. Urine, saliva, blood, serum and semen or breast milk (when available) were obtained from participants every other day during their first week of illness. Weekly samples were collected thereafter, until samples became negative twice. All samples were initially tested by real-time RT-PCR. We detected Zika RNA in urine up to 21 days (6-21 days) in some participants, whereas in paired whole blood we were able to detect Zika RNA only up to 5 days. Detection of ZIKV RNA in blood was variable with excretion times ranging from 1 to 9 days after the onset of symptoms. All samples are undergoing viral isolation procedures to confirm the presence of live virus. Results from this follow up study will contribute to our understanding of Zika transmission potential and to develop guidance for optimal sample types and collection times.

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ZIKA VIRUS NS1 PROTEIN MODULATES THE BARRIER FUNCTION OF HUMAN PLACENTAL EXPLANTS AND HUMAN TROPHOBLAST CELL LINES

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Zika virus (ZIKV) was recently introduced into the Americas, causing massive epidemics, and is associated with birth defects when infection occurs during pregnancy and with neurological complications in adults. ZIKV has been shown to replicate in the human placenta, and we recently proposed two potential routes for vertical transmission of virus from mother to fetus. We have also shown that the flavivirus nonstructural protein 1 (NS1) disrupts the endothelial barrier function, leading to endothelial hyperpermeability *in vitro* and vascular leakage in mouse models. Here, we investigate the effect of NS1 proteins from ZIKV

(American strain) and the closely related West Nile virus to modulate the barrier function of human placenta explants *ex vivo* and trophoblast cell lines *in vitro*. Using chorionic villus explants from different gestational ages (7-9 weeks), we showed that recombinant ZIKV NS1 but not WNV NS1 increases the permeability of explants after 24 hours of treatment, as determined by the amount of fluorescently labeled-dextran (Dx-A680) detected inside chorionic villi. Further, explants exposed to unpurified ZIKV or purified ZIKV plus ZIKV NS1 protein showed significantly more permeability compared to explants exposed to purified ZIKV alone (no NS1). In vitro permeability experiments measuring trans-epithelial electrical resistance (TEER) across human trophoblast cell lines (JAR) cultured on transwell inserts indicate that ZIKV NS1 also modulates the barrier function of these polarized cell monolayers. Ex vivo and *in vitro* studies are ongoing to define the role of ZIKV NS1 in virus dissemination and infection of target cells either in the villus core of human villus explants or human monocytic cells plated on the basolateral side of cultured JAR monolayers in transwells. These results suggest a new potential role for ZIKV NS1 in viral transmission from mother to fetus via modulation of the placental barrier function.

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ZIKA VIRUS TRANSMISSION IN SRI LANKA

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Zika virus (ZIKV) is a mosquito borne Flavivirus that has garnered broad international attention in 2015-2016 as it rapidly spread through Latin America, causing severe birth defects and neurological disease. While the ZIKV case burden has decreased, many critical questions remain. The epidemiology of ZIKV in Asia is an area of uncertainty with major implications for understanding the recent epidemic in Latin America. While ZIKV activity is reported in many countries in the Asian continent, the complete epidemiology and patterns of transmission have not been well-defined. We employed advanced serologic techniques such as antibody depletion and micro neutralization to assess type-specific antibody responses to Zika virus from archived human specimens collected from a pediatric DENV vaccine cohort study conducted between 2008-2010 in Sri Lanka. Preliminary results demonstrate past and ongoing ZIKV transmission in the cohort. We will present data on the age specific seroprevalence and annual incidence of Zika virus infection in the cohort. We will discuss the implication of our data for understanding the Zika virus activity in the Indian sub-continent.

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EVALUATION OF ZIKV POTENTIAL TO INFECT VERTEBRATES AND MOSQUITOES IN AN URBAN-SYLVATIC INTERFACE IN COLOMBIA

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Despite Zika virus (ZIKV) being zoonotic in origin, there is not much information about animal reservoirs for ZIKV in the Americas, and the role they might play in the virus maintenance and transmission. The aim of this study was to evaluate the potential of ZIKV to establish a sylvatic transmission cycle in Santander, Colombia. We conducted active surveillance of mosquitoes, native primates, as well as other non-human sylvatic vertebrates in the locality of Bocas del Carare, at established field sites in both urban and sylvatic areas during 50 nights. We performed fieldwork during five periods between March 2017 and March 2018. Trapping efforts were focused on abundant wildlife and domestic animals, as these likely have a role as reservoirs or amplifiers if they are hosts. We collected 24 mosquito species belonging to 10 genera, including *Aedes aegypti* as well as other suspected genus such as *Mansonia, Culex* and *Coquilletidia.* We obtained around 350 urban and 250 sylvatic samples including domestic animals, livestock and poultry, as well as wild animals belonging to classes Mammalia, Reptilia and Amphibia. Whole blood and mosquito samples were screened for ZIKV antigen by panflavivirus PCR and confirmed by a ZIKV specific PCR. Preliminary testing results revealed that 20% of vertebrates were positive by the panflavivirus PCR while no positives for mosquito pools were obtained. Remaining laboratory analysis and confirmatory testing is underway. The limited study data analyzed to date shows no evidence of the establishment of a ZIKV sylvatic cycle in Colombia. Once all data are examined and analyzed, the results of this study will directly contribute to the scientific literature by facilitating a better understanding of ZIKV's ability to establish a sylvatic cycle outside of the abundant human transmission.

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INCIDENCE OF GUILLAIN-BARRÉ SYNDROME IN LATIN AMERICA AND THE CARIBBEAN FOLLOWING THE 2015-2016 ZIKA EPIDEMIC

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Guillain-Barré syndrome (GBS), a severe autoimmune disorder, is the most common cause of acute flaccid paralysis. Between 20%-30% of patients require mechanical ventilation. Mortality rates range from 3%-7% and are higher in low-resource settings. The median global incidence of GBS is estimated at 1.1/100,000 (range 0.8-1.9). However, this is based on studies from Europe and North America. Many countries reported increases in GBS cases following the 2015-2016 Zika epidemic. Recent data from Latin America and the Caribbean (LAC) present an opportunity to assess the GBS incidence in the region for the first time. We conducted a systematic review of the incidence of GBS and searched 9 scientific databases from 1980-2017 using pre-defined search-terms. We included publications reporting primary GBS incident cases within well-defined populations. A detailed protocol is available at PROSPERO (CRD42018086659). Of 6568 identified publications, we screened 4093 titles/abstracts after removing duplicates; of 118 full-text articles reviewed, 24 met inclusion criteria. The majority (62.5%, n=15) was published after 2015. While 3 were multi-country studies; 7 were from Caribbean, including Puerto Rico; 7 from Brazil; 5 from other South American countries; and 2 from Central America and Mexico. Six analyzed the incidence of GBS in children and 2 in adults only. Sources of data included national surveillance systems (GBS disease became notifiable in 2016), hospitalization records, and the Polio Eradication Surveillance System. Background rates of GBS varied from 0.3 per 100,000 persons in Natal, Brazil to 2.65 in El Salvador and 1.31 in Honduras. GBS incidence rates increased 2 to 9.8 times from baseline during the Zika epidemic. Trends were inconclusive for chikungunya and dengue. This is the first review summarizing the GBS incidence in LAC. Central American countries appear to have higher background incidence rates of GBS than the Southern

Cone and the Caribbean. GBS incidence seems to increase during arboviral epidemics, particularly Zika. GBS poses an additional burden to healthcare systems, particularly in low-resource settings.

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ZIKA VIRUS INFECTION IN OLIGOSYMPTOMATIC AND ASYMPTOMATIC CLOSE CONTACTS IN PERU

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The first Zika virus (ZIKV) infection was detected in Peru in February 2016. As of epidemiological week 7 of 2018, 441 cases of Zika have been reported in the country, 210 cases less than the previous year for the same period of time. Given that approximately 80% of cases are asymptomatic, there is potentially a significant level of underreporting in this data. In an effort to account for this, the aim of this study was to determine the prevalence of ZIKV infection in asymptomatic or oligosymptomatic close contacts of confirmed ZIKV infection through cluster investigations. This study was nested in a surveillance of febrile and exanthematous acute illness in Yurimaguas (lat -5.92; long -76.09), a Peruvian Amazon city with approximately 68,115 habitants. When a ZIKV infection was detected and the participant allowed informing their laboratory results to their household contacts, the participant was visited in their house. The household close contacts were defined as persons who have been living in or around the same house for the last 30 days. We invited all close contacts older than 5 years old to participate in the study. We followed each house weekly for 21 days. After the informed consent was obtained, if any sign or symptom suggested active infection, we took a 10 mL blood sample. If we found no symptoms, we obtained a 2 mL blood sample. All samples were processed by Trioplex Real-time RT-PCR Assay (CDC). Since December 2017 to March 2018 we detected 20 ZIKV infections, from those 16 clusters were identified with 37 household close contacts enrolled. ZIKV infections were found in 2 from 6 oligosymptomatic and 4 from 31 asymptomatic participants. Two asymptomatic ZIKV positives developed symptoms two days after the enrollment. Our study shows that the number of ZIKV cases is greater than those observed in the health facilities, which could range from 13% in asymptomatic cases to 33% in oligosymptomatic ones among the index case close contacts.

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ARBOVIRUS CO-INFECTIONS PROLONG DISEASE DURATION AND DELAY VIRAL CLEARANCE

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ZIKV and CHIKV co-infections (ZIKV/CHIKV CO) occur at variable rates in areas of arbovirus co-transmission. Nonetheless, there are scarce data on clinical and virological findings during long-term follow–up. 23 laboratory-confirmed ZIKV and CHIIKV co-infected individuals were followed – up for two years. Short duration disease (ZIKV/CHIK CO short) was defined as ≤ 13 days post-onset (do) and prolonged (ZIKV/CHIK CO pro) as > 14 do. RT-PCR for detection of ZIKV and CHIKV RNA was performed in serial samples of sera, whole blood and urine longitudinally collected. Demographic findings included nine female and 14 male (mean age of 48.8 ± 12.7 years old). In 6 /23 (26.1%) individuals, ZIKV/CHIK CO presented as a short (mean: 8.3 ± 4.5 do; 95% CI: 3.563-13.1) and, 18/23 (78.26%) a prolonged duration disease (mean: 95.1 ± 78.96 do; 95% CI: 54.52-135.7). Comparison between groups showed a statistically significant difference ($p < 0.0001$). Viral clearance assessed by RNA detection showed that ZIKV clearance occurred in 11 ± 6.54 days (95% CI: 10.2 – 88.2) in ZIKV/CHIK CO short and, 49.2 ± 70.5 days (95% CI: 4.134-17.87), in the prolonged duration infection group ($p < 0.015$). CHIKV clearance extended for 31.8 ± 29.6 (95% CI: 16.03-47.59) and 10 ± 7.95 days (95% CI: 1.657-18.34) in the ZIKV/CHIK CO prolonged and short duration disease, respectively ($p < 0.05$). Arbovirus co-infections present as short and prolonged duration diseases. In the last group, co-infection delays both ZIKV and CHIKV clearance in serum or urine. Prolonged ZIKV/CHIK CO might contribute to progression to chronic ZIKV infection.

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CO-CIRCULATION AND CLINICAL MISDIAGNOSIS OF ZIKA, DENGUE, AND CHIKUNGUNYA LED TO UNDERESTIMATION OF THE 2015-2016 ZIKA EPIDEMIC IN THE AMERICAS

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During the 2015-2016 Zika epidemic, two other arboviruses - dengue and chikungunya - were also in circulation in many countries in the Americas. Published results from a clinical study in northeastern Brazil showed that clinical case definitions used throughout the region at this time were highly prone to misdiagnosis, due to imperfect sensitivity (all < 87%) and specificity (all < 79%). Given the importance of clinical surveillance data for informing estimates of the epidemic's dynamics, our aim was to produce revised estimates of the magnitude of the Zika epidemic in light of clinical misdiagnosis in areas with co-circulation of two or more of these viruses. Our approach was based on a series of equations that define fundamental mathematical relationships among the numbers of disease episodes attributed to each of these three viruses on the basis of case definitions and the numbers of disease episodes that truly were caused by each of these three viruses. Using these relationships, we identified combinations of diagnostic sensitivity and specificity that were compatible with weekly case reports of all three diseases at a national level from 2014-2017 across all PAHO countries. For each country, this yielded a probabilistic estimate of the weekly incidence of symptomatic cases that truly were caused by each of Zika, dengue, and chikungunya viruses. Of the nearly 8 million cases of dengue and chikungunya reported in the Americas in 2014-2017, we estimate that 319,730 (95% CI: -364,141-1,440,888) may have been caused by Zika virus (ZIKV) infection instead. This implies an 80% chance that at least some portion of reported dengue and chikungunya cases were caused by ZIKV infection. Across the region as a whole, these findings revise estimates of the Zika epidemic from 745,572 to 1,137,302 cases. The majority of these misdiagnoses come from countries with high incidence of all three viruses. These findings have significant implications for quantifying the magnitude and spatial distribution of ZIKV infections, which is of critical importance for projecting risk of future Zika epidemics in the Americas and for planning prospective cohort studies.

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VACCINATION FOR JAPANESE ENCEPHALITIS IN THE PHILIPPINES: A COST-EFFECTIVENESS ANALYSIS

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Japanese Encephalitis (JE) is a mosquito-borne viral infection causing a type of "brain fever," especially in children. JE kills up to 30% of victims and leaves up to half of survivors with permanent brain damage. In the Philippines, efforts are underway to provide a safe and efficacious live

attenuated JE vaccine (CD-JEV) to children through a national vaccination routine immunization (RI) strategy. To inform decision-making, the cost-effectiveness of JE vaccination was compared to a strategy of no vaccination. A Markov model was developed from the payer perspective to assess potential health outcomes and costs associated with introduction of CD-JEV, assuming 82% coverage and 93% efficacy. The model simulated a child from time of vaccination or no vaccination through five health states (no JE, acute JE, asymptomatic JE, post-acute JE, and death) over one-year cycles. Costs of illness ($859/acute care case), vaccines ($0.50/dose), and routine service delivery ($0.95/dose) were established via in-country clinical and financial records from local government programs and health facilities. Transition probabilities and incidence data were derived from literature with a base case incidence of 10.6/100,000. Disability weights were applied to consider quality of life, and costs and outcomes were discounted at 3%. One-way and probabilistic sensitivity analyses evaluated model uncertainty. Model results suggest that CD-JEV introduction is likely to be highly cost-effective compared to no vaccination when introduced via RI with a cost per life year saved of $182 (95% credible range [CR]: $46-$315). Cost-effectiveness ratios below per capita gross domestic product are often viewed favorably, and this value is only 6% of per capita gross domestic product. Vaccination was estimated to cost $1,233/averted JE case (95% CR: $383-$2544), $150/disability adjusted life year averted (95% CR: $55-$397) and $7,200/death averted (95% CR: $2,068-$16,418). In general, implementation of JE vaccination through a routine immunization strategy is predicted to improve health outcomes for the population and reduce long term costs due to JE cases averted.

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RAPID DIAGNOSIS OF CHIKUNGUNYA VIRUS BY MOLECULAR TECHNIQUES RT-PCR AND RT-LAMP IN FEBRILE PATIENTS FROM MACHALA, ECUADOR AND TUMBES, PERU

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Chikungunya, along with other tropical diseases such as dengue and zika, is a global level public health issue transmitted via bites from mosquitos of the genus *Aedes aegypti* and *A. albopictus.* Not all diagnostic laboratories have access to molecular testing based in PCR. The present work consisted of the implementation of the isothermal amplification technique "RT-LAMP" and its comparison to RT-PCR, the technique used in the diagnosis of chikungunya. For this analysis, 16 serum samples were taken from febrile patients suspected to have chikungunya infections in Machala, Ecuador and Tumbes, Peru. Serum samples were screened via RT-PCR for the chikungunya gene fragment viral glycoprotein E1 (401pb). Samples were sent for sequencing and virus strains were found to be homologous to Caribbean genotypes (99%) as well as, to a less degree, to those of Africa (94% Congo), India (93%) and Brazil (93%). RT-PCR revealed 5 positives cases (31.25%) while RT-LAMP detected 11 positive cases (68.75%) in the 16 serum samples analyzed. In conclusion, the technique RT-LAMP was revealed to be applicable in the diagnosis of serum samples of patients from the Ecuador-Peru region with symptoms of mosquito-borne viruses. This fast, sensitive and specific test can be rapidly implemented in any diagnostic laboratory thus improving said laboratory's capacity to detect viruses in patients.

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IMPACT OF JAPANESE ENCEPHALITIS VACCINE INTRODUCTION, UTTAR PRADESH, INDIA, 2006-2017

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Japanese encephalitis (JE) virus is the leading cause of viral neurological disease and disability across temperate and tropical zones of Asia. Several vaccines exist to prevent JE disease, including the live attenuated SA-14-14-2 Japanese encephalitis vaccine (CD-JEV) manufactured by the Chengdu Institute of Biological Products (CDIBP). Previous trials with CD-JEV have shown that it is safe and effective. India introduced CD-JEV in 2006, in selected states, and eventually expanded to national use in routine immunization (RI). Efforts to measure the impact of CD-JEV introduction in Uttar Pradesh (UP), India, one of the first states to introduce CD-JEV, was assessed by analyzing immunization data in comparison to serum and/or CSF IgM Elisa-confirmed JE disease in seven high-risk districts of UP. Vaccination data assessed included campaigns targeting children 1-15 years old, and RI targeting 9-12 month olds in these districts annually. Analysis showed that JE incidence dropped from 43/1,000,000 in 2010 to near 0 in 2011, and then varied from 15-7/1,000,000 in the period 2012-2016. JE vaccine RI coverage ranged 10-60% over this period. Campaigns in these seven districts in 2006 and 2010 reached >95% coverage and are likely responsible for most of the decline in disease incidence. Data analysis for three other district clusters that conducted single JE vaccination campaigns in 1-15 year olds in 2007, 2008, and 2009, reached over 90% coverage, followed by RI coverage between 22-60%. Analysis of immunization efforts in each of these three clusters failed to show an impact on disease, unlike the initial district cluster. However, linear regression analysis of JE incidence trends in all 36 districts demonstrated a significant decline overall (p=0.00001). We conclude that a single campaign with high coverage followed by low RI coverage is not sufficient to reduce JE occurrence without catch-up campaigns.

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*A*SSESSING THE IMPACT OF HOUSE ABANDONMENT ON *AEDES AEGYPTI*-TRANSMITTED DISEASE RISK TWO YEARS AFTER THE 2016 EARTHQUAKE IN BAHÍA DE CARÁQUEZ, ECUADOR

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In 2016, a devastating earthquake (magnitude 7.8) struck the province of Manabí in coastal Ecuador, resulting in severe destruction, morbidity and mortality. Following the disaster, the risk of *Aedes aegypti*-transmitted illnesses (e.g., dengue, chikungunya, Zika) increased due to damaged housing, increased water storage, increased exposure to infectious mosquito bites, and limited access to healthcare. Following the earthquake, a major epidemic of Zika fever occurred, with 85% of cases in Ecuador reported within 100 km of the earthquake epicenter. Zika is a global health concern because it can cause a spectrum of congenital

complications. Two years after the earthquake, many damaged homes have been abandoned at the edges of inhabited communities, and these areas do not receive mosquito control by the public health sector. We hypothesize that abandoned areas have greater vegetation cover and shade, and provide larval habitat and refuge for adult *Ae. aegypti* in 3 urban and 3 periurban sites in Bahía de Caráquez, Ecuador. Here, we examine the effect of the spatial distribution of abandoned homes in a post-disaster scenario, vegetation cover and neighborhood housing conditions on larval habitat, average number of mosquito larvae/pupae/adults per site, and the prevalence of arboviruses (identified by RT-PCR) in pools of adult *Ae. aegypti*. These findings improve our understanding of how abandoned sites following natural disasters may amplify human arbovirus infection risk, information that will inform interventions to reduce the risk of infectious diseases in affected communities.

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USE OF DISPERSION INDEX TO IDENTIFY KEY CONTAINERS RESPONSIBLE FOR *AEDES AEGYPTI* BREEDING IN SELECT COMMUNITIES OF GUATEMALA

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As part of the Zika Community Response (ZICORE) project in Guatemala, we quantified household containers that could become *Aedes aegypti* breeding sites in order to gather entomological data to target Zika prevention and control interventions. As the water supply fluctuates, households store water and often have non-useful containers outside where rain water also accumulates. In 2017, a round of pupal surveys was conducted in 32 Guatemalan communities. Water containers were inspected for *Aedes* larvae or pupae, which were counted when present. A dispersion index (DI) was used to determine if the sample size needed adjustment. A total of 821 households (HHs) were visited, 40% of which contained pupae (HH index). Use of DI allowed for basins and barrels to be identified as key breeding sites for *Aedes* in 18 out of the 32 communities visited. Small and medium containers kept in the front yards or backyards were identified as key breeding sites in 8 communities. Used tires kept outdoors were identified as key breeding sites in 3 communities. These data were used to guide the implementation of social and behavior change communication and community mobilization activities to reduce or eliminate key containers, such as mass disposal of used tires in targeted communities. In contrast to other indices, DI was used to effectively determine container productivity and identify key *Aedes* breeding sites that needed to be targetedand removed. Such data-driven vector control activities have the potential to increase cost effectiveness and impact in the reduction of Zika transmission.

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EVALUATION OF COMMUNITY-LEVEL VECTOR CONTROL ACTIVITIES AND *AEDES AEGYPTI* EGG DENSITY INDICES IN GUATEMALA

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The Zika Community Response (ZICORE) project in Guatemala aims to improve surveillance of the *Aedes* vector by using entomological data as the basis for low-cost community-level vector monitoring as well as social and behavior change interventions. In 2017, household-level *Aedes aegypti* breeding site monitoring was conducted in 44 communities. Ovitraps were installed in selected households and egg counts were recorded weekly. In these communities, 4,457 trap readings were positive out of 8,004 readings. A moving average was used to adjust for seasonality to enhance vector monitoring and identify communities of high entomological risk to be targeted for community-level clean-up campaigns. Utilizing weekly ovitrap monitoring results, the ZICORE project has implemented community-level campaigns to prevent *Aedes aegypti* breeding, such as: -Breeding sites eliminated in targeted communities in the 90th percentile or above for ovitrap egg counts. Activities included disposal of non-useful containers and scrubbing of sinks and useful containers as part of the ZICORE-VELITA (*Voltear, Eliminar, Limpiar y Tapar*) protocol, which describes in Spanish the steps to be taken with household items that have the potential to become mosquito breeding sites (in English: flip, eliminate, clean and cover). -Elimination of solid waste identified as potential *Aedes* breeding sites, including tires and non-useful containers of varying sizes. Included strong municipal government support, such as sponsored trash removal vehicles to dispose of waste. Communities below the 90th percentile for ovitrap egg counts did not receive intensified community-level interventions and will be analyzed as controls. This analysis is useful for assessing any correlation between community-led clean-up campaigns and *Aedes aegypti* egg counts in intervention versus control communities.

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URBANIZATION INCREASE THE POPULATION ABUNDANCE OF EARED DOVE (*ZENAIDA AURICULATA*), AMPLIFYING HOST OF ST. LOUIS ENCEPHALITIS VIRUS (SLEV, FLAVIVIRUS)

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Human-ecosystem interactions have changed in the last 10.000 years producing a greater environmental invasion and changes in the use of land cover for the exploitation of natural resources. Among the immediate environmental effects we can refer to global climate change, habitat alteration, changes in the assembly of species and their human interchange that promote the emergence of zoonotic diseases. In Argentina, SLEV emerged as a human pathogen since 2005 when a human encephalitis outbreak occurred in Córdoba city. Eared Dove (*Zenaida auriculata*) and *Culex quinquefasciatus* mosquito are the main host and vector species, respectively. The main goal of this study was to evaluate the effect of urbanization over the abundance and production of nests of Eared Dove. Using Landsat 8 satellite images of high-resolution, indexes related to urban landscape (i.e. indicators of built surface, vegetation and productivity, bodies of water, complexity and dominance of landscape elements) were obtained. Córdoba city area was classified into three levels of urbanization (high, medium and low). Abundance of Eared Dove and number of nests were recorded in fixed-width transects in 30 randomized sites in the city (10 for each urbanization level). The distribution of the abundance was analyzed by a Generalized Linear Model (GLM) with negative binomial distribution of errors, log link function. The statistical significance (α= 0.05) of the terms was determined through ANOVA. Built surface was the only tested variable that explained significantly the abundance of Eared Dove. On the other hand, the variable nests of Eared Dove was significantly explained by built surface, high vegetation and distance between low grasses. Our study reveals a positive effect of urbanization over the abundance of Eared Dove. Eared Dove represents the second most abundant bird species and produces 15 times more SLEV infectious mosquitoes than other bird species. The expansion and colonization of urban areas by Eared Dove can be one of the factors causing the emergence of SLEV in Argentina.

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FITNESS OF ZIKA VIRUS MUTATIONS CIRCULATING IN THE AMERICAS

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The recent Zika virus (ZIKV) epidemic spread throughout the Americas and infected millions of people causing severe fetal and neurological disease. The emergence of similar mosquito-borne RNA viruses like West Nile and chikungunya has been attributed to genetic adaptations to their environment, but the dramatic spread of ZIKV, and the origin of it's disease severity remain unexplained. While there have been recent publications describing the functional effects of ZIKV NS1 and prM mutations, there has yet to be a comprehensive study to determine the effects of genetic variants which arose during the ZIKV outbreak, and whether or not they alter viral fitness. Our preliminary data comparing replicative and competitive fitness between ZIKV isolates collected throughout the Americas demonstrated phenotypic variability, suggesting a genetic component. To further test this hypothesis, we reverse engineered 12 clade-specific ZIKV genetic mutations detected during the epidemic into a recent ZIKV isolate (PRVABC-59). We will evaluate the replicative fitness of each mutation across several relevant primary and continuous human, primate, and mosquito cell lines. In addition, we will use competitive fitness assays to detect subtle functional differences of individual mutations against the infectious clone containing the wild type sequence. Finally, we will infect *Aedes Aegypti* mosquitoes to detect mutations that influence vector competence and transmission. Our proposed system will provide insights into the genetic determinants of Zika virus disease and emergence. Moreover, the studies outlined here will provide a framework for evaluating functional virus evolution during disease outbreaks.

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ENABLING HEALTHCARE ACCESS FOR HEPATITIS C THROUGH COMMUNITY-BASED SCREENING AND LINKAGE TO CARE

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Although HIV has received great attention, a parallel epidemic - that of HCV - has been ignored until recently. Globally, HCV infects 71 million people and causes 400,000 deaths annually. New Orleans is currently suffering from a Hepatitis C epidemic comparable to regions with the highest prevalence of HCV worldwide. This disease disproportionally affects marginalized populations who lack healthcare access. With 80% of infected individuals unaware of their diagnosis, early detection is the primary barrier to eradicating this silent epidemic. This project seeks to address this shortcoming by bringing screening directly to the community, focusing on high-risk individuals. The aim of this project is to improve access to timely healthcare by linking HCV infected individuals to a network of specialist treatment. The study site includes community-based medical student run clinics in the city of New Orleans. The impact of our program will be presented based on outcome measures including detected HCV prevalence, linkage to care rates, and the number of patients successfully cured. Voluntary HCV screening using a rapid antibody test began in March 2015 and has now expanded to include eight homeless shelters, food pantries, and rehabilitation facilities across Orleans Parish. The patient-centered medical home model is utilized to coordinate care across multiple specialty services. Patient navigators guide our vulnerable population through the complex treatment cascade and maximize linkage to care. From March 2015 to April 2017, 245 (28%) of 861 patients screened have tested HCV positive. Of the 221 patients currently in our treatment cascade, 131 are RNA confirmed and 83 have received a hepatic ultrasound. Ten patients have begun treatment and four are cured of HCV. The most up-to-date results will be presented. Our program can provide "lessons learned" to the audience as a model for screening marginalized populations, and in addition, coordinating their care through a multi-step evaluation and treatment cascade. This model could be replicated elsewhere to improve healthcare access by bringing services directly to a community in need.

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COINFECTION OF PLACENTAS FROM CONGENITAL ZIKA VIRUS INFECTION WITH HUMAN CYTOMEGALOVIRUS IN BABIES WITH AND WITHOUT MICROCEPHALY

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Zika virus (ZIKV), a mosquito-borne flavivirus, caused the 2015-2016 epidemic linked to severe birth defects in Brazil and Central America, countries where over 95% of the population is seropositive for human cytomegalovirus (HCMV), a herpesvirus that also causes congenital infection and disease. Spread of maternal infection to the fetus can result in congenital ZIKV syndrome (CZS), a spectrum of neurological and neuromuscular defects including microcephaly. ZIKV RNA is detected in the brains, placenta and amniotic fluid of affected babies. Whether HCMV is a co-factor that could contribute to severe congenital anomalies has not been studied. We reported that Nicaraguan ZIKV strains infect cytotrophoblasts in cell columns, Hofbauer cells (fetal macrophages) in villus cores of anchoring villi from explants of first-trimester placentas and primary amniotic epithelial cells, suggesting routes of transmission. Here we examined placentas from 19 cases of congenital ZIKV infection from the Nicaraguan Zika Positives Study of pregnant women confirmed as rRT-PCR ZIKV+ by the Ministry of Health or with positive serology and history of Zika symptoms during pregnancy. Fetal diagnosis was confirmed at delivery and follow-up of infants is ongoing. Over 100 biopsy specimens from chorionic villi, basal and parietal decidua and amniochorionic membranes were examined for ZIKV antigens and pathology. Fifty-five specimens were also immunostained for HCMV antigens. Immunohistochemistry detected ZIKV NS3 protein in cells in basal and parietal decidua, the chorion and occasionally villus cores. HCMV antigens were also found in 5 of 19 placentas, including 2 of 6 cases of microcephaly, in cells of the amniotic membranes, chorion and basal and parietal decidua. Although villitis was mostly absent, indicators of chronic vascular underperfusion, Tenney-Parker changes and villus aggregation were found. Increased Hofbauer cell densities and villus edema were observed in placentas from cases of microcephaly. Our results suggest that ZIKV and HCMV coinfection should be diagnosed and the contributions of each virus to pathology and birth defects determined.

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GROWTH AND DEVELOPMENTAL OUTCOMES OF CONGENITAL ZIKA INFECTION DURING FOLLOW-UP OF A CHILD COHORT FROM SALVADOR, BRAZIL

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A large proportion of children were exposed to Zika virus (ZIKV) during the 2015 epidemic in Northeast Brazil yet did not develop severe birth defects, such as microcephaly. To determine whether these children have adverse growth and development outcomes as they complete their 2nd year of life, we recruited a cohort of children who were born between October 2015 and January 2016 from a maternity hospital of Salvador, Brazil. We evaluated growth, development and neurological outcomes by performing anthropometric measurement, Bayley Scales of Infant Development III and Hammersmith Infant Neurological Exam (HINE), respectively. Children received clinical evaluations that included audiometry (OAE, ABBR), ophthalmological exam, and when indicated, examinations with a pediatric neurologist and imaging studies. The Blockade of Binding (BoB) assay, which uses the ZIKV35 anti-NS1 monoclonal antibody, was performed to evaluate ZIKV exposure. Among the 513 children who were born during the study period, 55 (11%) had microcephaly. Of the 458 children who were born without microcephaly, we have performed outcome measurements of 208 (45%) and have completed BoB analysis to date for 72(35%). Of these 72 children, 28(39%) had serologic evidence of ZIKV exposure. These children with serologic evidence of ZIKV exposure had an increased proportion of ophthalmologic (17% vs. 3%, p=0.105) and auditory abnormalities (12% vs. 0%, p=0.098) in comparison to the 44 without evidence of exposure, albeit these differences were not significant. We are completing follow-up of the cohort and the serological evaluations to obtain more precise estimates. Although our analyses are preliminary, these findings suggest that children who were exposed to the virus who were not identified to have defects at birth may have significant disabilities as they enter the 2nd year of life.

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KINETICS OF ANTI ZIKA-IGG ANTIBODIES DURING FOLLOW-UP OF INFANTS EXPOSED TO ZIKA VIRUS *IN UTERO*

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Zika virus (ZIKV) infection during pregnancy is a cause of congenital microcephaly, which involves severe fetal brain defects and has also been associated with other adverse birth outcomes. Currently there is no validated laboratory test to detect *in utero* exposure to ZIKV in infants. Detection of IgG antibodies in infants beyond 8-months could be a proxy for ZIKV exposure *in utero*. In this study, infants who were born during a Zika outbreak (from October 1, 2015 to January 31, 2016) in Salvador, Brazil, were evaluated by ZIKV nonstructural protein 1 (NS1)-based blockade-of-binding (BOB) ELISA. The study population encompassed 234 mother-infant pairs from the General Hospital Roberto Santos (HGRS). Of 234 infants, there were follow-up longitudinal samples for 55 infants (23%). These infant longitudinal samples were further classified into four groups based on Microcephaly (MC), IgM ELISA and PCR-confirmation status at birth: MC^+ and IgM^+/PCR^+ (15 samples), MC^- and IgM^+/PCR^+ (4 samples), MC^+ and IgM^-/PCR^- (9 samples), MC^- and IgM^-/PCR^- (27 samples). The ZIKV infection status of corresponding mother samples was confirmed by Plaque Reduction Neutralization Test (PRNT) and NS1 BOB ELISA. We observed 80% positivity rate in the NS1 BOB assay for infants in the MC^+ and IgM^+/PCR^+ group while only 44% of the infants scored positive in the MC^+ and IgM^-/PCR^- group. Interestingly, the positivity rate of 55% in the MC^- and IgM^-/PCR^- group infants indicates the possibility of ZIKV exposure in these infants at a later time-point after birth. We observed that overall duration of detectable IgG antibodies against ZIKV NS1 did not wane even after disappearance of maternal antibodies (6-9 months). Our survey of infants by NS1 BOB ELISA in Salvador could help in rigorous follow-up of infants born to women with potential exposure to ZIKV. In addition, screening by NS1 BOB assay will also inform us about those cases where infants were exposed to ZIKV at later time-points. Therefore, this study helps in understanding the long-term implications of ZIKV outbreak in Salvador and also in developing appropriate care strategies for affected children.

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IMPLICATIONS OF CONGENITALLY ACQUIRED AND EARLY EXPOSURE TO ZIKA VIRUS INFECTION ON CHILD NEURODEVELOPMENT

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The full spectrum of adverse developmental outcomes in ZIKV-exposed fetuses and infants is not fully understood. We are monitoring a prospective pregnancy cohort in León, Nicaragua to design algorithms for ZIKV surveillance in pregnant women and to better understand outcomes. Our cohort of 253 pregnant women were recruited Jan-Apr, 2017; all have given birth. Outcomes include 3 cases of documented microcephaly, 1 of anencephaly, and 3 stillbirths. At each parent-infant visit, at 3, 6, 12, & 18 months, staff collect demographic, socioeconomic, and medical data via parental questionnaires and record infant anthropometric measurements. They assess infant neurocognitive development using the Mullen Scales of Early Learning (MSEL), a validated neurodevelopmental assessment, translated into Nicaraguan Spanish. The 5 Scales assess gross & fine motor, visual reception, and receptive & expressive language. The Mullen standardized Cognitive Composite combines standardized scores from the last 4 scales. The infants will also have formal ophthalmologic & otoacoustic tests. Every 3 months, samples of the infants' blood are assayed for maternal antibody titers & incident ZIKV infections. In lab testing on the first 120 cord blood specimens, 75 (62.5%) were positive on ZIKV neutralization tests, supporting ZIKV-exposure during pregnancy. We are still compiling results of analyses of ZIKV infection status and presence/magnitude of neurodevelopmental deficits, including visual & auditory results, of these exposed/infected and unexposed infants. We use multivariate regression and analyses to estimate the mean differences in standardized T-scores for each of the Mullen Scales and the MSEL Composite between exposed/infected and unexposed infants, and to estimate the associations between the groups & the odds of scoring below average on the Composite. For multivariable analyses, we use generalized estimating equations to account for repeated observations of the same infants. The results of this prospective analysis will describe the impact of ZIKV infection/exposure on infant neurodevelopment in a way not yet done to date.

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EXPLORING THE DIFFERENCES IN IMMUNE RESPONSES BETWEEN SURVIVORS AND NONSURVIVORS OF *ZAIRE EBOLA VIRUS* CHALLENGE IN CYNOMOLGUS MACAQUES VACCINATED WITH A RECOMBINANT SUBUNIT BASED VACCINE

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In 2014 the *Zaire ebolavirus* outbreak in West Africa highlighted the urgent need for an efficacious vaccine. Now, nearly two years after its end, several vaccine candidates are still under preclinical and clinical development. These candidates use a variety of different platforms and the search for markers that correlate with protection is of great interest. Our lab is developing a *Zaire ebolavirus* vaccine based on insect cell expressed recombinant protein subunits. We are currently testing our vaccine in cynomolgus macaques, in which formulations containing the surface glycoprotein (GP) either alone, or in combination with the matrix proteins VP24 and VP40 are protective. Throughout the course of vaccination and challenge, blood samples are collected, and this collection of samples from survivors and nonsurvivors within the same vaccination group gives us a unique opportunity to thoroughly examine immune response differences between animals that are fully protected against Ebola virus disease (EVD) and those that are not. To understand these differences, we have taken PBMCs, stimulated them *ex vivo* with either homologous whole antigen or peptide pools and analyzed cytokine production from their CD4+ and CD8+ T cells and expression of surface activation markers using flow cytometry, ELISpot assays and luminex based multiplex immunoassays (MIA). Collected data have shown that our vaccine candidate primarily elicits a CD4+ T cell response, as indicated by the secretion of cytokines such as IL-4 and TNF-α by these cells upon antigen and peptide pool stimulation. Currently we are extending the analysis of the data collected by these methods after antigenic stimulation of PBMCs from immunized animals to identify differences in the responses between survivors and nonsurvivors to help us define potential correlates of protection.

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STANDARDIZATION OF A FOUR-PLEX FORMAT REVERSE TRANSCRIPTION REAL TIME PCR FOR THE SIMULTANEOUS DETECTION OF ZIKA, CHIKUNGUNYA, DENGUE AND YELLOW FEVER IN PERU

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Mosquitos transmitted diseases have been increasing in the world in the last years. Peru due to its tropical location is one of the most affected areas, where infections by Zika, Chikungunya, Dengue and Yellow Fever viruses are the main problem. Several methods to diagnose these four diseases are currently used in the country such as ELISA, culture and single RT-PCR. However, these methods are time consuming, costly and expensive. A platform that can detect multiple vector-borne viruses at once is required. We put together 15 primers and 7 probes in a single reaction to build a qRT-PCR to detect ZIKV, CHIKV, DENV and YFV simultaneously. We collected 640 serum samples which were previously tested using the RT-PCR (gold standard) individually following the method indicated by the CDC-US. The samples were also tested using the ZCDY RT-PCR multiplex and the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were obtained. Cohen's Kappa was determined to measure the concordance between the two methods. Our ZCDY RT-PCR method could detect 282 positives to one of the four etiologies from 640 samples analyzed. These results were also compared with the single RT-PCR routinely used (CDC-US) at the CNSP/INS Peru and it showed a sensitivity of 98.61, a specificity of 99.69, a PPV of 99.80 and a NPV of 97.90. The Cohen's Kappa was 0.980 in a 95% C.I. of 0.966 - 0.994. Based on these results, the ZCDY qRT-PCR method showed to be a powerful molecular tool for rapid, specific and simultaneous detection of etiologies associated with acute febrile syndromes that allows a proper and timely diagnosis of these important infectious diseases.

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DEVELOPMENT OF THERMOSTABLE FILOVIRUS VACCINES BASED ON RECOMBINANT INSECT CELL EXPRESSED SUBUNITS

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Ebola Virus Disease (EVD) is the most prominent example of filovirus disease. Transmission from wild animals into the human population typically causes outbreaks of limited scale in endemic regions, therefore other public health threats usually garner more attention. This changed when the *Zaire Ebolavirus* (EBOV) outbreak in several West African countries claimed more than 11,000 lives and revealed the true epidemic potential that filovirus infections may possess. Despite significant progress with the clinical development of several EBOV vaccine candidates during and after the West African outbreak, no broadly protective vaccines targeting other filoviruses have received regulatory approval. We are developing a trivalent vaccine based on recombinant filovirus glycoproteins (GP) from EBOV, Marburg virus (MARV) and *Sudan ebolavirus* (SUDV) produced using the *Drosophila* S2 cell expression system. The immunogenicity of highly purified recombinant subunits alone or in combinations was evaluated in mice and guinea pigs resulting in strong antigen-specific IgG titers as well as cell mediated immune responses after two or three immunizations. Recombinant vaccine candidates were tested successfully for protection in the mouse and guinea pig models of EBOV and MARV. Studies in two species of non-human primates demonstrate that vaccination with formulations of recombinant EBOV subunits and an emulsion-based adjuvant consistently produces high IgG titers. Vaccination further prevents viremia subsequent to EBOV challenge and protects animals from EVD. Thus we have defined a recombinant subunit EBOV vaccine candidate that forms the basis for further pre-clinical and clinical development of a trivalent filovirus vaccine. Ongoing formulation optimization in our laboratory focuses on thermostabilization of recombinant subunits and adjustment of dosing schedules following promising efficacy testing against EBOV of a single shot recombinant subunit vaccine. These results should enable clinical development of safe and efficacious, field-deployable vaccine candidates for protection against Ebola and Marburg Virus Disease.

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SPATIOTEMPORAL HETEROGENEITY IN THE DISTRIBUTION OF CHIKUNGUNYA AND ZIKA VIRUS CASE INCIDENCES AND RISK FACTORS DURING THEIR EPIDEMICS IN BARRANQUILLA (COLOMBIA) BETWEEN 2014 AND 2016

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The chikungunya and Zika viruses have recently emerged as global infections with consequential disability adjusted life years (DALYs) and economic burden. This study aimed to explore the spatiotemporal heterogeneity in the occurrence of chikungunya (CHIKV) and Zika (ZIKV) virus outbreaks in Barranquilla, Colombia during 2014 and 2016 and

to identify socioeconomic, demographic and environmental factors that influenced their distribution. We investigated the spatial distribution of CHIKV and ZIKV incidence across Barranquilla neighbourhoods and explored the potential for clustering. Incidence data were fitted using multiple Bayesian Poisson models based on a suite of explanatory variables as potential risk factors and multiple options for random effects with: i) no random effects, ii) independent random effects, and iii) spatially correlated random effects, implemented through a conditional autoregressive model (CAR). A best-fitted model was used to analyse the case incidence risk for both CHIKV and ZIKV throughout Barranquilla and to identify risk factors during their epidemics. Neighbourhoods in the northern region of Barranquilla, which also houses the highest socioeconomic strata, were hotspots for the outbreaks of CHIKV and ZIKV. Additional hotspots occurred in the southwestern and central regions of the CHIKV and ZIKV outbreaks, respectively. Multivariate CAR models identified strong evidence that higher socioeconomic strata and residing in detached houses were risk factors for ZIKV case incidence. This study provides an evidence-based framework that public health programmes can use to efficiently target specific regions of Barranquilla that are likely to drive future outbreaks of CHIKV and ZIKV infections. Furthermore, we clearly showed that living in high socioeconomic strata was a risk factor for ZIKV case incidence. These novel findings challenge the logic that these infections are driven by social vulnerability—such as low socioeconomic strata, high residential densities, and poor education—and merits further study both in Barranquilla and throughout the tropical and subtropical regions of the world.

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TRENDS IN INFLUENZA AND OTHER RESPIRATORY VIRUSES IN SOUTHERN PUERTO RICO, 2012-2017

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Influenza seasonality has been described in temperate countries, where influenza activity typically peaks between November and March in the Northern Hemisphere, and between April and September in the Southern Hemisphere. While peaks in influenza activity may be observed in tropical regions, increased surveillance efforts are needed to better define seasonality. We used data from the Sentinel Enhanced Dengue Surveillance System (SEDSS) to describe trends in influenza and other respiratory viruses in southern Puerto Rico during May 2012 to December 2017. Patients presenting to the emergency department at Saint Luke's Episcopal Hospital in Ponce and Guayama, Puerto Rico with onset of fever ≤7 days of presentation were eligible. Nasal swabs were tested for influenza A and B viruses, adenoviruses, respiratory syncytial virus (RSV), coronaviruses, parainfluenza viruses and metapneumovirus by RT-PCR. To identify months with the largest relative frequency of cases in comparison with other times throughout the year, we calculated a monthly case proportion, defined as the number of test-positive cases reported in a given month as a percentage of the total number of cases for each virus reported in the calendar year. We defined a month as having viral activity if the cases represented a fixed threshold of 10% or more of total reported positive cases in the year in two or more years from 2012 through 2017. Among 19,228 participants, 24% were recruited in 2012-2013, 24% in 2014-2015 and 53% in 2016-2017. Overall, 26% of specimens were positive for any respiratory virus: 9% influenza A, 4% influenza B, 4% adenovirus, 4% RSV, and 6% other. In 2012-2013, Influenza A and B viruses occurred year-round showing no clear seasonality. In 2014-2017, influenza activity was identified from December through February. Adenoviruses had increased activity in June through August, and RSV October through December. Understanding the trends in respiratory virus circulation is helpful to target prevention and control strategies, such as vaccination. Current US recommendations are for influenza vaccination before the end of October, which may be appropriate for Puerto Rico.

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A RECOMBINANT VESICULAR STOMATITIS VIRUS EXPRESSING THE JUNIN VIRUS GLYCOPROTEIN PROTECTS GUINEA PIGS FROM LETHAL JUNIN VIRUS CHALLENGE

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Junin virus (JUNV) is a highly virulent arenavirus and the causative agent of Argentine hemorrhagic fever (AHF). It is considered a significant biodefense concern and NIAID category A pathogen due to its potential for aerosol transmission and mortality rates upwards of 30%. Currently, a single live-attenuated vaccine (Candid #1) is available for prevention of AHF in Argentina, however, this vaccine has a number of significant limitations which preclude it from receiving Food and Drug Administration (FDA) approval in the United States. Specifically, Candid #1 retains a low level of neurotropism and has an instable attenuated phenotype, with a single in-vivo passage yielding isolates up to 100-fold more virulent. In this study, we look at developing a vaccine alternative which overcomes these limitations and has promise for FDA licensure as a biodefense vaccine. Vesicular stomatitis virus (VSV) based vaccines against hemorrhagic fever viruses have shown promise as demonstrated by a recent successful Phase III clinical trial against Ebola virus in Guinea. VSV infection is typically asymptomatic or causes mild disease in humans and has low seroprevalence in human populations making it an ideal vaccine platform. In this study, a recombinant VSV expressing the JUNV surface glycoprotein (rVSVΔG-JUNVGP) was cloned, recovered, and characterized. Two groups of 6 guinea pigs each received a prime dose of rVSVΔG-JUNVGP thirty-five days before lethal JUNV challenge. One group received a boost dose fourteen days before challenge. Survival for animals receiving the prime versus prime-boost injection was 17% and 83%, respectively. Importantly, none of the surviving animals had detectable viremia at days 7, 14, or 35. No evidence of JUNV was found in the tissues of survivors including the liver, spleen, or brain at the study end. Additionally, no significant lesions or viral antigen was observed in the liver, spleen, or brain of surviving animals via histology.

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DIAGNOSTIC NEEDS FOR LASSA FEVER OUTBREAK DETECTION, CLINICAL CARE, AND VACCINE DEVELOPMENT

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Lassa fever, caused by arenavirus Lassa virus (LASV), is an acute viral hemorrhagic disease that affects up to 300,000 individuals and causes up to 5,000 deaths per year in Western Africa. There are at least 4 geographically distinct lineages of LASV circulating in the region. Early detection of LASV infection is difficult as the clinical course can range from 2-21 days, with 80% of those infected having asymptomatic or mild disease, and the majority of patients presenting at a health center already progressed to severe stage. Currently available LASV diagnostic methods are difficult to operationalize in low resource health centers and may be less sensitive to detecting all known or emerging LASV strains. To understand and prioritize the diagnostic gaps, we conducted a landscape and developed use cases for: 1) diagnostics for outbreak and non-outbreak clinical care in low resource hospitals and clinics, and 2) diagnostic support for surveillance and vaccine development in LASV-affected countries. In general, there is a lack of fully validated and regulated commercial assays for LASV, with many of the tests unable to detect all LASV lineages (pan-LASV). For outbreak response, diagnostics are needed for point-of-care (POC) and near-patient testing that enable pan-LASV pathogen detection as well as differentiation from other endemic causes of fever. POC and near-patient nucleic acid tests (NATs) are critical for rapid diagnosis, with a secondary role of immunoassays (IAs) for community triage; these tests

are also important for non-outbreak clinical care. For surveillance and vaccine development, pan-LASV detection is important for understanding LASV incidence and prevalence, especially for vaccine clinical trials. The 2017-2018 Lassa outbreak in Nigeria has clearly identified diagnostics needed for: 1) POC and near-patient NATs for rapid detection of pan-LASV and differentiation from other fever-causing pathogens, 2) improved IAs to support surveillance and vaccine development, and 3) POC IAs for community-based screening and triage.

1669

ISOLATION OF CHIKUNGUNYA VIRUS FROM HUMANS IN CHIAPAS, MEXICO

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Chikungunya virus (CHIKV) is an emerging arthropod-borne virus introduced into the Americas in 2013. CHIKV is transmitted to humans by *Aedes aegypti* and *Ae. albopictus* mosquitoes, causing fever and severe arthralgia. In Mexico, human cases of CHIKV have been reported since 2014. However, part of CHIKV outbreaks has not been described due to the lack of diagnostic assays. An outbreak of acute undifferentiated fever occurred from July to August 2015 in two localities in the central valley region of Chiapas, Mexico. Seventy-four human sera samples were collected from patients experienced fever, arthralgia, and headache. Sera samples were tested by cell culture on C6/36 cells and immunofluorescence assays. Seventeen out of seventy-four samples (22.9%) reacted against only CHIKV antibody and RNA extracted from the cell supernatants was positive for a generic reverse transcription polymerase chain reaction (RT-PCR) for Alphaviruses, and further identified as CHIKV by sequencing. Analysis of the envelope-1 gene clustered the CHIKV isolates in the Asian lineage. This study reports by the first time the presence of CHIKV in the central valley of Chiapas, Mexico.

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GENOMIC EPIDEMIOLOGY OF RABIES VIRUS IN THE EASTERN UNITED STATES

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Rabies virus (RABV) remains enzootic in the United States in wild mammals, with distinct variants in raccoons, skunks, foxes, and bats. Passive surveillance of RABV is conducted largely by state laboratories and seeks to monitor and mitigate rabies spillover into humans, domestic animals, and new host species. RABV variant information is used to monitor regional transmission dynamics and shifts to new hosts, and informs treatment management for animal exposures, but variant typing or full genome sequencing is not regularly performed. We will describe current rabies virus diversity, and introduce a new training partnership between MA DPH and the Broad to enhance laboratory sequencing capabilities, using rabies as a training set. Over twenty participants from seven state labs attended the training workshops in 2017-18 and generated over 80 new RABV genomes collected from eight mammal species. These sequences reveal new RABV subclades structured by region as well as individual but unsustained cross-species transmission. Expanded laboratory sequencing in state public health labs open new opportunities to establish coordinated networks for genomic surveillance, with immediate application for rabies monitoring in the U.S.

1671

ISOLATION OF BACTERIOPHAGES FROM THE PERUVIAN AMAZON RIVER BASIN AGAINST CLINICALLY RELEVANT MULTI-DRUG RESISTANT (MDR) *KLEBSIELLA PNEUMONIA* IN SUPPORT OF PHAGE THERAPEUTIC DEVELOPMENT

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Bacteriophages kill bacteria in a specific manner and were recently used in the successful treatment of a moribund patient suffering from a systemic MDR *A. baumannii* infection in the U.S., using personalized cocktails via a library-to-cocktail approach. The continued advancement of phage therapeutics is dependent on the expansion of diverse lytic phage libraries against clinically relevant bacteria. Here we harvested phages from the Amazonian River Basin in Iquitos, Peru, using local MDR *K. pneumoniae* (KP) clinical isolates, and interrogated their host-ranges against a larger panel of MDR KP clinical strains. 10 clinical MDR KP strains with different resistance profiles; isolated from hospitals in Iquitos from 2011 to 2017, were used to isolate phages from local sewage. Phage-rich water (300 ml) from a sewage canal was mixed with 3% w/v TSB-powder, inoculated with 5 different exponential MDR KP strains, and grown overnight at 37°C shaking. Two such cultures were generated totaling 10 MDR KP strains used for phage isolation. Centrifuged supernatants were sterile filtered and serial dilutions spotted onto individual lawns of the 5 MDR KP isolates and incubated at 37°C overnight. Agar plugs of well isolated plaques were re-suspended in PBS and filter sterilized. Preliminary host-ranges were defined using similar spot-plates with the 10 original host MDR KP strains, and an additional 10 MDR KP isolates. 10 total KP phages were isolated using the two phage harvesting cultures. These phages covered 8 of the 20 total MDR KP strains. 8 of the 10 phages only infected the host strain on which each was isolated. 2 of the 10 phages showed broader activity: KP07ϕ1 formed plaques on 7 of the 20 MDR KP strains, and KP07ϕ2 formed plaques on 3. In this study we readily isolated both "narrow" and "broad" host-range phages against MDR KP clinical isolates from a remote source. Isolating diverse phages with unique host ranges is critical for the development of a robust phage library capable of supporting personalized therapeutic cocktails. Our initial results justify ongoing expansion of geographically broad and diverse phage libraries against MDR bacterial pathogens.

1672

CYTOMEGALOVIRUS-RELATED CHANGES IN T CELL PHENOTYPE ARE ASSOCIATED WITH REDUCED VACCINE RESPONSES IN YOUNG ADULTS IN THE UK AND SENEGAL

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During concurrent Phase I clinical trials of viral-vectored Ebola vaccine candidates in healthy young adults (aged 18-50 years) in the UK (n=16) and Senegal (n=40), we observed significantly reduced humoral responses in Senegalese vaccinees compared with the UK cohort. Although often noted that vaccine responses are reduced in developing countries, there are very few studies that investigate the potential mechanisms underlying this phenomenon. A higher pathogen burden, particularly with chronically infecting pathogens, may be a factor associated with dysregulated or suppressed immune responses. Volunteers were vaccinated with a chimpanzee adenovirus expressing Zaire Ebola glycoprotein (ChAd3-

EBO Z) and boosted one week later with modified vaccinia virus Ankara, also expressing Zaire Ebola glycoprotein (MVA-EBO Z). Using a multiplex assay, we assessed the serostatus of individuals in each cohort for 18 different pathogens that cause chronic, latent or repeated infections. All of the Senegalese and 50% of the UK cohort were positive for CMV IgG. CMV seropositivity was associated with significant diferences in the global T cell repertoire with a shift towards late-differentiated memory T cells expressing CD57 and killer cell lectin-like receptor G1 (KLRG1). In CMV+ individuals in the UK $CD57^{+}KLRG1^{+}$ cells formed 2.5% of the total $CD4^{+}$ subset and 16.9% of the total $CD8^{+}$ subset compared to just 0.06% of $CD4^{+}$ T cells and 4.0% of $CD8^{+}$ T cells in CMV- individuals. T cell repertoires in Senegalese individuals were comparable to UK CMV+ individuals. The proportion of $CD57^{+}KLRG1^{+}$ $CD4^{+}$ T cells was associated with CMV IgG titres in both cohorts. These populations were not associated with any other pathogen tested and negatively correlated with vaccine responses in both cohorts. This study suggests that CMV, which has previously been associated with immunosenescence and reduced vaccine immunogenicity in elderly populations, can impact vaccine responses in young adults and may have a particularly marked impact in developing countries where CMV seropositivity is almost universal.

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A GLOBAL SUCCESS STORY: THE INCREDIBLE DECLINE OF POLIOMYELITIS INCIDENCE, PREVALENCE, AND MORTALITY. RESULTS FROM THE GLOBAL BURDEN OF DISEASE STUDY 2017

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Wild poliovirus continues to circulate in only three countries globally, representing an enormous decrease in its burden due to a global effort to eradicate the disease. The Global Burden of Diseases, Injuries, and Risk Factors Study 2017 (GBD 2017) is a systematic, scientific effort to measure health loss globally and reintroduced poliomyelitis as a cause of death and disability. We estimated the historical incidence and mortality due to poliovirus from 1980 to 2017 including the lifelong prevalence of paralysis and disability. Acute paralysis due to poliovirus incidence was modeled using notification and surveillance data. We used a cohort model to track the prevalence of acute and permanent paralysis due to poliomyelitis as well as of post-polio syndrome for every geography, both sexes, and all ages. We estimated 4,430,000 prevalent cases of paralytic polio in 1990 (95% Uncertainty Interval [UI] 3,022,000-5,899,000). We estimated that there was a 99% decline in poliomyelitis incidence between 1980 and 2017. Based on a meta-analysis of the case fatality ratio of acute poliomyelitis, we estimated that there were 5,020 (95% UI 3,300-7,300) deaths due to poliomyelitis in 1990. Lifelong disability due to poliomyelitis represents a significant burden of disease, accounting for 1,611,000 years lived with disability (YLDs) in 1990 (95% UI 980,000-2,372,000) and 962,000 YLDs in 2017 (95% UI 589,000-1,394,000). Global eradication of wild poliovirus may be within reach and will represent an enormous public health achievement, particularly when viewing its significant historical burden.

1674

FRUIT BAT ECTOPARASITES OF BUNDIBUGYO DISTRICT, UGANDA HOST DIVERSE RHABDOVIRUSES: IMPLICATIONS FOR VECTORBORNE TRANSMISSION OF "BAT-ASSOCIATED" VIRUSES

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Bats are the natural reservoir for diverse, highly virulent emerging (-) ssRNA viral pathogens such as Rabies virus, Marburg virus, and Nipah virus. The role that ectoparasites play in the transmission and maintenance of bat viruses is poorly understood, but the recent discovery of a bat fly rhabdovirus related to "bat-associated" viruses of the Ledantevirus genus suggests that ectoparasites may play a role. The relationship of Kanyawara virus to the eponymous Le Dantec virus, first isolated in Senegal from the serum of a patient with fever and hepatospenomegaly, suggests that further research into the diversity of Ledanteviruses is warranted. As part of a study of the ecology of emerging bat viruses in Bundibugyo District, Uganda we collected Nycteribiid bat flies - highly specialized hematophagous dipterans - from Myonycteris sp. fruit bats. We used next-generation sequencing to explore the diversity of bat fly rhabdoviruses in an emerging infectious disease hot spot. We identified diverse viruses that dramatically expand the known diversity of bat fly rhabdoviruses, and provide insight into the relationship between bat ectoparasite rhabdoviruses and "bat-associated" viruses.

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DETERMINING THE EFFICACY, SAFETY, AND SUITABILITY OF DISINFECTANTS TO PREVENT EMERGING INFECTIOUS DISEASE TRANSMISSION

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The scale of the 2014-2017 West African Ebola Virus Disease outbreak overwhelmed international response capacity. This led to inconsistencies in international guidance documents, particularly around chlorine disinfection of surfaces and hands to prevent ongoing transmission, which lacked a basis in evidence. To provide the necessary evidence-base for international disinfection recommendations, which in general recommend the use of 0.5% chlorine solution to disinfect non-living things (e.g. surfaces, dead bodies) and 0.05% solution to disinfect living things (e.g. hands), three research strands were conducted: 1) impacts of source chlorine chemistry of the four chlorine compounds used in Ebola contexts (HTH, NaDCC, NaOCl, gNaOCl); 2) efficacy of surface cleaning recommendations on stainless steel, nitrile, and heavy duty tarp surfaces using the Ebola surrogate bacteriophage Phi6; and, 3) safety and efficacy of the recommendation to use 0.05% chlorine solutions for community handwashing, also using Phi6 on hands. Strand 1 research found source chlorine compound chemistry impacts chlorine solution shelf-life (4 hours-30 days) and testing of chlorine solutions with one reliable test strip or portable iodimetric titration is recommended to ensure solution accuracy. Strand 2 research found surface cleaning with 0.5% chlorine solutions with a 15-minute exposure time is efficacious at reducing transmission risk across all surface types tested. Strand 3 research found community handwashing with chlorine solutions is as safe and efficacious as handwashing with soap and water or sanitizer, and offers a benefit of reducing pathogens in rinse water stored in buckets. Across all studies, the source chlorine compound calcium hypochlorite performed particularly well. The research was successful at providing information to align inconsistent international guidelines. Further research is needed to proactively establish the efficacy, safety, and suitability of disinfectants and sterilizers for the seven viral pathogens considered likely to cause severe outbreaks with few/no medical countermeasures.

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MAPPING ROTAVIRUS DIARRHEA IN CHILDREN AT THE 5 X 5 KM SCALE ACROSS AFRICA, 2000 - 2016

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Rotavirus is a ubiquitous diarrheal pathogen and is the leading cause of diarrhea mortality across the African continent. The 2016 GBD Study estimates that rotavirus was responsible for approximately 129,000

deaths in sub-Saharan Africa among children under 5 years old in 2016. By integrating a Bayesian geostatistical mapping effort powered by over 191 household-level surveys with the 2016 GBD Study, we have created, for the first time, high-resolution (5x5km) estimates of the prevalence, incidence, and mortality associated with rotavirus across the African continent from 2000-2016. Diarrhea mortality has decreased over the past 16 years for a number of reasons, and countries that have introduced the rotavirus vaccine have seen additional decline. However, health gains have not been equitably distributed between within countries. For example, the rotavirus mortality rate decreased by over 85% in several first administrative subdivisions in Angola from 2000 to 2016, whereas countries such as Swaziland saw a limited decrease in burden during that time period. Moreover, our results highlight inequalities within countries. For example, Nigeria observed the largest within country variation, with mortality rates nearly six times greater in the country's north eastern region compared the southern coast. The continued reduction of rotavirus diarrhea burden in Africa hinges upon addressing local inequalities by directing resources to the areas where the largest gaps remain. Estimates at such a granular scale are imperative for accurately targeting interventions and policies in areas that may otherwise be masked by maps that aggregate to the country or first administrative subdivision level.

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ASSOCIATION OF ADENOVIRUSES 40-41 AND AICHIVIRUS TO DIARRHEA IN MEDICALLY-ATTENDED CHILDREN IN PERU

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Diarrheal disease is still a leading cause of mortality among children under five. The disease is caused by a variety of agents including bacteria, parasites, and virus; but frequently no etiological agent is identified, suggesting that other viral agents may be involved. Adenovirus and aichivirus has been reported as etiology of diarrhea but its attribution to the diarrhea in children is limited. A case-control study was conducted to evaluate the association of human adenovirus 40-41, and aichivirus with acute gastroenteritis in children younger than five years of age seeking for medical attention at the national children's hospital in Peru following universal Rotarix™ vaccine implementation. Stool samples were analyzed using qPCR. Positive samples were tested for adenovirus 40-41 by conventional PCR while aichivirus positive samples were amplified by conventional PCR and sequenced. Human adenovirus 40 was present in 2.44% (4/164) of cases and 0.61% (1/164) of controls, while Adenovirus 41 was present only among cases (6.71%, 11/164). The OR for adenovirus 40-41 was 16.41 (95% CI: 2.145-36.86). Aichivirus was present in 2.44% (4/164) of cases and in 1.23 (2/164) of controls (OR=2.03, 95% CI:0.37-11.21); however, all aichivirus qPCR positive samples were negative by conventional PCR. Human adenoviruses 40-41 are an important cause of diarrhea among children with medically-attended diarrhea in Peru.

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ISOLATION, SUBTYPE DETERMINATION AND ALTENUATION OF LASSA FEVER VIRUS FROM WILD RODENTS IN SOUTHWEST NIGERIA

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The project is focused on vector ecology and virus epidemiology, skills in studying emerging viruses Molecular characterization of isolates from a wider geographic area to fully understand the diversity of the LASV strains and its impact on disease distribution and risks. Such information would be useful for developing efficient viral detection technologies, for example, enabling design of PCR primers and antibodies specific for a broad range of LASV types. These diagnostic tests are extremely relevant to disease surveillance, monitoring and evaluation of interventions to prevent primary LASV epidemics in humans. More extensive information about sequence diversity affecting the antigenicity of the virus or the function of its RNA-dependent, RNA polymerase may help in the development of vaccines and antiviral drugs. It will also lead to deeper understanding of the biology and pathogenesis of LASV Reconcile age-related surveillance trends through an immune-epidemiologic hypothesis, age-related patterns in cross-reactive strains humoral and cell-mediated immunity that may correlate with outcomes.determine presence of homologous and cross-negative neuraminidase-inhibiting(NI) antibodies against viral strains in sera. Results from this study will add to the current, the existing body of knowledge about Lassa Hemorrhagic fever. Further investigations may open potential therapy/vaccine candidates. The study is relevant to public health policy and practice in combination therapy approach to enhance immunity towards prevention, treatment and control of Lassa fever infection.

1679

7-HYDROXYSTAUROSPORINE, UCN-01, IS AN EFFECTIVE INHIBITOR OF LIPID ENVELOPED VIRUS REPLICATION

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Apoptotic mimicry utilized by lipid-enveloped viruses for infection is dependent on external leaflet viral phosphatidylserine (PS) expression and host cell kinase activity involving TIM proteins, AXL, and TYRO3. In addition, it has been shown that matrix proteins Ebola VP40 and HIV-1-Gag induce external leaflet expression of PS. UCN-01 is a staurosporine FDA approved for phase II clinical trials for multiple neoplasms, which acts as a non-specific kinase inhibitor and has been shown to disrupt PS localization to the plasma membrane by redistributing to endomembranes. By utilizing UCN-01 at lower concentrations than used for anti-cancer clinical trials it is hypothesized that viral replication in multiple lipid enveloped viruses can be inhibited in two ways: first, by inhibiting matrix protein localization to the plasma membrane and viral budding by localizing PS to endomembranes from the plasma membrane, and second, by inhibiting viral infection which utilizes apoptotic mimicry by reducing available PS on viral particles and by also inhibiting host cell kinase activity of receptor proteins known to interact with PS. Cellular toxicity of UCN-01 was reproduced from the literature and concentrations from 50-400nM were determined to be within the acceptable range in the cells being studied. Using these concentrations UCN-01 was shown to reduce plasma membrane localization of Ebola VP40 and HIV-1-Gag significantly using confocal imaging of recombinant GFP tagged viral proteins. Changes in budding were then assessed in Marburg VP40 and Ebola VP40 using western blot with significant increases in budding at 200nM and 300nM in Ebola VP40. Live Chikungunya virus and Ebola virus was shown to be inhibited by UCN-01 treatment. TEM and SEM of Ebola VP40 and Marburg VP40 producing cells treated with UCN-01 will be performed in addition to localization assays utilizing fluorescent probes for Ebola VP40, Marburg

VP40, and HIV-1-gag. UCN-01 inhibition on kinase activity of AXL and TYRO3 will also be performed in-vitro. Finally, live virus studies will be expanded to include Dengue virus, HIV-1, a second Ebola virus strain, Marburg virus, and Zika virus.

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ATORVASTATIN REDUCES MALARIA LIVER STAGE DEVELOPMENT IN A RODENT MODEL

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Malaria parasites use a complex metabolic machinery to grow inside host cells such as red blood cells and hepatocytes, but lack the enzymes to synthesize the sterols they need for multiplication inside these cells. Within a hepatocyte a single parasite produces >10.000 daughter parasites, representing one of the fastest multiplication rates among eukaryotic cells. To meet the metabolic demand for this rapid proliferation, the parasite scavenge sterols from its host cell. Given the world-wide exponential increase in metabolic diseases, and the concomitant use of lipid lowering drugs, we used the *Plasmodium berghei* rodent malaria model to investigate the effects of such metabolic drugs on parasite multiplication in the liver. C57BL/6 mice were treated with atorvastatin (AT), atorvastatin in combination with ezetimibe (AT+EZ) or alirocumab (AL) for three weeks after which they were infected with either sporozoites or blood stages of a transgenic *P. berghei* parasite expressing luciferase. Treatment with AT or with AT+EZ altered the lipid metabolism of mice, resulting in weight loss, a reduction in plasma cholesterol and plasma triglycerides levels and reduced liver cholesterol content. However, treatment with AL did not result in an reduction of liver lipid content despite reduced plasma cholesterol levels. A lower liver lipid content paralleled reduced parasite liver loads as measured by *in vivo* imaging of luciferase expressing parasites. Mice treated with AT+EZ had impaired parasite development in the liver resulting in 55% and 86% reduction in blood stage infection, monitored with blood smears, compared to control mice. As expected, the mice treated with AL did not reveal significant differences in parasite liver loads despite the reduced levels of plasma lipids. AT and AL did not affect blood stage parasite multiplication. In conclusion, treatment with commonly used lipid-lowering medication such as atorvastatin with or without ezetimibe significantly reduced parasite liver loads. This study provides a proof-of-principle for how manipulations in the host metabolism can affect the replication potential of malaria parasites.

1681

CASE OF SYMPTOMATIC *PLASMODIUM VIVAX* INFECTION WITH PARASITE DENSITY HIGHER THAN COMMONLY OBSERVED IN DUFFY BLOOD GROUP NEGATIVE PATIENT IN MALI, WEST AFRICA

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Plasmodium vivax was assumed not to be able to infect the red blood cells of Duffy blood group negative people particularly in Central and West Africa. In the last decade, *P. vivax* has been observed across Africa, including in areas inhabited predominantly by Duffy-negative populations. Previous studies suggested that *P. vivax* invasion and reproduction are less efficient in Duffy blood group negative people, with very low parasite densities reported. Subsequently infection is generally milder than that observed in Duffy-positive individuals. Even so, our recent data have shown that chronic carriage of asymptomatic *P. vivax* infection in Duffy-negative individuals can be associated with anemia. In addition we report a case of *P. vivax* infection in a febrile Duffy blood group negative individual with high parasite density. Blood samples were collected during outpatient examination in health centres during malaria transmission season in northern Mali, West Africa. Quantitative PCR was used to detect *Plasmodium* species and quantification. A PCR RFLP using Styl and Banl endonuclease digestion was performed to determine Duffy blood group phenotype. Of 1320 individuals sampled, 316 detected *P. falciparum* infections. Two febrile cases of *P. vivax* mono-infection occurred in a 29-year-old Duffy-positive male with parasite density of 126 parasites/uL and in an 11-year-old Duffy-negative male with parasite density of 4670 parasite/uL. Our data indicates that *P. vivax* density could be higher than generally assumed in Duffy-blood-group negative patients in sub-Sahara Africa.

1682

EXPLORATION OF CROSSTALK BETWEEN INFLAMMATION, COAGULATION, AND OXIDATIVE STRESS HOST RESPONSES IN MALARIA-INDUCED MIDGESTATIONAL PREGNANCY LOSS IN A RODENT MODEL

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Placental malaria (PM) is a major complication of *Plasmodium falciparum* malaria that impacts hundreds of thousands of pregnancies annually, contributing to pregnancy loss, preterm delivery, and infant low birth weight. Mouse models for malaria infection during pregnancy have been instrumental in our current understanding of PM, but this understanding remains incomplete. We have developed a mouse model of infection during pregnancy using the murine-infective species P. chaubaudi chaubaudi AS. Infection initiated at gestation day 0 in mice deficient in either tumor necrosis factor or tissue factor function and intact C57BL/6J control mice treated with antioxidant and anticoagulant drugs were used to probe host responses to malaria infection during pregnancy. While unmanipulated control mice lose their pregnancies, tumor necrosis factor- and tissue factor-deficient and drug-treated mice experience improved embryo viability at mid-gestation. These findings suggest that by disrupting the function of key host mediators of severe malaria, it may be possible to mitigate negative pregnancy outcomes associated with malaria. Through strategic use of genetically modified mice, combined with studies of gene expression, tissue staining, and therapeutic intervention, this work investigates the crosstalk between three major host responses to infection - inflammation, coagulation and oxidative stress - and characterizes how their interplay may contribute to PM pathogenesis and associated poor pregnancy outcomes.

1683

ZINC AND OTHER METAL CONCENTRATIONS IN UGANDAN CHILDREN WITH SEVERE MALARIAL ANEMIA COMPARED TO COMMUNITY CHILDREN

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Metals, including zinc and copper among others, can affect immune function and physiologic processes in children, and may predispose to infection or to increased disease severity. To investigate how metal levels may affect risk of severe malaria, we measured the concentrations of 17 metals in whole blood in Ugandan children with severe malarial anemia (SMA, n=199) and compared results to age-matched, healthy community

children without acute illness (CC, n=21), using inductively coupled mass spectrometry. Concentrations were compared between SMA and CC, adjusted for age, sex, socioeconomic status and nutrition. The data were randomly split between a training set (40%) and a validation set (60%). Two WQS indices of deciled-scored concentrations were estimated for the study group comparisons using generalized weighted quantile sum (WQS) regression with a logit link. The resulting WQS index in children with SMA compared to CC showed a mixture effect with significantly higher levels of tin, copper, and manganese and significantly lower levels of selenium, zinc, molybdenum, magnesium, cadmium and cobalt (P values for both mixtures <0.0001 in the validation data set). Results suggest that SMA dysregulates multiple biologically active metals which could play a role in acute and chronic SMA related morbidities. Future studies will investigate the associations of these metal concentrations with inflammatory markers and with measures of acute and chronic morbidity in these children, such as neurodevelopment.

1684

EXTRACELLULAR FLUX ANALYSIS REVEALS THE MAJOR METABOLIC SHIFT AND DIFFERENTIAL SUBSTRATE UTILIZATION FOR OXPHOS ACTIVITY DURING GAMETOCYTE DEVELOPMENT

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The causative agent of malaria, *Plasmodium falciparum*, has a complicated life cycle consisting of multiple developmental stages in the human host and the mosquito vector. Earlier reports have indicated that blood-stage parasites in the human host rely on glycolysis as their primary energy source, while mosquito stages fully depend on mitochondrial oxidative phosphorylation (OXPHOS). These observations suggest that malaria parasites achieve a huge metabolic shift from human to mosquito stages, but raise the question of when and how this shift occurs. Previously, we developed a robust bioenergetic assay for blood-stage parasites utilizing an Extracellular Flux Analyzer. It enables simultaneous investigation of mitochondrial respiration and glycolysis in a physiologically relevant microenvironment with readouts of an oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). With this assay system we demonstrated a robust glycolytic but an inefficient OXPHOS activities in schizonts (late asexual stage). We now report a dramatic increase of OXPHOS over the course of gametocytes development (sexual stage). We investigated differential substrate utilization for OXPHOS at various developmental stages in gametocytes. Among six tested substrates [glycerol-3-phosphate (G3P), dihydroorotate, glutamate, malate, succinate and pyruvate], we found that G3P gave the highest OCR increase in early stage gametocytes (day 6 and 8) similar to schizonts. Responses to both G3P and malate decreased over the course of development and we observed that pyruvate increased the OCR in late stage gametocytes (day 10 and 12). In contrast, pyruvate had no effect on OCR in schizonts. These results suggest that parasites significantly change their metabolic pathways to achieve more efficient ATP production by OXPHOS in order to support successful transmission to mosquitoes. Further details of substrate utilization as well as fatty acid ß-oxidation will be discussed. Our findings may suggest the mitochondrion OXPHOS pathway as a unique target for transmission blocking therapeutics.

1685

DISSECTING THE MECHANISMS OF MALARIA INDUCED ANEMIA IN RODENT MALARIA MODELS

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Malaria induced severe anemia is likely multifactorial, arising from clearance of infected and uninfected red blood cells and an inhibition in erythropoiesis, the production of new red blood cells. However, the exact molecular mechanisms contributing to both red blood cell clearance and inhibition of erythropoiesis are still widely unknown. Using *Plasmodium yoelii* parasites N67C strain and 17XNL strain, we are dissecting the molecular mechanisms of malaria induced anemia. From microarray analysis, the N67C strain has decreased expression of erythropoietic associated genes at day 4 post infection. In contrast, the 17XNL strain has an initial inhibition of erythroid associated genes but later in the infection shows an increase in expression. Therefore, using these two rodent models, we aim to further measure and characterize the effect of malaria infections on erythropoiesis in the host. Using flow cytometry analysis of the bone marrow and spleen, we found differences of cell populations of progenitor cells and late stage cells of hematopoiesis and erythropoiesis when comparing infected mice to non-infected mice. In particular, the N67C strain had decreased cell frequencies for proerythroblast and other early cell stages of erythropoiesis on day 4 post infection. Our aims are to elucidate the molecular mechanisms contributing to the changes in hematopoiesis and to advance our understanding of malaria induced anemia.

1686

AN ER-RESIDENT HSP40 IS REQUIRED FOR THE ASEXUAL DEVELOPMENT OF THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Malaria remains a significant global health burden. The parasitic disease kills hundreds of millions of people every year, with infection by *Plasmodium falciparum* associated with the most severe cases of malaria. All of the clinical symptoms of malaria result from the asexual replication of *Plasmodium* parasites within human red blood cells (RBCs); thus, an understanding of the mechanisms used by the parasite to survive within the RBC is critical. The Endoplasmic Reticulum (ER) is an organelle central to parasite biology, and its function is required for parasite survival. The ER serves as the starting point for protein trafficking to other organelles and to the host RBC, and stress response signaling from the ER is associated with parasites surviving treatment with the frontline anti-malarial Artemisinin. ER-resident chaperones support ER function and are therefore ideal candidates for exploring the parasite's biology. To this end, we have generated a conditional knockdown parasite line for PfJ2, a putative ER-resident Hsp40 expressed throughout the asexual cycle. Using this parasite line, we have confirmed that PfJ2 is an ER-resident protein and is essential for parasite survival inside the RBC. Specifically, knockdown of PfJ2 results in delayed parasite development during the trophozoite stage before failure to complete schizogony to form new invasive parasites. ER functions, such as protein trafficking and stress response, will be assayed during PfJ2 knockdown to investigate the chaperone's role in these processes. Additionally, PfJ2 uniquely contains both a J-domain and a thioredoxin-like domain, and we will explore how these domains contribute to PfJ2 function. By elucidating the role this essential chaperone plays in parasite biology, we will gain a better understanding of the mechanisms used by the parasite to survive in the RBC and cause disease.

1687

MALIAN CHILDREN WITH HEMOGLOBIN C OR S TRAIT RECOGNIZE FEWER EXTRACELLULAR PFEMP1S THAN CHILDREN WITH HEMOGLOBIN AA, BUT DIFFER IN RECOGNITION OF CD36-BINDING PFEMP1S

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Hemoglobin (Hb) C trait, like Hb S trait, protects against severe malaria in children and, in some Malian populations, against milder infections. Hb C trait alters the display of *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1), a family of parasite proteins expressed on the erythrocyte surface that mediates binding to endothelial receptors. Parasitized erythrocytes from individuals with hemoglobin C attach inefficiently to host endothelial receptors, potentially limiting host tissue sequestration and immune evasion. We hypothesized that sera from children with Hb C trait recognize fewer PfEMP1s than sera from children with Hb AA, given the altered PfEMP1 presentation in these individuals. We probed sera from Malian children with a protein microarray with 179 protein fragments from three variant surface antigen (VSA) families. Sera from children with uncomplicated malaria who had Hb C trait (N=14; ages 10-76 months) recognized fewer PfEMP1s than sera from similarly infected children with Hb AA (N=53; 7-98 months). This was true for both CD36-binding PfEMP1 fragments (14.0% of fragments recognized vs 68.6%, χ^2 test, P=3.4x10^{-13}) and non-CD36-binding PfEMP1 fragments (17.3% vs 75%, P=3.6x10^{-9}). Both groups recognized intracellular PfEMP1 fragments (81.8% vs 90.9%, P=0.38) and non-PfEMP1 VSAs (88.9% vs 88.9%, P=1) equally. Sera from uninfected children with Hb S trait (N=8; 6-55 months) recognized fewer PfEMP1s than sera from uninfected children with Hb AA (N=56; 7-76 months). This was true for CD36-binding PfEMP1 fragments (26.7% vs 47.7%, P=0.005), non-CD36-binding PfEMP1 fragments (11.5% vs 50.0%, P=2.1x10^{-5}), and intracellular PfEMP1s (13.6% vs 90.9%, P=2.9x10^{-7}). Interestingly, sera from these Hb S children recognized more CD36-binding PfEMP1 fragments than sera from uninfected children with Hb C trait (N=5; 8-31 months) (26.7% vs 8.1%, P=0.001). These results suggest that altered PfEMP1 presentation with Hb C or S trait reduces host immune recognition of these parasite proteins and that Hb C and S trait may affect PfEMP1 surface presentation in distinct ways that differentially alter humoral immune responses.

1688

REVERSIBLE BRAIN SWELLING IN EXPERIMENTAL CEREBRAL MALARIA

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Recently, reversible brain swelling has been identified in cerebral malaria patients using magnetic resonance imaging (MRI). It is unknown, if similar pathology occurs in experimental cerebral malaria (ECM). We thus examined immunized C57BL/6 mice after single vaccination with radiation-attenuated sporozoites (RAS) and naïve C57BL/6 mice upon infection with *Plasmodium berghei* ANKA- expressing mCherry. All mice were assessed according to the Rapid Murine Coma and Behavioral Scale (RMCBS) score once naïve mice showed ECM. We further analyzed brain pathology by using high resolution small animal MRI in order to assess brain swelling and blood-brain barrier disruption (BBBD), immunohistochemical staining for microglia, single plane illumination microscopy (SPIM) to locate parasites microscopically and correlative light and electron microscopy (CLEM) to study parasites on a nanoscopic level. In contrast to naïve mice, that showed extensive BBBD and brain swelling, all immunized mice survived the challenge and showed either reversible brain swelling and mild BBBD or no pathological signal alteration on MRI. Furthermore, less microglial activation was found in immunized mice compared to naïve mice. However, along the rostral migratory stream microglial activation appeared to be similar in both groups. Distinct brain areas with higher parasite density could be observed in both groups, although parasite distribution was more disseminated in naïve mice. By employing CLEM, we demonstrated that parasites have a high propensity towards the endothelium. In summary, we showed for the first time that reversible brain swelling also occurs in a mouse model of ECM by using advanced imaging techniques. We further identified hot spots of parasite accumulation and inflammation. This model may present a valuable tool to understand the mechanisms of reversible brain swelling in ECM.

1689

MALARIA IN PREGNANCY INCREASES THE RISK OF PRETERM BIRTH IN ASSOCIATION WITH LONGITUDINAL CHANGES IN ANGIOGENIC, METABOLIC, AND INFLAMMATORY PATHWAYS

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Malaria in pregnancy is associated with adverse birth outcomes, however the underlying mechanisms are poorly understood. Tight regulation of angiogenic, inflammatory and metabolic pathways are essential for healthy pregnancies. In this study we test the hypothesis that malaria disrupts these critical pathways in mid-pregnancy leading to preterm birth (PTB). Here we conduct a secondary analysis of a randomized trial of malaria prevention in pregnancy, to investigate the effect of malaria infection on the kinetics of biomarkers of angiogenesis and inflammation during pregnancy and their association with PTB. We assessed plasma biomarkers longitudinally over pregnancy in a cohort of HIV negative women (n=1755). Pregnancies were ultrasound dated and women were sampled at 13 to <24 weeks (Visit 1), 28 to <34 weeks (Visit 2), and/or 34 to <37 weeks (Visit 3). Biomarkers were analyzed by Luminex multiplex assays. Malaria prevalence was high with at least one episode of PCR-positive *Plasmodium falciparum* infection occurring in 69% (n=1210) of women,

and at least one smear-positive infection occurring in 28% (n=499) of women. Malaria infection before week 24 was associated with increased relative risk of PTB (adjusted RR, 95% CI: 1.29, 1.03-1.61, p=0.026). At all three visits over the course of pregnancy, malaria infection was associated with higher levels of CRP, IL-18BP, sTNFRII, and sEng. Using linear mixed effects modeling, PCR-positive malaria during the second trimester was associated with altered kinetics of CRP, CHI3L1, IL-18BP, sICAM-1, sTNFRII, sEng, Angptl3, and Leptin levels over pregnancy (χ^2 >11.0, p<0.01 for all). These data show that malaria infection in the second trimester of pregnancy is associated with altered kinetics of critical mediators of angiogenesis and inflammation over the course of pregnancy and an increased risk of PTB. Effective malaria treatment early in pregnancy may be required to prevent altered placental vascular development and poor birth outcomes.

1690

INVESTIGATING THE ROLE OF VASCULAR ALPHA GLOBIN IN EXPERIMENTAL CEREBRAL MALARIA

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Alpha globin was recently discovered in the endothelium of resistance arteries, where it regulates diffusion of nitric oxide (NO) through its interaction with endothelial nitric oxide synthase (eNOS) at the myo-endothelial junction (MEJ). During malarial infection, people with one or two deletions of the alpha globin gene are protected from severe complications, raising the question of whether this protection is conferred by changes to vascular endothelium, and if so, whether this pathway may present a novel target for a new adjuvant therapy in cerebral malaria. We are using dual genetic and pharmacologic approaches to disrupt the interaction between vascular alpha globin and eNOS in a murine model of experimental cerebral malaria (ECM) by infection with *P. berghei* ANKA. The genetic approach utilizes deletion of the alpha globin gene locus *Hba1* within the vascular endothelium; *Hba1* deletion increases arteriolar NO bioavailability, and our preliminary data shows robust expression of *Hba1* in cerebral arteries. For the pharmacologic approach, infected mice are treated with daily I.P. injections of HbaX, an alpha globin mimetic peptide. HbaX binds specifically with eNOS and blocks its association with alpha globin, effectively increasing NO bioavailability *in vivo* in healthy and hypertensive mice. Primary outcomes for the genetic and pharmacologic approaches in this ongoing study (completion expected August 2018) include: rapid murine coma score; time to onset of ECM symptoms and survival; laser speckle imaging of cerebral blood flow; assessment of blood brain barrier permeability; *ex vivo* reactivity of isolated cerebral arteries; and plasma and tissue measurement of NO bioavailability. We anticipate that this study will be highly informative towards understanding whether the protective effects of alpha globin deletion seen in malaria patients are conferred by changes within the vascular endothelium. Additionally, this study is interrogating whether targeting alpha globin to increase endogenous NO production within the cerebral vasculature can improve survival, cerebral blood flow, and vascular reactivity in a murine model of ECM.

1691

THE ASSOCIATION OF BLOOD TRANSFUSION WITH OUTCOME AMONG AFRICAN CHILDREN HOSPITALIZED WITH *PLASMODIUM FALCIPARUM* MALARIA

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Infection with *Plasmodium falciparum* leads to severe malaria and death in approximately 445,000 children each year in sub-Saharan Africa. Blood transfusion may benefit some patients with malaria but could potentially harm others. To determine the hemoglobin concentrations at which blood transfusion is associated with improved survival of children admitted to hospital with *Plasmodium falciparum* malaria, we analyzed admissions to six tertiary care hospitals participating in the Severe Malaria in African Children network. Odds of in-hospital death associated with transfusion were estimated using site- and severity-adjusted analyses. Generalized additive models were used to estimate optimal hemoglobin transfusion thresholds. 25,893 pediatric patients were admitted to hospital with *P. falciparum* malaria, and 8,513 (32.8%) received a blood transfusion. Transfusion was associated with decreased odds of death in site-adjusted analysis (OR=0.82 [95%CI 0.71-0.94]). This association became stronger after adjustment for increased disease severity of transfused patients (OR=0.50 [95%CI 0.42-0.60]). Among all patients, transfusion was associated with improved survival when the hemoglobin was up to 77 g/L (95%CI: 65-110). Among those with impaired consciousness, transfusion was associated with improved survival at hemoglobin concentrations up to 105 g/L (95%CI: 71-115). Among those with hyperlactatemia, the association with improved survival persisted at even greater hemoglobin concentrations (lower bound 95%CI: 90 g/L). In conclusion, whole blood transfusion was strongly associated with improved survival among African children with *P. falciparum* malaria. Among those with impaired consciousness or hyperlactatemia, transfusion was associated with improved survival at hemoglobin concentrations well above the currently recommended transfusion threshold.

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MORPHOLOGICAL CHARACTERIZATION AND IDENTIFICATION OF CONSERVED *PLASMODIUM* BLOOD STAGE PROTEINS IN C*OLPODELLA* SP., FREE-LIVING RELATIVES OF APICOMPLEXANS

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Colpodella sp. are the closest free-living ancestors of the apicomplexan phylum which contains important human pathogens such as *Plasmodium falciparum* and *Toxoplasma gondii*, causative agents of malaria and toxoplasmosis, respectively. *Colpodella*-like parasites infecting erythrocytes were reported in a human infection marked by low natural killer cells and anemia. *Colpodella* sp. possess a pseudoconoid, rhoptries, micronemes and in some species trichocysts at the apical end of the trophozoite. In a process similar to merozoite invasion in *P. falciparum*, contents of the rhoptries are emptied during myzocytosis. In this study, we investigated the morphological characteristics of *Colpodella* sp. using different staining techniques for light microscopy. Transmission electron microscopy was also performed for ultrastructural characterization. Antibodies specific to *Plasmodium* blood stage proteins were used in immunofluorescence assay. *Colpodella* trophozoites and cysts were stained by Giemsa, Wright's, Sudan IV, Picro-Sirius, Alum Carmine and hematoxylin and eosin (H&E) staining. We compared the clarity of morphological characteristics such as delineation of flagella, cytoplasmic structures, cyst features and the attachment junction formed during myzocytosis. Trophozoites and cysts were distinguished by all dyes except Alum Carmine and Sudan IV. Antibodies recognized several blood stage antigens including the high molecular weight rhoptry protein RhopH3 and SERA1. DNA sequencing of a polymerase chain reaction (PCR) amplified product obtained using primers targeting the *P. falciparum* RhopH3 gene confirmed the presence of RhopH3 in *Colpodella* sp. A combination of staining, immunological

and molecular protocols can be used to further investigate *Colpodella* sp. to gain insights regarding the potential for Colpodellid protists to be opportunistic human pathogens.

1693

DO ALPHA GLOBIN GENE VARIANTS AFFECT ENDOTHELIAL FUNCTION IN PATIENTS WITH SEVERE MALARIA?

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The human alpha globin genes *HBA1* and *HBA2* are highly polymorphic, with deletions and sequence variants that decrease the transcription or synthesis of functional alpha globin protein causing a spectrum of alpha thalassemia syndromes. While some variants have been associated with decreased susceptibility to malaria, precise mechanisms of protection remain to be elucidated. Recently, alpha globin was discovered to be expressed in vascular endothelial cells where it regulates nitric oxide signaling. Genetic or pharmacologic inhibition of alpha globin expression increased nitric oxide signaling in intact vessels, therefore we hypothesize that human polymorphisms that inactivate alpha globin will increase endothelial nitric oxide signaling. This is relevant to the pathogenesis of malaria where the loss endothelial nitric oxide signaling may contribute to adhesion of infected blood cells, occlusion of small vessels, and impairment of vasoregulation. To address the hypothesis that alpha globin regulates endothelial nitric oxide signaling, we will genotype alpha globin gene mutations in a case control study of moderately severe and severe malaria patients infected with *Plasmodium falciparum*, *Plasmodium vivax* or *Plasmodium knowlesi* from Timika, Indonesia and Sabah, Malaysia. We will genotype the -3.7, -4.2, --SEA, and --FIL deletions and the Constant Spring and Adana sequence variants. We will use a droplet digital PCR approach to quantify precisely the number of variant alleles at this duplicated tandem gene locus. We will analyze the association of alpha globin gene deletions/variants with previously performed functional measures of vascular endothelial function: peripheral arterial tonometry and skeletal muscle reperfusion kinetics. In addition, we will examine biomarkers of endothelial activation such as angiopoietin-2 which is regulated by nitric oxide and elevated in severe malaria. These studies will extend our understanding of the role of endothelial alpha globin in nitric oxide signaling and determine what impact alpha globin variants have on endothelial function during severe malaria.

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THERE IS RIBOSOME STALLING IN *PLASMODIUM FALCIPARUM*

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Ribosomes are main players in protein synthesis, and their function can be interrupted due to damaged mRNA, insufficient availability of tRNAs and amino-acids or genetic errors. Most of the organisms have evolved mechanisms to recognize stalled ribosomes and initiate pathways that involve conserved eukaryotic proteins Pelota in mammals (Dom34 in yeast) and Hbs1. In many translation-inhibiting stress conditions, free ribosomal subunits reassociate to form a large pool of non-translating 80S ribosomes. The subunits of these inactive ribosomes need to be mobilized for translation restart upon stress relief. In yeast, the Dom34-Hbs1 complex, have been shown to split ribosomes stuck on mRNAs. Interestingly, to date in *Plasmodium falciparum* genome putative PELO gene is annotated but not HBS1L. In our recent work, we have shown that polyA stretches longer than 12 adenosines in a row cause stalling in most eukaryotes resulting in mRNA decay and protein degradation. Surprisingly, the same sequences are efficiently translated in *P. falciparum*. To induce ribosome stalling we used the fact that *P. falciparum* must acquire isoleucine exogenously. *P. falciparum* is auxotrophic for most amino acids. Degradation of host erythrocyte hemoglobin is the source of most of the amino acid except for isoleucine because this amino acid is not present in adult human hemoglobin. When isoleucine is withdrawn from the culture medium of intraerythrocytic *P. falciparum*, the parasite slows its metabolism, and isoleucine-starved parasites remain viable for 72 h. During the parasite hibernation, the ribosomes are stalled. Using CRISPR/Cas9 technique, we tagged PELO gene with 2HA tags. Successfully tagged PELO gene was expressed in *P. falciparum*. Using polysome profiling, we showed the interaction of Pelota protein with stalled ribosomes in cultures deprived of isoleucine compared to control culture supplemented with it. The open question is does Pelota have interaction partner because in most eukaryotes most the partner in crime is Hbs1 or it works on its own.

1695

IS COMPLICATED MALARIA AN IMPORTANT CAUSE OF ILLNESS AMONG ADULTS IN KAMPALA, UGANDA?

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Malaria is one of the most important diseases in the world with more than 3 billion people at risk of infection. The largest burden is still in Sub-Saharan Africa. In Uganda, malaria, has persisted as the most important cause of mortality and morbidity despite years of efforts towards its reduction and eventual eradication. Worldwide, most of the cases of severe malaria have been documented in children but adults are not spared either. We set out to out to find out the extent to which patients on the adult (12 years and above) medical ward in our hospital exhibit features of severe malaria. Diagnosis was based on clinical signs and positive Rapid Diagnostic Test (RDT) and blood slide for malaria. We analysed charts of patients who were admitted from January to March 2018. Out of a total 133 admitted,22 had features of complicated malaria as below: Seven (31%) had thrombocytopenia,6 (27%) had anemia,4 (18%) had respiratory distress,4 (18%) had altered mentation,2(9%) had hypoglycemia,1 (4.5%) were in shock and 1 (4.5%) had renal insufficiency. All were treated for malaria using standard intravenous artesunate dosing. Twenty cured,1 was referred and 1 died. The above findings indicate that indeed complicated malaria is an important cause of morbidity and even mortality among adult patients in Kampala, a high endemicity area.

1696

PREVALENCE OF MOLECULAR MARKERS ASSOCIATED WITH ARTEMISININ, LUMEFANTRINE AND AMODIAQUINE RESISTANCE IN PRE-TREATMENT ISOLATES FROM TWO THERAPEUTIC EFFICACY MONITORING SITES IN GUINEA, 2016

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In Guinea, artemisinin-based combination therapy is the first-line antimalarial treatment for uncomplicated *Plasmodium falciparum* infection. There are limited data on the prevalence in Guinea of polymorphisms in the *pfk13* gene (artemisinin resistance) and the *pfmdr1* gene (amodiaquine

and lumefantrine resistance). Since 2015, the National Malaria Control Program has implemented yearly therapeutic efficacy studies rotating between four sentinel sites in the country. We isolated DNA from 444 samples from study participants, including 421 pretreatments and 22 day-of-late treatment failure samples collected as part of the 2016 round of therapeutic efficacy studies in Mafèrinyah and Labé Health Districts. The *pfk13* and *pfmdr1* gene were amplified, sequenced and analyzed for polymorphisms. Of the 397 samples successfully analyzed, the majority of pretreatment (99%, 369/374) and all day-of-late treatment failure (100%, 22/22) samples were wild type for *pfk13*. Five pretreatment samples (1%, 5/374) carried synonymous mutations in the Kelch-propeller domain, including three in the artemether-lumefantrine arm (L429L, E509E and V510V) and two in the artesunate-amodiaquine arm (C469C and G496G). All five patients with *pfk13* mutations were slide-negative on Day 3 and finished 28-day follow-up with adequate clinical and parasitological response. The NFD haplotype was the predominant *pfmdr1* haplotype, present in 48% (189/391) of pretreatment samples, followed by the NYD haplotype, present in 33% (128/391) of pretreatment samples. Efficacy studies in 2016 in Mafèrinyah and Labé Health Districts showed genetic evidence of susceptibility to artemisinins, consistent with clinical outcome data showing 100% Day 3 clearance rates. Despite the absence of non-synonymous *pfk13* mutations, a substantial proportion of pretreatment isolates had *pfmdr1* haplotypes previously associated with decreased lumefantrine susceptibility. Continued molecular monitoring is required at country level to ensure timely detection of antimalarial drug resistance and resistance-associated mutations.

1697

GENOMIC PROFILE OF *PLASMODIUM FALCIPARUM* PARASITES LACKING HISTIDINE-RICH PROTEIN (*PFHRP2*) FROM ERITREA

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Recent emergence of *Plasmodium falciparum* lacking *hrp2* may have diverse consequences for the parasite. Deleterious mutations can generate a strong selection pressure to balance the detrimental effects through compensatory genetic changes. It has been predicted that as transmission declines the parasite population shifts towards parasites lacking *hrp2*, presumably due to the selective pressure of HRP2-RDT results determining the likelihood of treatment. *Hrp2*-deleted parasites have been shown to have lower multiplicity of infection compared to *hrp2*-harbouring parasites, but it is not clear whether such parasites also have lower genetic diversity and whether they are more likely to harbour wild-type alleles of drug resistance-associated genes. In this study, we compared genomic data for *hrp2*-deleted and *hrp2*-normal samples from Eritrea. *P. falciparum* DNA extracted from dried blood spots was selectively whole genome amplified and sequenced on an Illumina platform, leading to the first genomic data of *P. falciparum* parasite isolates from Eritrea. Several genes of antimalarial drug resistance markers including *pfcrt*, *pfmdr1*, *pfdhfr*, *pfdhps*, *pfk13*, *pfAP2μ*, *pfubp1*, *pfATP6* and *pfnhe-1* were compared between the two parasite groups. We also analysed genes associated with cerebral malaria (CM), as *hrp2* has been reported to play a role in manifestation of CM. Finally, the genetic diversity within and between the two parasite groups, including variant antigen repertoires, was examined. Drug resistance marker alleles as well as other novel mutations from both *hrp2*-deleted and *hrp2*-positive parasite isolates will be reported. Initial findings show that both parasite groups carried the chloroquine-resistant haplotype *pfcrt* CVIET and the sulfadoxine-pyrimethamine resistance-associated allele *dhps* 540E. The implication of the findings on malaria control and elimination will be discussed.

1698

EFFICACY OF ARTESUNATE-AMODIAQUINE IN THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN MADAGASCAR

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In Madagascar, artesunate-amodiaquine (ASAQ) are the first line treatment recommended in uncomplicated falciparum malaria. Since 2006, only two clinical drug efficacy studies have been conducted to assess the efficacy of ACTs. In this context, new data about the efficacy of the drug combinations currently used to treat malaria are needed. Cohorts' studies of therapeutic efficacy of ASAQ were conducted in six sites in different epidemiological patterns, whose, Vangaindrano, Tsiroanomandidy in 2012, Mandoto, Marovoay in 2013, Ifanadiana, Maevatanana in 2016. The 2009 WHO protocol for monitoring antimalarial drug efficacy was followed. 358 patients were enrolled. Their ages ranged from 6 months to 56 years (median: 8 years). Patients enrolled in Vangaindrano and Ifanadiana were all aged <15 years. The mean of the parasite density at day 0 was 17,440 parasites/µl of blood (95%CI: 5,164-29,734, range: 510-356,250). The combination was well tolerated. Few adverse events in gastrointestinal transit, in the central nervous systems and in musculoskeletal system were observed, whose, 11,45% (41/358) patients, 2,51% (9/358) and 2,51% (9/358) respectively. The median hemoglobin concentration, among patients with available data, significantly increased from 10,61 g/dL on day 0 to 11,92 g/dL on day 28. The overall efficacy of ASAQ after PCR correction was 98,2% (95% IC: 90,6 - 99,7). According to years, the PCR-corrected day 28 cure rates were 94,4% (95% IC: 88,4 -97,4) in 2012; 100% (95% IC: 96,9 - 100) in 2013 and 100% (95% IC: 96,6 - 100) in 2016. There was no case of early treatment failure (ETF); two cases of Late Parasitological Failure (LPF), determined by msp1/msp2genotyping, the first was a true recrudescence, the second was classified as reinfected and one case of Late Clinical Failure (LCF) were observed and also was classified as reinfected. ASAQ remains highly efficacious in uncomplicated falciparum malaria in Madagascar. According to our results, the increased incidence of falciparum malaria cases observed recently seems not related to clinical treatment failure of the first line treatment promoted since 2006.

1699

ARTEMISININ RESISTANCE? MIND THE TRAFFIC ...

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Artemisinin susceptibility in *Plasmodium falciparum* is modulated by mutations in the gene *pfk13*, which encodes a kelch propeller domain protein of unknown function. Reduced susceptibility is demonstrated *in vitro* by elevated parasite survival after short exposures to physiologic concentrations of drug in the early ring stage. Using CRISPR-Cas9 genome editing, we provide the first evidence of a similar but K13-independent *in vitro* artemisinin resistance caused by a single base change in the locus encoding the AP-2 adaptor complex mu-subunit (*pfap2mu*). Through extensive fluorescence and electron microscopy and proteomics, our functional characterisation of PfAP2mu validates that gene as encoding a clathrin-independent, non-canonical AP-2 trafficking factor that interacts with K13 and other important factors at a distal face of the ER and is essential for asexual parasite survival. We provide evidence that disruption of trafficking in early rings initiates an ER-based stress response that underlies artemisinin resistance and induced dormancy. A model depicting a role for ER trafficking components in ring-stage artemisinin action is proposed and implications for controlling multi-drug resistance in natural parasite populations will be discussed.

1700

TARGETED DEEP AMPLICON SEQUENCING ANALYSIS OF *KELCH 13* AND *CYTOCHROME B* GENES IN *PLASMODIUM FALCIPARUM* ISOLATES FROM ERITREA

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The emergence in Southeast Asia of *Plasmodium falciparum* parasites resistant to artemisinins poses a threat to malaria control and prevention strategies. Several polymorphisms in the *P. falciparum kelch 13* (*k13*) gene have been linked to artemisinin resistance (ART-R). Similarly, polymorphisms in *cytochrome b (cyt b)* gene have been associated with atovaquone resistance (ATQ-R), which is a component drug in malarone, used for prophylaxis and as one of the primary treatments for travelers diagnosed with *P. falciparum* in the U.S. and Europe. Molecular surveillance for drug resistance markers is valuable in developing guidelines for treatment and prophylaxis in the U.S and other countries. Next generation sequencing (NGS) have helped to develop a multiplex targeted genome based method at CDC for the characterization and early detection of drug resistance polymorphisms, including mixed infection genotypes. In this study, we used this high-throughput, standardized molecular surveillance system, Malaria Resistance Surveillance (MaRS), to screen 148 *P. falciparum* isolates from Eritrea for polymorphisms in *k13* and *cyt b*. The blood samples were collected from malaria patients attending the referral hospitals and health facilities located in the study area. No mutations associated with ART-R or ATQ-R were observed. However, eleven non-synonymous and two synonymous polymorphisms that have not been linked to ART-R were observed in *k13*. Seventy of 148 (48%) had a polymorphisms in the *k13* gene, of which 4 of 148 (2%) were minor alleles (with an allele frequency at or below 50 percent). The K189**T** polymorphism was found in 59 of 148 (40%) of samples. The D281**V** polymorphism was found in 8 out of 148 (5%) of samples. The remaining 9 non-synonymous polymorphisms were observed in 4 or fewer samples. No non-synonymous polymorphisms were observed in the *cyt b* gene. Only the synonymous V127**V** and L230L polymorphisms were found in one sample each. As demonstrated in the presented study, continued monitoring using MaRS for early detection of drug resistance associated polymorphisms will be useful to develop treatment and prevention guidelines for malaria.

1701

DISTRIBUTION AND ORIGINS OF PFCRT MUTATIONS ASSOCIATED WITH PIPERAQUINE RESISTANCE IN CAMBODIA

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Resistance to both the artemisinins and key partner drugs, such as piperaquine, has become well-established in regions of Cambodia and has spread to some neighboring countries. We have recently shown that the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) F145I mutation is associated with reduced susceptibility to piperaquine, tends to occur on a background of amplified *plasmepsin II* (*pfpm2*), and confers an additional degree of resistance above that of amplified *pfpm2* alone. To better understand the timing of the emergence of this mutation and other PfCRT mutations associated with piperaquine resistance, we examined parasites from 469 clinical infections collected from eight provinces during past and ongoing studies in Cambodia, to estimate the distribution of PfCRT mutations over time and their origins. Parasites were available from northern provinces of Cambodia from 2009-2014, from western provinces from 2009-2011, and from the south in 2011-2012. We used quantitative PCR, or read coverage for samples with whole genome sequence available, to estimate *pfpm2* copy number. PfCRT F145I was genotyped by pyrosequencing. In this dataset, amplified *pfpm2* was observed in <1% of samples in 2009 and increased in frequency over time, reaching a prevalence of 75% in Northern Cambodia by 2014. PfCRT F145I was first observed in the Oddar Meanchey province in 2013 (25% prevalence) and was present in 33% of parasites sampled from that study site in 2014. Of the 23 infections with the PfCRT F145I mutation, 21 occurred on a background of amplified *pfpm2*. PacBio amplicon sequencing is underway to sequence the entire *pfcrt* gene to estimate the prevalence of other PfCRT mutations reported to be associated with piperaquine resistance, in both archived and more recent *P. falciparum* infections. Markers flanking and in linkage disequilibrium with the *pfcrt* gene will be examined to determine the origins of observed mutations. This work will provide insights into the emergence and spread of PfCRT mutations contributing to piperaquine resistance.

1702

ACQUISITION OF POINT MUTATIONS IN *DIHYDROPTEROATE SYNTHETASE* GENE DRIVE COPY NUMBER VARIATIONS OF GTP-CYCLOHYDROLASE *1* (*GCH1*) GENE IN GHANAIAN *PLASMODIUM FALCIPARUM* ISOLATES

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Sulfadoxine-pyrimethamine (SP) is used for Intermittent Preventive Treatment in pregnant women (IPTp) as well as for seasonal malaria chemoprophylaxis (SMC) in children in Ghana. *Plasmodium falciparum* resistance to SP has been linked to point mutations in the parasite's dihydropteroate synthase *(pfdhps)* and dihydrofolate reductase *(pfdhfr)* genes which encodes the protein targets of SP action. There is recent evidence from South East Asia of the amplification of GTP cyclohydrolase 1 (*pfgch1*) gene, which encodes for the first enzyme in the parasite *denovo* folate synthesis pathway in parasites which harbor the SP resistance point mutations including I164L. The point mutations make the parasites less fit, but the acquisition of multiple copies of the *gch1* gene may compensate for this fitness cost or directly enhance SP resistance. This study determined the association between *pfgch1* copy number variations (CNV) and SP resistance molecular markers in clinical isolates in Ghana. The findings are important for policy makers of malaria chemotherapy in Ghana. Two hundred and two (202) blood samples collected from children aged 14 years and below with uncomplicated malaria, presenting at health centres in Accra, Kintampo, Cape-Coast and Navrongo were used for this study. Quantitative real-time PCR was used to estimate *pfgch1* copy number and nested PCR followed by Sanger sequencing for detection of mutations in *pfdhps* and *pfdhfr* genes. It was observed that 92.6% (187/202) and 7.4% (15/202) of the parasite isolates harbored

single and double copies of the *gch1 g*ene respectively. SP resistance markers, I51, R59 and N108 were observed except L164, R50 and T163. A significant correlation was observed between the *gch1* CNV and *pfdhps* E540 (P=0.001) and G581 (P=0.002), but none for the *pfdhfr* mutations. Findings from this study have that *pfdhps* E540 and G581 correlated with increased *gch1* copy number may imply that *gch1* may compensate for the fitness cost of parasites harboring these mutations. Continuous monitoring of the *gch1, pfdhfr and pfdhps* genes is recommended and further studies to discover component drugs to target the *gch1* gene product is required.

1703

DRUG SENSITIVITIES AND MECHANISMS OF RESISTANCE IN UGANDAN *PLASMODIUM FALCIPARUM* ISOLATES TO LEAD ANTIMALARIALS IN THE MEDICINES FOR MALARIA VENTURE DEVELOPMENT PIPELINE

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New antimalarial drugs are urgently needed. To characterize the Medicines for Malaria Venture pipeline of lead antimalarials, we assessed the *ex vivo* drug sensitivity of fresh *Plasmodium falciparum* clinical isolates from Tororo and Busia districts in eastern Uganda and analyzed sequences of parasites with altered sensitivity. *Ex vivo* drug sensitivities were assessed with 72 h growth inhibition (IC50) assays on 188 clinical isolates collected from 2015-2017. Mean IC50 values were generally near those reported for laboratory isolates, yet outliers were identified. Positive correlations between IC50s were seen for compounds with shared targets, notably predicted inhibitors of PfATP4 (KAE609, SJ733, and PA92), PfDHODH (DSM265, DSM421, and DSM632), and PfPI4K (UCT048 and UCT943). For outliers, defined as an IC50 diverging by at least 2.5-fold from the geometric mean IC50, we sequenced putative drug targets/resistance mediator alleles. Notable outliers were seen for inhibitors of PfATP4 (KAE609, SJ733, and PA92), PfCYTb (ELQ300), PfDHFR (P218), PfCARL (KAF156), PfPI4K (UCT048 and UCT943), and V-type H^+ -ATPase (AZ253). For PfATP4, a G223S single nucleotide polymorphism (SNP) was found in outliers for PA92, SJ733, and KAE609. A SNP at this same allele, G223R, was previously selected in laboratory strains with a KAE609 analog. For PfCYTb, the isolate showing the highest ELQ300 IC50 exhibited a novel A205V mutation, predicted to be in the ELQ300 binding site. For PfDHFR, SNPs N51I, C59R, S108N, and I164L associated with resistance to pyrimethamine, were highly prevalent, with I164L more common than in other tested parasites in this region, and associated with the highest IC50s for P218. Sequencing of *pfpi4k*, *pfcarl, pf V-type H^+-ATPase*, and other polymorphisms is ongoing. Data available to date show variations in sensitivity to new antimalarial leads in parasites now circulating in Uganda; some of these variations appear to be explained by genetic polymorphisms in predicted drug targets. Regular, sustained study of the sensitivity of field parasites to new compounds will be essential in establishing next generation antimalarial therapies.

1704

IDENTIFICATION OF A DUAL LIVER-ASEXUAL STAGE ANTIMALARIAL TARGETING PFDHODH

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Continued malaria elimination and eradication efforts require the development of new drugs acting on all stages of the parasite lifecycle. Compounds that target liver stage development are particularly valuable as chemopreventative agents. In addition to providing prophylactic protection, the parasite might be less capable of evolving resistance to liver-stage drugs. During the liver stage a significantly smaller parasite population is exposed to drug pressure, reducing the probability of selecting for resistant forms. To address the need for liver-stage active compounds, a *P. berghei* - hepatic cell culture system was used to screen a commercially available library. Confirmed hit compounds were further screened for activity against asexual blood stage parasites to aid in target identification efforts. Interestingly, a number of molecules were only active against liver stages suggesting that they affect unique targets that are not essential during asexual development. Here we present target identification studies for one of the dual liver and asexual-active molecules, MMV1454442. In preliminary functional assays, MMV1454442 had markedly reduced activity in transgenic parasites that express *S. cerevisisae* dihydroorotate dehydrogenase (ScDHODH), suggesting a mitochondrial mode of action. To further elucidate the target of this compound, *in vitro* resistant lines were generated and whole genome sequencing used to identify genetic variants contributing to the resistance phenotype. Resistant clones had either a point mutation resulting in an F188I amino acid change in *P. falciparum* DHODH or evidence of increased copy number encompassing this locus. Interestingly, MMV1454442 represents a new scaffold of DHODH inhibitors and would not have been predicted to be a DHODH inhibitor based on structure alone. Further studies to understand the interplay of diverse DHODH inhibitors and resistance mutations are ongoing. DHODH is a well-validated antimalarial target, and this study highlights the importance of this pathway particularly in developing chemotherapeutic interventions that target pre-erythrocytic stages of the lifecyle.

1705

DISAPPEARANCE OF CHLOROQUINE RESISTANT *PLASMODIUM FALCIPARUM* IN EASTERN UGANDA

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Standard treatment for malaria in Africa has changed from chloroquine (CQ) and/or sulfadoxine- pyrimethamine to artemisinin-based combination therapies (ACTs) over the last 10-15 years, accompanied by reemergence of CQ-sensitive parasites. In Uganda, the first-line treatment changed to artemether-lumefantrine in 2004, with gradual implementation thereafter; alternative regimens are artesunate-amodiaquine and dihydroartemisinin-piperaquine. We assessed *ex vivo* drug sensitivity and molecular markers to gain insight into drug sensitivity over time in eastern Uganda. As previously reported, the geometric mean *ex vivo* IC_{50} for CQ of 408 *P. falciparum* isolates collected in Tororo in 2010-13 was 248 nM (cut-off for CQ resistance 80 nM). We reinitiated regular assessment of drug sensitivities in Tororo and Busia districts in 2016. The geometric mean IC_{50} for 182 isolates collected during 2016-17 was 20 nM. Trends toward increased CQ sensitivity continued, with the percentage of isolates with IC_{50} > 80 nM decreasing from 31% to 16% to 10% to 8% over 6 month intervals in 2016-17. Molecular data showed tight correlation between the *pfcrt* 76T mutation and CQ IC_{50}> 80 nM. Isolates were generally sensitive to monodesethyl amodiaquine, piperaquine, lumefantrine, and dihydroartemisinin, with improved sensitivity to monodesethyl amodiaquine and piperaquine, decreased sensitivity to lumefantrine, and no change for dihydroartemisinin since 2010-13. Sensitivities to these drugs did not change noticeably over 2016-17. Molecular markers associated with decreased sensitivity to artemisinins (K13), lumefantrine (*pfmdr1* amplification), and piperaquine (*plasmepsin 2* amplification) have been uncommon in Uganda, and not clearly associated with *ex vivo* drug sensitivity. Overall, parasites now circulating in eastern Uganda are sensitive to a range of antimalarials, and the large majority are now sensitive to CQ. Trends toward increasing resistance to ACTs in SE Asia are of great concern, but current widespread improved drug sensitivity now offers an opportunity for drug-based interventions to better control malaria in Africa.

1706

EFFECT OF THE QUINOLINE RING SUBSTITUTION PATTERN ON THE ACTIVITY OF REVERSED CHLOROQUINE COMPOUNDS

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Antimalarial drug resistance is a serious global problem, and so there remains an ongoing need for new antimalarial drug candidates that will be active against drug-resistant parasites. Historically, chloroquine (CQ) was one of the most important antimalarials, but its utility is now greatly limited by chloroquine resistance (CQR). We have already shown that attachment of a chemosensitizer (reversal agent, RA) to the side chain of a chloroquine-like moiety results in molecules active against CQR strains of *P. falciparum* malaria; we have termed these molecules reversed chloroquines (RCQs). We here examine the effect of the quinoline ring system substitution pattern upon *in vitro* antiplasmodial activity of the RCQs. The compounds presented here include those bearing a substituent in the quinoline 2-, 5, 6-, 7-, and/or 8-position, and include those with chloro, bromo, iodo, fluoro, nitro, trifluoromethyl, methyl, and methoxy substituents. For RCQs, 2-, 5-, and 8- substituents were found to decrease *in vitro* antiplasmodial activity against *P. falciparum* relative to 7-chloro substitution, whereas 6- and 7- substituted compounds with the various substituents have in many cases similar activity to that of 7-chloro substituted RCQs. Little difference was observed between 6- and 7- substitution, or between chlorine and a methyl group in position 6. In most cases these effects on activity are directionally similar to those observed for chloroquine analogs without an attached reversal agent, but the magnitude of the effect is generally smaller, suggesting that the activities of RCQs are less affected by modifications to the quinoline ring system than are chloroquine analogs without an attached reversal agent.

1707

MOLECULAR SURVEILLANCE FOR ARTEMISININ RESISTANCE ASSOCIATED *KELCH 13* MUTATIONS IN *PLASMODIUM FALCIPARUM* SAMPLES FROM THE STATE OF RORAIMA, BRAZIL

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Roraima State is located in the northern region of Brazil bordering both Venezuela and Guyana and is part of the Guiana Shield. Although the majority of autochthonous malaria cases in Roraima are predominately *Plasmodium vivax* infections, recent observations have revealed an increase *in P. falciparum* cases originating mainly from Guyana and Venezuela, with a high proportion of these due to miners moving between these regions. Given that the C580**Y** polymorphism in the *kelch 13* propeller domain of *P. falciparum*, a confirmed marker of artemisinin resistance, was found in about 5% of parasites in Guyana, there is a concern that this mobile population could contribute to the spread of drug resistant parasites in the region. In order to determine if *kelch 13* mutations associated with artemisinin resistance are present in Roraima State, we initiated a molecular surveillance study. In 2016-2017, a total of 429 samples were collected from three sites, namely: Pacaraima (at the border with Venezuela; 129), Boa Vista (the state capital; 243) and Rorainopolis (57). Dried blood spots were collected for molecular diagnosis and characterization of drug resistance markers. Among these 429 samples, there were 136 PCR confirmed *P. falciparum* positive samples which were sequenced for known polymorphisms in both the *kelch 13* propeller domain and the *Pfmdr-1* gene using the Sanger sequencing method. We did not observe any mutations in the *kelch 13* propeller domain in the 126 samples that were successfully sequenced. A total of 115 samples were successfully sequenced for *Pfmdr-1* gene; we observed two *Pfmdr-1* mutant genotypes: 184**F**/1042**D**/1246**Y** (triple-mutant) and 184**F**/1034**C**/1042**D**/1246**Y** (quadruple-mutant) at frequencies of 83% and 17%, respectively. These results suggest that artemisinin resistance mutations found in Guyana have not spread to Roraima State of Brazil. However, given the high mobility of people in this state and the neighboring countries, continued molecular surveillance is essential to detect any potential migration or local emergence of artemisinin resistant mutation in this region.

1708

THE ACT PARTNER DRUG MOLECULAR SURVEYOR: ONLINE MAPPING DATABASE FOR *PLASMODIUM FALCIPARUM* DRUG RESISTANT MOLECULAR MARKERS

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Comparing the prevalence of *pfmdr1* and *pfcrt* molecular markers of antimalarial drug resistance across studies can be challenging due to the abundance and heterogeneity of studies. The purpose of this project was to develop a free online tool, which provided a standardized visualization of the published literature on molecular markers of ACT partner drug resistance, and a downloadable database. A global geospatial database of a selection of *pfmdr1* and *pfcrt* single nucleotide polymorphisms, and *pfmdr1* and *plasmepsin2* copy number variations associated with antimalarial drug resistance was developed, populated with data extracted from the literature published from 2001 to date. We conducted a literature review and designed a standardised method to extract data from publications, made critical decisions on visualisation, and created an online application for displaying the information within 6 months. We present the ACT Partner Drugs Molecular Surveyor database, which currently includes prevalence information linked to point locations, derived from over 570 sites in 76 different countries. The database currently includes results from over 90,000 samples from 453 publications. WWARN launched the ACT Partner Drugs Molecular Surveyor in March 2015 and it has attracted nearly 2,000 unique visitors since then (http://www.wwarn.org/tracking-resistance/act-partner-drug-molecular-surveyor). The database supporting the tool is fully accessible, providing users with a rich resource to explore and analyse. The informatics framework used for developing the tool can be easily adapted to other data, as demonstrated by the subsequent launch of our K13 artemisinin marker surveyor. Extracted data have also been used to build continuous maps for specific areas of the world where more intense marker prevalence mapping is taking place, also demonstrating the usefulness of a centralised standardised database. Further analyses of the ACT Molecular Surveyor dataset will be presented.

1709

EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN KLOUEKANMEY AND DJOUGOU, REPUBLIC OF BENIN

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In 2008, artemether-lumefantrine (AL) was introduced as first-line treatment for uncomplicated *Plasmodium falciparum* malaria in Benin. Following the World Health Organization recommendation to regularly monitor antimalarial efficacy, we conducted an efficacy study of AL for uncomplicated *P. falciparum* malaria in Klouekanmey and Djougou. Febrile patients 6-59 months old with *P. falciparum* monoinfection and 2,000-200,000 parasites/µl were treated with a 3-day course of AL. Clinical and parasitological response was monitored for 28 days. We differentiated recrudescence from reinfection using microsatellite genotyping and calculated uncorrected and corrected adequate clinical and parasitological response (ACPR) rates. We performed molecular analyses for PfK13 propeller and Pfmdr1 gene mutations, associated with artemisinin and lumefantrine resistance, respectively. In Klouekanmey, among the 115 patients who completed follow-up, 2 (1.7%) infections occurred on Days 21 and 25, yielding an uncorrected ACPR of 98.3% (95% confidence interval [CI]= 93.9-99.8). In Djougou, among 120 patients with complete follow-up, there was 1 (0.8%) early treatment failure (signs of severe malaria on Day 1) and 11 (9.2%) infections identified from Day 21 to 28, uncorrected ACPR of 90.0% (95% CI= 83.2-94.7). None of the recurrent infections in Klouekanmey and one in Djougou were classified as recrudescence by genotyping; microsatellite-corrected ACPRs of 100.0% (95% CI= 96.8-100.0) and 98.2% (95% CI= 93.6-99.8), respectively. No PfK13 gene mutations were observed at enrollment. Considering mutant and mixed (mutant and wild type) Pfmdr1 alleles, we observed prevalence of 11.2% and 58.9% for the 86Y and 184F mutations, respectively. AL remains efficacious for falciparum malaria in these two sites in Benin; however, we found a high frequency of the Pfmdr-1 Y184F mutant allele. Our study also revealed high incidence of recurrent infections in Djougou during the 28-day follow-up, highlighting the importance of considering clinical, parasitological, and molecular data together to adequately estimate treatment efficacy and drug resistance.

1710

USE OF DRUGS IN MANAGEMENT OF UNCOMPLICATED MALARIA IN RURAL, URBAN HEALTH CENTERS, AND REFERRAL HOSPITALS IN DEMOCRATIC REPUBLIC OF THE CONGO

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The Democratic Republic of Congo (DRC) is still heavily affected by Malaria despite the adoption of the preventive measures and powerful drugs that have produced tremendous drop in malaria morbidity and mortality worldwide. Inappropriate use of these drugs may contribute to this underperformance. A retrospective review of patients' medical files containing a prescription of an antimalarial in a twelve months period was carried out in 2014 to assess the prescription of drugs in malaria. In each of the former 11 provinces of DRC, one Rural Health Center (RHC), one Urban Health Center (UHC) and one Referral Hospital (GRH) were selected and in each of them, 100 patients' files selected. In 2300 files (69.7%), uncomplicated malaria was the indication for antimalarial prescription. Descriptive analyses were performed. Antimalaria prescription was based on positive malaria tests in 51.5% of cases, without test in 37% and despite negative tests in 11%. RHC relied more on positive tests than GRH (62.7%vs 32.8%; p< .00001) and UHC (62.7% vs 56.7%; p= .015588). GRH prescribed antimalarials to patients with negative tests more than RHC (16.0% vs 4.4%, p<0.00001). The recommended drugs were more prescribed in RHC than GRH (67.3%vs 39;7%; p <0.00001). Quinine and the injectable artemether and alfa-beta arteether, were more prescribed in GRH where they represented 52% of prescriptions. Apart from antimalarials, patients received an average of 3.1 other drugs with no specified indication in 51% of cases. Antibiotics were prescribed to around 68% of patients in all settings. Anthelminthic (p<0.00001) and vitamins (p <0.00001) were significantly more prescribed in RHC. Anemia drugs, were prescribed more in RHC (p=.001848) and GRH (p<0.00001) than in UHC. The use of drugs in uncomplicated malaria is characterised by a low adherence to recommended policies and polypharmacy. Referral Hospitals adhere less whereas Rural health centers adhere more to national policy but prescribe more concomitant medications. Determinant of this inappropriate use of drug and the difference between health facilities need to be assessed.

1711

PLASMODIUM FALCIPARUM ARTEMISININ *IN VIVO* EFFICACY MONITORING AND MOLECULAR DRUG-RESISTANCE SURVEILLANCE IN A PRE-ELIMINATION SETTING IN SABAH, MALAYSIA

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Artemisinin derivatives are the WHO first-line treatment for severe and uncomplicated falciparum malaria. Spreading artemisinin drug-resistance threatens Southeast Asian malaria elimination goals. In Malaysia, the Ministry of Health is implementing drug response surveillance of artemisinin treatment efficacy, but there is only limited evaluation of *Plasmodium falciparum*-molecular artemisinin-resistance markers. This was a multi-centre open-label study conducted in three adjacent district hospitals in northwest Sabah, Malaysia, to assess clearance rates of *P. falciparum* parasitaemia in patients with acute uncomplicated malaria. Febrile non-pregnant patients with a positive *Pf*-HRP2 rapid diagnostic test, microscopic monoinfection with *P. falciparum* and a parasite count of 1,000-100,000/µL were enrolled, with *Plasmodium* species confirmed by PCR. Patients received oral artesunate alone (total target dose 12mg/kg) for 3 days, followed by oral mefloquine. Blood slides were taken 6-hourly to calculate microscopic parasite clearance. Molecular surveillance of 20 SNPs in the *P. falciparum* K13 propeller domain associated with artemisinin-resistance were evaluated by PCR, including in additional patients with falciparum malaria enrolled in a prospective tertiary-referral hospital study for western Sabah. From 2012-15, 59 patients with falciparum malaria were enrolled in the artemisinin *in vivo* efficacy study. Median age was 21 years (IQR 12-31; range 3-62), with 15 (25%) being children ≤12 years. Median parasite count at presentation was 9,074/µL. The median parasite clearance time was 24 hours (IQR 18-42; range 6-72), and median time to 50% parasite clearance was 4.2 hours. All patients were microscopy-negative for parasites by 72 hours. For the molecular surveillance study, a total of 264 patients with falciparum malaria from western Sabah were evaluated for K13 drug-resistance markers, with all SNPs found to be wild-type. In conclusion, artemisinin derivatives remain highly efficacious for the treatment of falciparum malaria in Sabah, Malaysia, with no K13 drug-resistance markers detected.

1712

EVALUATION OF ACCURACY OF MAGNETO-OPTICAL METHOD FOR THE DETECTION OF MALARIA PARASITES

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Malaria is a global public health problem and is prevalent in tropical and subtropical regions. Laboratory techniques to confirm clinical suspicion of malaria have traditionally relied on microscopy and more recently on rapid diagnostic tests (RDTs). Although inexpensive, microscopy is labor intensive and requires skilled personnel. RDTS are relatively easy to perform and do not need specialized facilities. However, accuracy of RDTs is of concern and most cannot reliably detect parasitemia lower than 100-200 parasites/µL. There is an acute need for improved malaria diagnostics that are not only cost-effective but also rapid and highly accurate. Our study evaluated a rapid (<1 minute) malaria detection device called Magneto-Optical Detection (MOD). The principle is based on using a magnetic field to detect hemozoin, a by-product of parasite digestion of hemoglobin. Upon being placed in an intense magnetic field, the iron-bearing hemozoin crystals align within the field, thereby affecting the opacity of the sample. The parasite level is determined by analyzing the resulting signal from a polarized light source passing through the test sample with and without the magnetic field. We are evaluating the diagnostic accuracy of the MOD as a point-of-care diagnostic for malaria, using samples collected from children enrolled in a longitudinal birth cohort study in Busia, Uganda, a high transmission setting. Study participants are ages 6 months to 1 year and have both passive and active (monthly) surveillance for malaria parasites. MOD results are being compared to microscopy, a standard case-management RDT (CareStart Pf HRP-2), and qPCR as a reference standard. Total enrollment is 300 children. Sixty children were tested until now, of whom 25 were tested at the time of acute febrile illness and 35 were tested at a routine asymptomatic visit. Preliminary results show the MOD to have similar sensitivity and much higher specificity than RDTs, and turn-around-time was significantly faster at one-minute than either microscopy or RDTs. Final results after completion of microscopy and qPCR will be presented at the conference.

1713

SHERLOCKING MALARIA: A SENSITIVE, ISOTHERMAL, NUCLEIC ACID-BASED DIAGNOSTIC CAPABLE OF DISTINGUISHING *PLASMODIUM* SPECIES

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Antigen-based malaria rapid diagnostic tests (RDTs) are inexpensive and convenient for field use, but lack the sensitivity and specificity of nucleic acid detection methods. We sought to develop a sensitive diagnostic that combines affordability, high sensitivity, and the ability to distinguish *Plasmodium* species by adapting the Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) method developed by Gootenberg et. al. (*Science* 2017) to detect *Plasmodium falciparum* and *Plasmodium vivax* at levels comparable to current PCR-based assays. This method combines isothermal recombinase-polymerase amplification (RPA), *in vitro* transcription, CRISPR RNA (crRNA) base-pairing, and collateral cleavage of fluorescent RNA reporters by LwCas13a to detect DNA or RNA targets. Previous SHERLOCK assays have achieved sensitivity to attomolar concentrations of nucleic acid, specificity to the single nucleotide level, and rapid read-out times of less than two hours. SHERLOCK has the additional advantage of being less resource-intensive than PCR, with the reaction being run at a constant temperature and read with a simple plate reader. Future directions include adapting our assay for field-deployable lyophilized and lateral flow platforms, as has been previously accomplished with SHERLOCK assays for other pathogens, as well as testing clinical isolates and developing crRNAs capable of detecting other malaria species and drug resistance alleles.

1714

TRACKING HEMOZOIN LEVELS OVER TIME IN SYNCHRONOUS CULTURES OF *PLASMODIUM FALCIPARUM* USING MAGNETO-OPTICAL DETECTION (MOD)

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Hemozoin, a by-product of malaria digestion of haemoglobin, is proposed as a biomarker for malaria. An open question is the time point after invasion when hemozoin becomes detectable. Previous studies have failed

to detect hemozoin in early ring stage parasites. This study evaluates the ability of magneto-optical detection (MOD) to detect parasites throughout a 48-hour incubation. To perform this test, a sample of 3D7 lab strain of *P. falciparum* was synchronized, washed (removing all exogenous hemozoin), and diluted to 5000 parasites/uL and 100 parasites/uL. The MOD device detected hemozoin in these 100% tiny ring stage parasites even before incubation. Then the culture was incubated at 37oC for 48 hours and sampled every 2 hours. A steady increase in hemozoin content in both the low and high parasitemias was observed. This study shows that early ring stage parasites do in fact have quantifiable levels of hemozoin that can be detected by MOD. The presence of hemozoin in all parasite stages demonstrates the usefulness of hemozoin as a biomarker. Future work will repeat the study for other species of malaria.

1715

EVALUATION OF A SINGLE SCREEN AND TREAT STRATEGY FOR PREGNANT WOMEN AT SELECT HEALTH FACILITIES IN LINDI REGION, TANZANIA

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Since 2014, Tanzania has been implementing a policy of single screen and treat (SST) of all pregnant women using HRP-2 based malaria rapid diagnostic tests (mRDT) at first antenatal care (ANC) visit, together with intermittent preventive treatment in pregnant women (IPTp). However, there is paucity of data to show its effectiveness in identifying women with malaria parasites and reducing the adverse effects of malaria in pregnancy (MiP). We evaluated the proportions of asymptomatic and symptomatic women identified by SST to determine its potential utility in reducing adverse effects of MiP. A cross-sectional study was conducted at 8 heath centres selected for high numbers of ANC attendees in two randomly selected districts of Kilwa and Lindi in Lindi Region, Tanzania. Pregnant women attending their first ANC visits were enrolled and retrospective data collected included gestation age, history of fever, mRDT results and treatment provided. From October 2017 to January 2018, 769 pregnant women were tested for malaria (20% in 1st trimester) and 106 (13.8%) had mRDT positive results. The positivity rates at the HF ranged from 0 - 29%; one HF had no asymtomatic women or malaria cases, three had positivity rates <4% (low prevalence), the other four had positivity rates ranging from 17 - 29% (moderate prevalence). Fifty-nine (8%) women reported fever within the past 48 hours; all symptomatic women had positive mRDT results. Forty-seven (6%) women with positive results were asymptomatic. All women with positive mRDTs and none of those with negative mRDTs were treated. In the HFs with low prevalence, only one malaria case was asymptomatic (13%, 1/8), while in those with moderate prevalence the proportion of asymptomatic cases was 47% (46/98), and ranged from 0 - 90%. While in low prevalence areas, SST detected primarily symptomatic women, who should have been identified and treated even in absence of SST policy, in moderate prevalence settings, SST detected a high proportion of asymptomatic women who would have otherwise gone undetected. In this high SP resistance setting, an evaluation of the impact of SST on birth outcomes may be warranted.

1716

MODELING HRP2 DYNAMICS AND THE IMPLICATIONS FOR A NEW ULTRA-SENSITIVE RAPID DIAGNOSTIC TEST (U-RDT)

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Of the rapid diagnostic tests (RDTs) routinely utilized to diagnose symptomatic *Plasmodium falciparum* malaria, the most frequently used detect HRP-2 antigen expressed by the parasites. In 2017 a new ultra-sensitive rapid diagnostic (U-RDT) was released with higher sensitivity at detecting HRP-2. The utility of this new diagnostic for detecting symptomatic and asymptomatic infections is currently being explored in field studies. Although the presence of the parasite and HRP-2 are correlated, HRP-2 typically persists for weeks after an infection has been cleared. We therefore sought to characterize HRP2 dynamics following treatment in order to quantify the likely relationship between malaria infection status and positivity by conventional RDTs and U-RDTs. A bi-phasic exponential decay model was fitted to repeated measures of HRP-2 concentration from non-immune treated individuals. We find that there is an initial rapid decay in the first 1.5 days followed by a subsequent second slower clearance. Combining this with data from the same individuals on their detectability by different diagnostics over time, we estimate that individuals will remain positive for approximately twice as long after treatment by the U-RDT compared to a conventional (HRP2) RDT. Cross-sectional data were then used to estimate the proportion of the infected population (using PCR positivity as the gold standard) that would be detected using the new U-RDT and to estimate the probability of detection by U-RDT and RDT for a given parasite density. For example, we estimate that for a parasite density of 100 parasites/ul, an individual has a 97% probability of being detected using the U-RDT but only 62% by a conventional RDT. Quantifying how the increased sensitivity of the U-RDT corresponds to detecting individuals (with differing levels of parasitaemia and HRP-2) in endemic populations is a vital first step in understanding the utility of the U-RDT in a range of use scenarios such as for surveillance or testing individuals prior to mass treatment interventions.

1717

PERFORMANCE OF A NEW MULTIPLEX FEVER PATHOGEN RAPID TEST TO DETECT MALARIA WHEN USED BY HEALTH WORKERS IN PERU AND NIGERIA

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To manage fever cases at the point of care (POC), new multiplexed rapid tests (RDTs) are needed that can identify non-malaria pathogens when malaria RDT results are negative. This is critically important for outbreak pathogens like Ebola. The ideal test would detect all relevant pathogens in each geography, including malaria, a major cause of fever globally. A new multiplexed RDT, the DPP® Fever Panel Antigen System (Chembio, USA), is designed to detect seven African pathogens (Ebola, Lassa, Marburg, Chikungunya, Dengue, Zika, and malaria) in <15 minutes. To evaluate the malaria component (HRP2 and pLDH for *P. falciparum* and any *Plasmodium* species, respectively) in comparison with microscopy and real-time PCR, FIND conducted field studies between March and October 2017, in 3,200 and 1,300 self-presenting febrile patients in Peru and Nigeria, respectively. Blood samples were collected for microscopy and PCR amplification

of *Plasmodium* mtDNA. Compared to microscopy, DPP® results from fingerprick blood in Nigeria showed good performance of the HRP2 test line to detect *Plasmodium* infections with 98.7% sensitivity [CI95%: 97.2-100.0%] and 88.6% specificity [CI95%: 86.5-90.6%]. The pLDH test line performance to detect any species was lower (70.8% sensitivity [CI95%: 68.0-73.6%] and 91.8% specificity [CI95%: 90.9-92.8%]), in Peru and Nigeria. These results compare favorably to the performance of high quality malaria RDTs used for on-site case management. In comparison to the SD Bioline Malaria Ag P.f/Pan (Alere, USA) in Nigeria, DPP® performance was 99.3% sensitivity [CI95%: 98.4-100.0%] and 96.4% specificity [CI95%: 95.2-97.6%] on the HRP2 test line. In Peru, DPP® performance was 71.2% sensitivity [CI95%: 68.1-74.4%] and 96.2% specificity [CI95%: 95.4-97.0%] on the pLDH test line, compared to the *Carestart*™ pLDH (Pf/PAN) RDT (Access Bio, USA). In sum, the DPP® Fever Panel Antigen System shows good performance for malaria detection in POC settings, comparable to available malaria RDTs. These studies constitute one step toward regulatory approval and the commercial availability of this new POC test for fever-causing infections in Africa.

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DETECTION OF *PLASMODIUM* INFECTION UTILIZING DRIED BLOOD SPOTS AND SIMPLIFIED MICROCAPILLARY CYTOMETRY

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Sample collection, storage and analysis often prove to be challenging for epidemiological studies and malarial disease monitoring in constrained settings. One approach to circumvent this is collecting blood via finger stick/venous draw spotting onto filter paper in the form of dried blood spots (DBS). The dried blood spots are often stored at ambient temperature/refrigerated/frozen conditions and allow for the easy transportation of a large number of samples to study sites for further analysis. These methods have been frequently applied to PCR/molecular detection but less often to multiple malarial antigens for compatibility with assays. Here we present results to detect *Plasmodium* infections after extraction from DBS and analysis on an affordable miniaturized flow cytometry platform, the Muse® Cell Analyzer utilizing a recently developed bead multiplexed bead based immunoassay for multiple *Plasmodium* antigens. This bead based assay allows for a highly sensitive, multiplexed distinction of *Plasmodium falciparum*, *Plasmodium vivax* and mixed infections. Utilizing blood spotted and stored on filter paper, stored at either room temperature or frozen , we were able to successfully detect both HRP2 and LDH antigens for *falciparum* based infection and LDH antigen for *vivax* based infection or all above antigens for mixed infection for positive samples across a broad range of parasitemia. Clear distinction was possible for positive, negative and mixed samples in the limited sample set studied. Data from longitudinal studies of storage of DBS and application to wider sample set will also be presented along with dilution of samples to determining the lower level of detection of the assay with dried blood spots. The combination of the Muse *P.f/P.v.* bead assay along with dried blood spot extraction and paired with simplified analysis on Muse® Cell Analyzer can provide comprehensive and sensitive solutions from collection, storage to analysis in an easy to use format that would be usable in diverse settings.

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TOWARDS A GLOBAL MAP OF *PLASMODIUM FALCIPARUM HRP2* DELETION FOR THE SELECTION OF OPTIMAL RDTS FOR MALARIA DIAGNOSIS

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Rapid diagnostic tests (RDTs) are an indispensable tool for diagnosis of clinical malaria and screening of asymptomatic carriers. The most sensitive RDTs for *P. falciparum* rely on detection of the HRP2 protein, but deletion of the *hrp2*-gene results in false-negative results. The extent of *hrp2*-deletion is not known in many countries where RDTs are used. It is also unclear whether the use of HRP2-based RDTs is a selective force strong enough to result in an increase of *hrp2*-negative parasites over time. Molecular monitoring of the extent of *hrp2* deletion is crucial to select the optimal diagnostic tools and to understand the spread of *hrp2*-negative parasites, but laboratory testing has been challenging. We have developed a highly-sensitive, high throughput method to screen for *hrp2* and *hrp3* deletion, based on droplet digital PCR (ddPCR). Our method is able to detect mixed infections with wild-type and *hrp2*-negative parasites. Analysis of samples from East Africa and other sites shows substantial differences between sites. Use of screening methods that cannot detect mixed infections of wild-type and *hrp2*-negative clones in regions with frequent multiple clone infection underestimates the true frequency of *hrp2*-deletion. In conclusion, screening by ddPCR greatly facilitates molecular surveillance of *hrp2* and *hrp3* deletion, and can inform malaria control programs on the optimal selection of diagnostic tests.

1720

MODELLING THE IMPACT OF AN ULTRA-SENSITIVE *PLASMODIUM FALCIPARUM* RAPID DIAGNOSTIC TEST (U-RDT): DETECTING ASYMPTOMATIC INFECTIONS AND THE POTENTIAL FOR OVERTREATMENT

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In 2017 a new ultra-sensitive *Plasmodium falciparum* rapid diagnostic test (U-RDT) with a limit of detection ten times lower than current RDTs was released. To better understand the potential utility of this novel tool, we used modelling to explore its value in different use case scenarios. We extended an existing malaria transmission model using data on the U-RDT to estimate how this 10x increased sensitivity translates to the proportion of the population and proportion of the infectious reservoir that would be detected in different transmission settings. In high transmission settings, we predict that this test would detect 95% of the infectious reservoir (compared to 55% with a conventional RDT). We then use the model to explore the impact of two asymptomatic infection detection interventions: i) active infection detection using the U-RDT in the households of symptomatic cases, where a combination of DHS and trial data are used to estimate the increased probability of infection in a household with symptomatic infection ii) Mass Screen and Treat, where preliminary results indicate that this diagnostic would reduce the number of years the intervention would be needed to interrupt transmission, for example, in a setting with an EIR of 1, 3 years of MSAT are needed to interrupt transmission compared to 8 years with a conventional RDT. We also consider the potential for overtreatment if the U-RDT is used for diagnosing symptomatic malaria. Individuals that have asymptomatic malaria infection or have recently been successfully treated for a malaria infection are likely to have positive HRP-2 concentrations (the antigen detected by the U-RDT) - if these individuals seek treatment for a non-malarial fever, there is an increased probability that they will be misdiagnosed as having malaria as their primary cause of fever. Data from challenge studies to estimate HRP-2 clearance after treatment and DHS data to estimate the prevalence of non-malarial fevers is used to estimate

the additional number of antimalarial doses that will be mis-administered if the U-RDT is used to diagnose symptomatic malaria in a range of transmission settings.

1721

MOLECULAR DIAGNOSIS FOR SURVEILLANCE OF ASYMPTOMATIC MALARIA IN THE PERUVIAN AMAZON

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Malaria in the Peruvian Amazon is an important public health problem that seeks to be reduced from this region in 2021 with the new "Malaria Zero Plan". There is a great need to know the best diagnostic strategies. We evaluated the diagnostic performance of the hemi-nested, multiplex polymerase chain reaction (PCR) comparing two different DNA extraction methods, to nested-PCR alongside Giemsa microscopy. One hundred and sixty-six participants from the community of Zungarococha (Iquitos, Peru) were recruited during cross-sectional surveys and screened for *Plasmodium* parasites by hemi-nested-PCR, nested-PCR assays and Giemsa microscopy. The isolation of DNA from blood was carried out using the DNeasy Blood and Tissue kits (QIAGEN) and the simple extraction buffer (400mM NaCl, 40 mM Tris pH 6.5, 0.4% SDS). *Plasmodium vivax* cases confirmed by Giemsa microscopy, nested-PCR, hemi-nested-PCR with the simple extraction buffer and hemi-nested-PCR with QIAGEN were 6.6%, 28.3%, 18.1% and 36.8%; and for *P. falciparum* cases were 3%, 4.2%, 1.3% and 3.6%, respectively. Only the hemi-nested-PCR with QIAGEN found one mixed infection (0.6%). Considering hemi-nested-PCR with QIAGEN as gold standard, microscopy was able to detect 17.7% and 57.1% of any Pv and Pf infection, respectively; while nested-PCR detected 69.4% and 28.6%; and hemi-nested-PCR with the simple extraction buffer 47.4% and 71.4% of the Pv and Pf cases, respectively. Both molecular methods showed higher specificity than 96%. Low density submicroscopic asymptomatic *Plasmodium* carriage is common in Zungarococha and microscopy diagnosis is less sensitive to detect human reservoir with low levels of parasite densities. Our data confirms that molecular methods should be used to identify parasites carriers to guide mass screening of malaria control and elimination programs. The easy and low cost of the simple buffer extraction should be considered for its application in laboratories of low resources.

1722

EVALUATION OF A MULTIPLEXED PLASMODIUM LACTATE DEHYDROGENASE BASED ASSAY USING SIMPLIFIED CYTOMETRY ON MUSE® CELL ANALYZER

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Malaria remains one of the leading cause of mortality rate at global level, prompting the need to develop effective and sensitive research and diagnostic strategies. Three antigens have been typically used for multiple tests in particular RDT's: *Plasmodium* Histidine Rich Protein 2 (HRP2), lactate dehydrogenase (LDH) and aldolase. However, RDT's are liable to several challenges and difficulties, including inadequate sensitivity, specificity and false-positive/negative outcomes. Recent studies have reported *P.f.* HRP2/3 gene deletions resulting in false negative results while the delayed clearance of the HRP2 antigen is not reflective of current infection. Due to these implications, there is current need for alternative antigens with high sensitivity performance as HRP2. We recently demonstrated the utility of the touch screen based cytometer, the Muse® Cell Analyzer and bead based assays for sensitive and multiplexed malarial antigen detection. In this study, we further expanded the approach by evaluating a multiplexed assay for detection of *P.f.* LDH, *P.v*. LDH and Pan LDH in parallel . The addition of the pan LDH marker allows for potential capability to detect the four major *Plasmodium* species. In data obtained on frozen blood samples, the assay shows capability to detect and identify *P. falciparum* and *P. vivax* LDH as well as *P. malariae* samples. Limitation in sample availability have not yet allowed for testing in *P. ovale* samples. Data from frozen blood samples across a range of parasitemia demonstrates good performance with the assay. Performance data across a wider range of parasitemia will also be presented. The multi-LDH assay has also shown good specificity for LDH type without cross reactivity. Dilution studies demonstrate low sensitivity of detection in the order of 5 parasites/uL for *falciparum* and *vivax* antigens. The availability of a simplified YES/NO robust LDH based assay on Muse® Cell Analyzer that can detect expanded plasmodium species provides a powerful tool for malaria applications in a sensitive easy to use format.

1723

CROSS REACTION OF ANTI-*PLASMODIUM FALCIPARUM* PLDH ANTIBODIES WITH *PLASMODIUM MALARIAE* PLDH USING THE RAPID DIAGNOSTIC TEST SD BIOLINE MALARIA PF/PF/PV (05FK123)

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Diagnosis of malaria is based on thick blood smear, still considered the reference test; however, its sensitivity depends on the expertise of the examiner. Rapid diagnostic tests (RDTs) dispenses expertise and can be performed at the point of care. In endemic areas, the laboratories lack facilities and in non endemic areas it is very difficult to maintain well trained personal. Molecular protocols can only be conducted in reference centers, leading to the search for accurate RDTs to be used in the routine, avoiding indiscriminate use of antimalarials. We describe a cross reaction of anti-*Plasmodium falciparum* pLDH antibodies with *P. malariae* pLDH using the RDT SD Bioline Malaria Pf/Pf/Pv (05FK123). Samples from positive patients by thick blood smear and nested PCR were tested: 10 positives for *P. falciparum*, 10 for *P. vivax* and 9 for *P. malariae*. The SD Bioline Malaria Ag Pf/Pf/Pv test is defined as a rapid, qualitative and differential test for the detection of histidine-rich protein II (HRP-II) antigen of *P. falciparum* (band T1), pLDH of *P. falciparum* (band T2) and pLDH of *P. vivax* (band T3) in human whole blood. Tests were conducted according to the manufacturer instructions. Among *P. falciparum* isolates, sensitivity was 100.0% (95% CI 72.2-100.0) considering both antigens and 90.0% (95% CI 59.6-100.0). For *P. vivax*, sensitivity was 100.0% (95% CI 72.2-100.0) for pLDH. Among *P. malariae* isolates, 88.9% (95% CI 56.5-98.0) cross reacted with anti-*P. falciparum* pLDH antibodies. Some advantages attributed to the SD Bioline Malaria Pf/Pf/Pv are the differential detection of *P. falciparum* and *P. vivax*, usefulness in regions where both species occur, ability of identifying false positives by HRP-II after treatment and usefulness in regions where HRP-II gene deletion is suspected. The cross reactions observed here point out to the need for developing of more specific TDRs, avoiding the misdiagnosis of *Plasmodium* species and the unsuitable treatment especially in resource-limited settings.

1724

REFINING OPERATIONAL STRATEGIES TO DETECT IMPORTATION AND ELIMINATE RESIDUAL *FOCI* OF MALARIA TRANSMISSION IN KWAZULU-NATAL, SOUTH AFRICA

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The South African province of KwaZulu-Natal (KZN) is extremely close to malaria elimination, with only 106 local cases in 2017, down from over 22,000 in the year 2000. A core intervention used by the KZN Malaria Control Program has been a multi-pronged approach to case detection, namely passive case detection, reactive case detection in response to local cases, proactive case detection in communities with a history of malaria transmission, and proactive case detection in border areas and amongst at-risk highly mobile populations. To reach elimination, the program conducted a survey between February and March 2018 aimed at (1) investigating potential drivers of transmission that may have been missed through other program activities, such as a potential for an asymptomatic reservoir, (2) investigating the potential need for interventions that relate to travel, travel pathways, and imported malaria, and (3) investigating the operational utility of a highly-sensitive RDT to identify a potential asymptomatic reservoir. To address these aims, the survey was conducted both at the household-level in communities as well as at known gathering places along borders, including border markets and taxi stands. Preliminary findings from this survey indicate that border market locations are a key location to target for active case detection (ACD). Based on these preliminary results, the proactive case detection in border markets and taxi stands yielded a positivity rate of 7% (65/890), compared to 0.19% (4/2,101) in at-risk communities, which relates closely with the rate of 0.012% (12/100,153) through routine ACD in 2016. In the survey, 95% of cases discovered (65 of 69) in the survey were at those border markets and taxi stands versus in the community. Of those 5% of cases discovered in the community (4 of 49), all had recent travel history to malaria endemic areas of Mozambique. All individuals who tested positive via the highly sensitive RDT tested positive by standard RDT. This analysis will provide detail about lessons learnt during ACD as the province nears elimination, and to inform its ACD strategy moving forward.

1725

IDENTIFICATION OF THE ZOONOTIC MALARIA SPECIES *PLASMODIUM KNOWLESI* IN ACEH PROVINCE, INDONESIA: DIAGNOSTIC SENSITIVITY, TRUE BURDEN AND IMPLICATIONS FOR ELIMINATION

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Plasmodium knowlesi is the cause of zoonotic human malaria in southeast Asia and its presence was reported for the first time in Aceh Province, Indonesia, in 2016. Due to morphological similarities with the three other human malaria species historically dominating transmission in this area (*P. falciparum, P. vivax* and *P. malariae*), it is possible that this species is routinely misidentified and its burden underestimated. Detailed evidence on the true burden of *P. knowlesi* infection relative to other species is crucial to provide appropriate case management guidelines and plan elimination strategies. This study used molecular methods to detect and identify *Plasmodium* species amongst all microscopy-positive malaria cases and a group of microscopy-negative controls presenting to health facilities or identified during reactive screening activities in two districts (Aceh Besar and Aceh Jaya Districts) in Aceh Province, from April 2017 and ongoing through September 2018. Malaria species identification methods by microscopy and loop mediated isothermal amplification (LAMP), were confirmed by nested PCR targeting the 18 small sub-unit ribosomal RNA (18S rRNA). The species-specific diagnostic sensitivity and specificity of microscopy and LAMP will be calculated using nested PCR as a gold standard. To date, nested PCR results confirmed that more than 70% of malaria cases were infected with *P. knowlesi*. Our finding suggests that this zoonotic infection is the most common cause of indigenous malaria in this area and commonly misidentified, which has important implications for diagnostic testing and case management.

1726

THE PREVALENCE AND DISTRIBUTION OF *PLASMODIUM* SPECIES AMONG CHILDREN AT MALABO REGIONAL HOSPITAL ON BIOKO ISLAND, EQUATORIAL GUINEA

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According to the World Health Organization (WHO), *Plasmodium falciparum* (Pf) infections account for about 99% of the total malaria cases in Africa. Thus antimalarial drugs and vaccines are mostly developed to target Pf infections. However, most malaria-endemic countries are increasingly recording mixed *Plasmodium* infections involving two or more of the species. Artemisinin-based combination therapies (ACTs), which are effective against blood stage infections, are recommended by WHO for the treatment of uncomplicated malaria caused by Pf. Relapses of *Plasmodium vivax* (Pv) and Plasmodium ovale (Po) blood stage can occur months after treatment of primary blood stage infections. Interactions of mixed *Plasmodium* species infections can influence the severity of the disease. Understanding the extent of mixed Plasmodium infections and species distribution on Bioko Island is important for the ongoing malaria vaccine trials in Equatorial Guinea. A total of 237 confirmed malaria cases were examined by microscopy to determine species-specific parasitemia and the prevalence of mixed infection at the Malabo Regional Hospital among children between one to 14 years old. Three species of *Plasmodium* were identified, *P. falciparum, P. malariae, and P. ovale*. Infections with *P. falciparum* alone accounted for 84.8% of the total cases, and that of *P. malariae* alone was 1.7%. Mixed infection of *P. falciparum* with *P. malariae* was 13.1%, while mixed infection of *P. malariae* with *P. ovale* accounted for 0.4%. Malaria vaccines and control strategies targeting only the dominant species could end up replacing the less dominant species. It is therefore important to establish the prevalence and the distribution of the different species of human Plasmodium parasites in control programs.

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THE HEMATOLOGICAL RESPONSE FOLLOWING CHLOROQUINE TREATMENT OF *PLASMODIUM VIVAX* WITH OR WITHOUT PRIMAQUINE: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Primaquine (PQ) is the only widely available treatment for the prevention of *Plasmodium vivax* relapse. Malaria causes anaemia that can be compounded by primaquine-induced haemolysis, particularly in glucose-6-phosphate-dehydrogenase (G6PD) deficient individuals. The aim of this study was to determine the haematological response following *P. vivax* malaria and the attributable haemolysis induced by primaquine. We conducted a systematic review to identify all prospective *P. vivax* therapeutic clinical trials published between January 2000 and March 2017. Individual patient data were pooled using standardised methodology and the haematological response estimated using a linear mixed effects model with non-linear terms, controlling for confounding factors. In total, 3,421 patients from 29 studies were included in the analysis, 99.2% (3,393/3,421) of which had normal or unknown G6PD status. In the 1,975 patients treated with chloroquine (CQ) alone, Hb fell to a nadir on day 2, before subsequently recovering rapidly and plateauing after day 7. Higher baseline Hb was associated with a greater absolute fall in Hb; in those with a baseline Hb ≤11.5 g/dL, 72.2% (182/252) had no fall in Hb during early follow-up. In the 1,446 patients treated with CQ+PQ, the Hb at day of nadir was only -0.13 g/dL (95%CI -0.27, 0.01; p=0.072) less than in patients treated with CQ alone. However, by day 42 the mean Hb was higher by 0.49 g/dL (95%CI 0.28 to 0.69; p<0.001) among patients treated with CQ+PQ compared to those treated with CQ alone. The Hb on day 42 was significantly lower (-0.72 g/dL, 95%CI -0.90, -0.54; p<0.001) in those patients with recurrent parasitaemia. In summary, the fall in Hb following the treatment of vivax malaria in patients enrolled in therapeutic clinical trials primarily relates to malaria, rather than treatment with PQ. Treatment with PQ leads to a higher overall Hb by day 42, likely due to prevention of recurrence.

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INTERMITTENT PREVENTIVE THERAPY WITH SULPHADOXINE-PYRIMETHAMINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE FOR THE PREVENTION OF MALARIA AND IMPROVEMENT OF BIRTH OUTCOMES

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In recent studies, intermittent preventive treatment of malaria in pregnancy (IPTp) with dihydroartemisinin-piperaquine (DP) was superior to sulfadoxine-pyrimethamine (SP) at reducing the risk of maternal malaria and placental malaria. However, previous studies were not sufficiently powered to look at differences in birth outcomes and SP was dosed less frequently than the updated recommendations of once a month. To address some of these limitations we conducted a double-blinded, randomized, controlled trial of 782 HIV-uninfected pregnant women in Busia District in eastern Uganda, an area where malaria is highly endemic and SP resistance is widespread. Participants between 12 and 20 weeks gestation were randomized in equal proportions to IPTp with monthly SP or monthly DP. The primary outcome was the risk of a composite adverse birth outcome defined as low birth weight (LBW), preterm birth, or small for gestation age among live births. Secondary outcomes included measures of malaria during pregnancy and at delivery, individual adverse birth outcomes, and measures of safety. Among enrolled women, 687 (87.9%) were followed through delivery. There was no significant difference in the risk of our composite adverse birth outcome between the DP and SP treatment arms (16.0% vs 18.2%, p=0.45). However, among primagravid women, there was a trend towards a lower risk of LBW in the DP arm compared to the SP arm (5.5% vs 14.3%, p=0.08). During pregnancy, DP was associated with a significantly lower incidence of symptomatic malaria (0.02 vs 0.52 episodes PPY, p<0.001), parasite prevalence (0.5% vs 30.8%, p<0.001), and anemia (9.9% vs 19.7%, p<0.001). At delivery, DP was associated with a lower risk of placental malaria by placental blood smear (0.3% vs 8.8%, p=0.001), placental blood LAMP (2.1% vs. 22.4%, p<0.001), and histopathology (28.4% vs 61.2%, p<0.001). Both regimens were safe and well tolerated. In our setting monthly IPTp with DP was safe and far superior to SP in the prevention of maternal malaria, maternal anemia, and placental malaria, however, this did not translate into a clear benefit for reducing the risk of adverse birth outcomes.

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A RANDOMIZED CONTROLLED TRIAL OF REGULARLY DOSED PARACETAMOL (ACETAMINOPHEN) TO REDUCE RENAL DYSFUNCTION IN *PLASMODIUM KNOWLESI* MALARIA VIA REDUCTION OF CELL FREE HEMOGLOBIN MEDIATED OXIDATIVE DAMAGE; PACKNOW

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Plasmodium knowlesi is the most common cause of human malaria in Malaysia and is present throughout SE Asia. Haemolysis associated acute kidney injury (AKI) is a frequent complication. Short and long term adverse consequences of AKI are well characterised; adjunctive therapies are therefore needed to reduce the impact of AKI in malaria. In falciparum malaria, cell free haemoglobin (CFHb) mediated oxidative damage and endothelial activation contribute to AKI. Paracetamol improves renal function in falciparum malaria, likely via inhibiting CFHb induced oxidative damage. The aim of PACKNOW is to assess whether regularly dosed paracetamol reduces the incidence and severity of AKI in knowlesi malaria by attenuating haemolysis mediated mechanisms. PACKNOW is a two arm, open label randomised controlled trial of adjunctive paracetamol versus no paracetamol in patients aged ≥5 years with knowlesi malaria, conducted over 18 months at 4 hospitals in Sabah, Malaysia. Enrolments finished in 2018, with 379 Pk patients randomised: 188 to regular paracetamol, and 191 to no paracetamol. Median age was 36 years, 85% were male. Thirty five patients (8.8%) had severe malaria on enrolment. AKI by KDIGO criteria was present on enrolment in 115 (30%) patients (60 in the control arm, 55 in the paracetamol arm); and 24 (6%) patients developed AKI after enrolment. The primary endpoint is change in creatinine from enrolment to 72 hours using enrolment creatinine as a covariate (by ANCOVA), stratified by CFHb. Secondary endpoints include longitudinal changes in markers of oxidative stress (plasma F_2-isoprostanes and isofurans), endothelial activation, and biomarkers of AKI over 72 hours. Laboratory analyses are underway; results from the PACKNOW study will be presented. Paracetamol is safe and widely available; if a renoprotective effect is demonstrated, this trial will support administration

of regularly dosed paracetamol to patients with knowlesi or falciparum malaria - species associated with significant haemolysis and AKI. Secondary outcomes will determine mechanisms of haemolysis induced AKI in malaria, and other haemolytic diseases.

1730

HEPATIC SAFETY OF REPETITIVE TREATMENT WITH PYRONARIDINE-ARTESUNATE (PA) AND ARTEMETHER-LUMEFANTRINE (AL) IN PATIENTS WITH UNCOMPLICATED MALARIA IN BOBO-DIOULASSO, BURKINA FASO

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Although its high effectiveness against malaria, PA has been associated with sparse cases of increased AST/ALT suggesting high risk of hepatotoxicity if used for retreatment in patients. In this study, we aimed to evaluate the real hepatic impact of the retreatment with PA versus AL in patients with uncomplicated malaria in Burkina Faso. From August, 2012 to November, 2015 patients with uncomplicated malaria were randomized into PA and AL arms and followed up during 2 years. Each subsequent episode was treated with the same ACT. Hepatotoxicity was assessed using spectrophotometric assay for AST/ALT, PAL, total and direct bilirubin measurement on days 0 (baseline), 3, 7, 28 and unexpected days during a 42-day follow-up. The risk of hepatic adverse events (HAE) was compared between 2 groups in each arm: first administration of study drug and retreatment groups. The associations between treatment modalities and the risks of HAE were determined. In PA arm, the overall risk of serious HAE (severity grade ≥ 3) was reduced in the re-treated group (1.99%) compared to the first administration group (6.82%), p = 0.005. In AL arm, this risk was similar between the re-treated and the first administration groups with 3% versus 3.38% respectively, p = 0.769. One adverse event complying with a Hy's law case was registered in the first administration group of PA arm. After adjusting for age, sex and hepatic biochemical parameters, the odds of adverse events attributable to increased concentrations of ALT were reduced by 70% (p=0.004) and 66% (p=0.018) in retreated patients from PA and AL arms respectively, compared to the groups of first drugs administration. Retreated patients from AL arm presented 80% less risk of increased ASAT like AE. Retreatment with PA had shown a better overall hepatic safety profile than that of its first administration. Retreatment with PA was as well tolerated by the liver as retreatment with AL.PA could be a good alternative for the treatment of repeated malaria episodes in endemic areas.

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EFFICACY AND SAFETY OF PYRONARIDINE-ARTESUNATE AND ARTEMETHER-LUMEFANTRINE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA AND MOLECULAR DETECTION OF RESIDUAL PARASITEMIA IN KENYAN CHILDREN

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Artemisinin-resistance is rapidly rising in South-East Asia and may spread to Africa. Therefore, alternative treatment options must become available. Pyronaridine-artesunate (PA) is a new artemisinin-based combination therapy. Efficacy and safety of PA was compared with artemether-lumefantrine (AL) to treat uncomplicated *Plamodium falciparum* malaria in children. Furthermore, monitoring parasite clearance dynamics after treatment is needed to determine whether responsiveness to artemisinin-based combination therapies (ACT) is changing in Africa. A phase III open-label randomized controlled non-inferiority trial was conducted in Western Kenya. Children aged 6 to 144 months and microscopically confirmed *P. falciparum* malaria were randomly assigned in a 1:1 ratio to receive PA or AL, dosed according to bodyweight, for three days and safety and efficacy of these combinations was assessed. In addition, parasite clearance was evaluated over 7 days following start of treatment by qPCR and direct-on-blood PCR Nucleic Acid Lateral Flow Immunoassay (db-PCR-NALFIA), a simplified molecular malaria diagnostic. ay-28 adequate clinical and parasitological response in the per-protocol population, PCR-corrected for reinfections, was 98.9% for PA and 96.4% for AL. Adverse events occurred in 41.6% and 34.4% of patients in the PA group and the AL group, respectively. Residual parasitemia (dayD 7) was detected by qPCR in 37.1% of AL-treated children and in 46.1% of PA-treated participants. Db-PCR-NALFIA detected residual parasites at day 7 in 33.3% and 30.3% of AL and PA-treated participants, respectively. qPCR determined parasitemia at day 7 was associated with increased prevalence and density of gametocytes at baseline and during follow-up. A positive db-PCR-NALFIA outcome at day 7 was associated with treatment failure, but this association was not found for qPCR. PA was well tolerated, efficacious and non-inferior to AL for the treatment of uncomplicated *falciparum* malaria in children and inclusion of PA in pediatric malaria treatment programs should be considered. To predict treatment outcome, db-PCR-NALFIA may be more suitable than qPCR.

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DRUG RESISTANCE ASSESSMENT OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA IN NINH THUAN PROVINCE, SOUTH-CENTRAL VIETNAM

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Plasmodium falciparum and *Plasmodium vivax* malaria resistance to the first-line treatment regimens of dihydroartemisinin-piperaquine (DHA-PPQ) and chloroquine (CQ), respectively, has been reported in southern Vietnam. Information on the distribution of DHA-PPQ and CQ resistance in different regions of the country is required to inform on antimalarial drug policy for the Vietnam Ministry of Health. Such information will also be needed for implementing malaria elimination strategies. To assist in identifying and monitoring for drug resistance MIPM, with support from IMPE, AAMI and NMRC-A conducted therapeutic efficacy trials in Phuoc Chien Commune (Ninh Thuan Province) in south-central Vietnam. In 2015-2016, 27 patients with *P. falciparum* mono-infections consented to be treated and after randomization received either 4 days of artesunate followed by 3 days of DHA-PPQ (n=13) or 3 days of artemether-lumefantrine (AM-LUM) (n=14). Sixteen patients received 3 days of CQ for *P. vivax* mono-infection treatment. In 2015 and 2016, malaria cases markedly decreased throughout Vietnam, which was also seen at Phuoc Chien Commune resulting in a low number of participants for the two studies. Nevertheless, the clinical and efficacy parameter data obtained after treatment, as well as molecular analysis of genetic markers of drug resistance and *in vitro* drug susceptibility of clinical *P. falciparum* isolates did not reveal reduced susceptibility to artesunate, DHA-PPQ or AM-LUM resistance. All falciparum patients were blood film negative by day 3 after starting treatment and no recrudescences were observed during a 42 day follow-up period. CQ-resistant *P. vivax* malaria did not appear to be present in the small number of patients evaluated (n=16) with two patients having a recurrence of vivax malaria on day 28 of follow-up, but with low drug concentrations. Treatment regimens were well tolerated and no serious adverse events were reported in both studies. Demographic,

clinical, molecular and *in vitro* data, as well as blood drug exposure concentrations will be presented and the application of the information for malaria elimination will be discussed.

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THE EFFECT OF DIFFERENT DOSING REGIMENS ON THE ANTIMALARIAL EFFICACY OF ARTESUNATE-MEFLOQUINE: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Artesunate-mefloquine (AS-MQ) is one of the leading artemisinin combination therapies (ACTs) used to treat uncomplicated falciparum malaria worldwide. Different dosing regimens have been used since it was first introduced, culminating in the creation of a fixed-dose combination which was prequalified by the World Health Organization in 2014. AS-MQ has been used extensively in Asia but there is only limited experience of its use in sub-Saharan Africa where malaria is predominantly a disease of under five year olds. Pooling individual patient data is a powerful tool for exploring the impact of mg/kg dosing on efficacy of antimalarial medicines in different patient groups, particularly young children who are often underrepresented in clinical drug development. In this analysis of data from patients treated with different regimens of AS-MQ we explore the relationship between mg/kg dose of mefloquine and timing of dosing and parasite recrudescence. A systematic search of the literature (PubMed, Embase, Web of Science) was conducted to identify all studies published between 1990 and 2017, in which patients were enrolled and treated with AS-MQ. Investigators were invited to share their data and participate to the study group. Individual patient data were pooled using a standardised methodology. Univariable and multivariable risk factors for parasite recrudescence were identified using a Cox's regression model with shared frailty across the study sites. The primary endpoint was the PCR-adjusted risk of *Plasmodium falciparum* recrudescence by day 42. Data from 37 studies from 19 countries from Asia, Africa and South America were shared, including 9445 patients with 211 recrudescent and 472 new infections reported. The majority of patients were adults (50), 15% were children younger than 5 years of age and 35% were children 5-15 year olds. Results of the analysis will be presented and discussed, focusing on the effect of dose on the risk of recrudescence.

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IMPACT OF CYP2D6 ON PRIMAQUINE AND TAFENOQUINE EFFICACY IN *PLASMODIUM VIVAX* MALARIA PHASE 3 CLINICAL TRIALS

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There is emerging evidence that metabolic activation of primaquine (PQ) by CYP2D6 is required for anti-hypnozoite activity. As tafenoquine (TQ) is in the same class as PQ, we examined the impact of CYP2D6 metabolism on PQ and TQ efficacy in two previously reported phase 3 clinical studies, DETECTIVE (NCT01376167) and GATHER (NCT02216123). Both arms were in combination with chloroquine. We derived CYP2D6 metabolizer class by summing the activity of a subject's two CYP2D6 *alleles with a value of 0 for a null allele, 0.5 for a reduced activity allele and 1 for a full function allele. Subjects with activity sums of 0, 0.5 to 1, and 1.5 to 2 were classified as poor (PM), intermediate (IM) and extensive (EM) metabolizers, respectively. Subjects who were confirmed recurrence-free or had a recurrence over 6 months post treatment were included in the analyses. Logistic regression models with geographic region as a covariate were fitted to assess the effect of CYP2D6 metabolizer class on recurrence-free efficacy by treatment arm and study. As there were ≤3 PM per arm by study, the PMs and IMs were combined and compared to the EMs. Allele frequencies varied by geographic region aligning with published regional frequencies. In the DETECTIVE PQ arm, recurrence risk increased with reduced activity (N=116, Odds Ratio (OR) = 2.36, p=0.032) where all 3 PM had recurrence. When PM were excluded from analysis, the effect in the DETECTIVE PQ arm was not significant (p=0.10). No evidence of an effect was seen in the DETECTIVE TQ arm (N=231, OR=1.05, p=0.44) in which 1 of 3 PM had recurrence. No effect was seen in the GATHER study for either arm (TQ arm N=134, OR=0.89, p=0.60; PQ arm N=65, OR=0.76, p=0.64). The 1 PM in GATHER was in the TQ arm and recurrence-free. In summary, neither study showed evidence of a CYP2D6 effect on TQ efficacy. These results suggest that PMs are more likely to suffer a recurrence with PQ treatment (3 of 3 PMs with a recurrence), but not with TQ treatment (1 of 4 PMs with a recurrence); however, a limitation of these findings is the small number of PM subjects and that we cannot distinguish relapses from new infections in this study.

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SAFETY, EFFICACY, TOLERABILITY AND PHARMACOKINETICS OF AZITHROMYCIN PLUS PIPERAQUINE AS A CANDIDATE FOR INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY

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Malaria in pregnancy remains a major cause of maternal anaemia, low birthweight and increased perinatal mortality. One of the strategies used to improve obstetric outcome is intermittent preventive treatment of malaria in pregnancy (IPTp). The World Health Organization-recommended IPTp is sulfadoxine-pyrimethamine (SP) but the spread of SP-resistant *Plasmodium falciparum*, and relatively short half-lives of the component drugs has prompted investigation into alternative therapies. Azithromycin (AZI) is one antimalarial compound that has received recent consideration given its known safety during pregnancy and efficacy against other pathogens, including sexually transmitted infections. The present study investigated the combination of AZI plus PQ as a candidate for future IPTp. One hundred and fifty pregnant Papua New Guinean women (>14 weeks gestation) were recruited and randomised to receive either AZI+PQ (1 g AZI plus 960 mg PQ phosphate) daily for three days, or a single dose of SP (1,500 mg SDOX and 75 mg PYR; current PNG IPTp regimen). Detailed follow-up assessment was performed on Days 1, 2, 3, 4, 7, 14, 21, 28, 42 and time of delivery for efficacy, safety and tolerability outcomes. Each participant recruited into the intensive pharmacokinetic (PK) sampling protocol (first 30 women in AZI+PQ treatment group) were randomly assigned to 7 of 18 time points (1, 2, 3, 6, 12, 24, 32, 40, 48 and 72 h, and Days 4, 5, 7, 10, 14, 21, 28 and 42) over the 42 day study period. Breast milk samples (fore- and hind-milk) for drug assay were collected from all participants for 28 days after the establishment of lactation. This study reports the first investigation of AZI+PQ as candidate IPTp or for pregnant women. Preliminary data demonstrate a good safety, efficacy and tolerability profile for AZI+PQP. PK analysis of all drug assay samples will be performed using liquid chromatography mass spectrometry with population PK modelling of plasma concentration-time data. The results of this study could have profound impacts on future IPTp regimens and women's health in malaria endemic regions.

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HEMOGLOBIN DECLINE IN G6PD NORMAL *PLASMODIUM VIVAX* PATIENTS TREATED WITH CHLOROQUINE AND TAFENOQUINE OR PRIMAQUINE IN THE PHASE 3 TAF112582 (DETECTIVE) STUDY

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Tafenoquine (TQ) is a novel 8-aminoquinoline in development for radical cure of *Plasmodium vivax* malaria but like all 8-aminoquinolines can cause haemoglobin decline in G6PD deficient individuals. TAF112582 (DETECTIVE Part 2, NCT01376167) was a phase 3 randomised, double-blind, placebo-controlled study. This multi-centre study recruited 522 G6PD normal patients with *P. vivax* malaria. Patients in all three treatment arms received chloroquine and were randomised to receive TQ 300mg single-dose (N=260), placebo (N=133) or primaquine (PQ) 15mg daily for 14 days (N=129). We report a *post-hoc* regression analysis of haemoglobin decline in the study to investigate a possible excess of haemoglobin declines from baseline observed in TQ group (5% TQ vs 1% placebo and 2% PQ patients with >3g/dL decline). The outcome of interest was maximum haemoglobin decline from baseline over the first 15 days post treatment. The covariates used in the final model were baseline haemoglobin, G6PD enzyme activity and baseline *P. vivax* parasite count. Other explanatory variables explored were geographical region, gender, age and baseline urea. Results: There was an overall decline in haemoglobin in all treatment groups. The maximum decline in haemoglobin was 1.22 g/dL (95% CI 1.13 - 1.30 g/dL) in the TQ arm, 1.02 g/dL (95% CI 0.90, 1.14 g/dL) in the placebo arm, and 1.11 g/dL (95% CI 0.98 - 1.23 g/dL) in the PQ arm. Haemoglobin declines in G6PD normal patients in the study were partly due to higher baseline haemoglobin and parasite count and lower G6PD enzyme activity. After adjusting for baseline haemoglobin, parasite count and G6PD enzyme activity, although there was a larger decline in haemoglobin in patients treated with TQ compared to placebo this treatment difference was only 0.19 g/dL [95% CI 0.04 - 0.34 g/dL], and thus not considered clinically significant.

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DEALING WITH INDETERMINATE OUTCOMES IN ANTIMALARIAL EFFICACY TRIALS: A COMPARISON BETWEEN COMPLETE CASE ANALYSIS, MULTIPLE IMPUTATION AND INVERSE PROBABILITY WEIGHTING

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Antimalarial clinical efficacy studies for uncomplicated *Plasmodium falciparum* malaria frequently encounter situations in which parasite molecular typing is unable to discriminate between parasitic recurrence, either new infection or recrudescence. The current WHO guideline recommends excluding these individuals with indeterminate outcomes in a complete case (CC) analysis. Data from the four artemisinin-based combination trial was used to compare the performance of multiple imputation (MI) and inverse probability weighting (IPW) with the standard CC analysis for dealing with indeterminate recurrences. A total of 3,369 study participants with molecularly defined parasitic recurrence treated with three artemisinin-based combination therapies were used to represent a complete dataset. A set proportion of recurrent infections (10, 30 and 45%) were reclassified as to missing using two different mechanisms: a completely random selection (mechanism 1); missingness dependent on treatment and transmission intensity (mechanism 2). The performance of MI, IPW and CC approaches in estimating the Kaplan-Meier (K-M) probability of drug efficacy was then compared. Performance measures (bias, relative bias, standard error and coverage) were reported as an average from 1,000 simulation runs. The CC analyses resulted in absolute overestimation of drug efficacy by up to 1.7% and were associated with reduced precision and poor coverage across all the missingness scenarios studied. Both MI and IPW method performed better (unbiased and greater efficiency) compared to CC analysis. The widely used CC approach overestimates antimalarial efficacy. The IPW and MI procedures provided efficient and unbiased estimation of antimalarial efficacy and should be considered when reporting the results of antimalarial clinical trials, especially in the areas of high transmission where the proportion of indeterminate outcomes could be large.

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THE PHARMACOKINETICS OF PRIMAQUINE AND ITS METABOLITES IN HEALTHY CAMBODIAN ADULTS WITH VARIOUS CYP2D6 GENOTYPES

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The Cambodian government declared elimination of *Plasmodium vivax* in 2025, which will require increased administration of primaquine for *P. vivax* radical cure. Recent research suggests the hepatic CYP450 2D6 enzyme to be responsible for metabolism of primaquine to the active metabolites required for hypnozoite clearance. The effects of different 2D6 genotypes on the pharmacokinetics of primaquine in populations living in vivax endemic areas are not well characterized. We previously reported on the various CYP2D6 genotypes prevalent in a subset of 40 Cambodians and found 55% allelic frequency of the reduced activity 2D6*10 allele, most often paired with a normal activity allele or another reduced allele. In 2017, we re-consented 27 of the 40 volunteers to participate in a primaquine pharmacokinetic study. Using the CYP2D6 AS-A scoring system, 1 volunteer was found to have a score of 2, 13 volunteers had a score of 1.5, 12 had a score of 1.0 and 1 Undetermined (UNDET) with a genotype of *5/*10 DUP, which could lead to scoring as either 0.5 or 1.0. Thus, all of the volunteers were predicted to be Extensive Metabolizers (EMs), except the UNDET volunteer, who could be predicted to be either an EM or Intermediate Metabolizer (IM) phenotype. Primaquine (PQ) and its metabolite levels were measured in blood and urine by UPLC-MSMS over the subsequent 24 hours after oral administration of single dose primaquine (15 mg) in order to correlate CYP2D6 genotype with metabolite concentrations and phenotype. Initial results show the plasma PQ C_{max} values were 65.2 61.6, and 101.2ng/ml for AS-A scores of ≥1.5, 1.0, and UNDET, respectively, which suggested the AS-A scores of 1.5 and 1.0 metabolized similarly and the UNDET had an IM phenotype. T_{max} results were similar for all 3 AS-A scores and to results seen previously (range 2-3.1 hours). Carboxyprimaquine, the major metabolite generated through the monoamine oxidase pathway, showed similar C_{max} values for all (452.0, 460.8 and 451.7ng/ml) and T_{max} of 7.7, 8.8 and 6 hours AS-A scores of ≥1.5, 1.0, and UNDET, respectively. Full pharmacokinetic analysis of primaquine metabolites in both blood and urine will be presented.

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TAFENOQUINE FOR MALARIA PREVENTION

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The development of tafenoquine for malaria prevention was completed by 60° Pharmaceuticals (60P) in collaboration with the U.S. Army in late 2017. Tafenoquine is currently undergoing priority review by the U.S. Food and Drug Administration for malaria prevention for up to six months in adults. Assuming regulatory approval is obtained, commercial launch is targeted for early 2019. Unlike the more well-known *Plasmodium vivax* indication where tafenoquine is given as a single dose to symptomatic individuals, the proposed label submitted by 60P to regulators involves continuous weekly dosing for up to six months in adults at risk of contracting malaria. Data from peer-reviewed clinical trials suggest that tafenoquine has activity against both *P. falciparum* and *P. vivax*. Structural, non-clinical, and clinical data from the literature suggest tafenoquine does not have a neurologic liability, but that G6PD testing is required to prevent inadvertent use in individuals with severe deficiency. Therefore, should tafenoquine receive marketing authorization, it may represent an improvement over the standard of care (mefloquine, chloroquine, daily drugs) in jurisdictions with robust G6PD screening capability. One can envisage, following the generation of additional clinical data, expansion of the proposed label to include children, dosing for up to 12 months, prevention of malaria amongst travelers between malaria-endemic countries, and outbreak prevention in countries declared malaria-free by the World Health Organization. 60P plans to conduct several post-marketing studies to allow these additional potential uses of tafenoquine for malaria prevention and eradication to be realized. The opinions expressed herein are the author's own and do not necessarily reflect those of the United States Army or Department of Defense.

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ASYMPTOMATIC MALARIA IN HOUSEHOLD MEMBERS OF FEBRILE CHILDREN ATTENDING CLINIC IN COASTAL TANZANIA

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Since asymptomatic malaria infections greatly outnumber symptomatic cases in low-endemic settings, submicroscopic parasitemias may be an important invisible reservoir in areas seeking malaria elimination. However, the relationship of asymptomatic to symptomatic cases is not well-established, with recent genetic studies suggesting that parasites that circulate asymptomatically may differ from those causing symptomatic infections. We used a clinic-based reactive case detection scheme to identify persons with asymptomatic parasitemia in coastal Tanzania, with the goal of examining the genetic relatedness of parasites causing asymptomatic vs. symptomatic infection. Asymptomatic persons accompanying febrile children presenting for care at the Yombo Clinic in Bagamoyo, Tanzania from March to April 2017 were screened for parasitemia by rapid diagnostic test (RDT) and peripheral blood smear, with DNA extracted from dried blood spots used for species-specific real-time PCR (qPCR) targeting the 18S ribosomal gene. Among 164 study participants who denied fevers/chills in the 3 days prior to enrollment, 71% were mothers, 21% were fathers, and 8% were other family members bringing their child to care. The median age was 32.5 (range 13-57), and 16% reported a history of malaria within the past year, while 75% reported malaria in their lifetime. We found no correlation between these factors and a positive malaria screening result. Malaria prevalence in the asymptomatic contacts was 3.1% (5/160) by RDT and 13.1% (21/160) by qPCR. Age, gender, parasite density, housing materials and bed net use in the febrile children were also not associated with positive malaria status in their asymptomatic contacts. We are employing hybrid capture-based target enrichment and multi-locus deep sequencing to investigate the genetic relatedness of parasites within and between households, and whether parasites causing symptomatic versus asymptomatic infections cluster separately.

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POTENTIAL FOR CELLPHONES TO ACCELERATE MALARIA ELIMINATION IN THE GREATER MEKONG SUBREGION, NE INDIA AND BANGLADESH

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Countries in the Asia-Pacific have committed to eliminating malaria by 2030. Ownership of cellphones and spatial coverage by cellphone service providers are rapidly expanding across the region. Cellphones are increasingly being used for a wide variety of public health purposes and research on malaria including sms and smartphone-based case reporting, tracking interventions and public health messaging, as well as newer techniques such as using call detail records to quantify the impact of population movement on malaria distribution and development of smartphone-based automated diagnosis. Many cellphone-based reporting systems for malaria require ongoing connection to the internet to upload data whilst some permit offline data storage for later uploading once sufficient signal strength is available. The utility of these techniques depends heavily on whether people with malaria have access to cellphones and on the coverage of cellphone services in malaria endemic areas. This study aimed to determine the patterns of cellphone usage among people with malaria and map cellphone signal strength in relation to malaria burden in Southeast and South Asia. Over 5000 people with confirmed malaria infection in Bangladesh, Cambodia, northeast India, Lao PDR, Myanmar, Thailand and Vietnam were surveyed about their usage of cellphones including whether they have a phone, if they share it and which service provider they use. In addition, publicly available data on over 2 million cell tower locations were analysed to determine the spatial distribution of cellphone service provision by different providers in relation to malaria burden in these countries. From this analysis, locations were identified where cellphones currently have the greatest potential to help accelerate malaria elimination in these countries and locations where improvement of cellphone service coverage would be of most benefit. Contributing study groups: Tracking Resistance to Artemisinin Collaboration Group; SpotMalaria GenRe-Mekong; Bangladesh Mobility Genotyping; Targeted Malaria Elimination.

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THE IMPACT OF MATERNAL MALARIA INFECTION DURING PREGNANCY ON INFANT MALARIA RISK: A COHORT STUDY IN WESTERN KENYA

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More than 125 million pregnant women are at risk for malaria annually. In Sub-Saharan Africa it is estimated that 1 in 4 women have placental malaria at the time of delivery. Placental malaria is associated with adverse maternal, fetal, and infant health outcomes. In Kenya, the Ministry of Health adopted Intermittent Preventive Treatment (IPT) for malaria and provision of insecticide-treated nets as a national strategy for malaria prevention in pregnancy, which has reduced the incidence of placental malaria. However, little is known about the effects of maternal asymptomatic parasitemia on infant outcomes. The objective of this study is to examine the impact of maternal untreated malaria parasitemia during pregnancy, detected retrospectively by qPCR, on infant illnesses, including malaria, through a longitudinal prospective cohort study conducted at Chulaimbo Hospital in the Kisumu District of Kenya, from June 2011 to July 2015. Demographic, medical, socioeconomic data and sera for peripheral and placental malaria were obtained. Infants were followed from birth to 24 months with scheduled follow-up appointments at 6, 10, 14, and 18 weeks, then every three months until 24 months of age. Diagnoses from scheduled follow-ups and sick visits were recorded. A total of 149 infants and mothers were included, of which 93 (62%) had a positive PCR for malaria during pregnancy. Maternal characteristics for both malaria infected mothers and non-malaria infected mothers were similar. Sixty (40%) infants were diagnosed with malaria during the follow up period. There were no significant differences observed between children diagnosed with malaria. Multivariate analyses showed no significant association between maternal asymptomatic parasitemia in pregnancy with infant diagnoses of malaria in the 2-year follow up period after adjusting for confounding variables including age of the mother, infant age, sex, birth weight and maternal education using multivariable logistic regression. Our data suggest that newborns may not be effected by maternal asymptomatic parasitemia during pregnancy and they are not at increased risk of malaria.

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PLASMODIUM FALCIPARUM MULTIPLICITY OF INFECTION IN MALAWI

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The likelihood of *Plasmodium falciparum* infection resulting in malaria illness is related to age, parasitemia, and parasite diversity. Multiplicity of infection (MOI), the number of unique parasite genotypes, may also contribute to the development of malaria disease. Previous studies in high transmission settings comparing symptomatic and asymptomatic infections have shown that high MOI infections are a risk factor for symptomatic illness, but MOI was not assessed after controlling for parasite density and age. Our aim was to examine the independent role of MOI on clinical disease. We hypothesized that increased MOI, after adjusting for parasite density, would be associated with symptomatic malaria. We conducted a two-year longitudinal cohort study of adults and children in a high transmission setting in Malawi; illness episodes were captured with active and passive surveillance. *P. falciparum* infection was detected using qPCR and all positive samples underwent genotyping using merozoite surface proteins (msp1, msp2) and glutamate-rich protein. Of 1,061 infections, 476 (44.9%) were symptomatic. Median MOI was 2 (IQR = 1, 3) and ranged from 1 to 10. Before adjusting for parasite density, higher MOI was associated with increased odds of clinical diseases (OR = 1.86, 95% CI: 1.36-2.55) and school-aged children had increased odds of symptomatic infection compared to children under five (OR = 2.25, 95% CI: 1.17-2.97). After controlling for repeated measures and parasite density, there was no association between MOI and presence of symptoms (OR = 1.23, 95% CI: 0.85-1.77). High parasitemia (>2,000 parasite/ul blood) was associated with an 80% increase in the odds of symptoms among children and a 60% increase in the odds of symptoms among adults. The strong association of parasite density and symptomatic illness may confound the effect of MOI or be due to improved sensitivity of genotyping when more parasites are present. In this high transmission setting, parasite density, but not MOI, was a strong predictor of clinical disease in the presence of *P. falciparum* infection.

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OPPORTUNITIES AND CHALLENGES TO STRENGTHEN DISEASE SURVEILLANCE TO SUPPORT MALARIA ELIMINATION IN THE PHILIPPINES

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The Philippines aims to eliminate malaria by 2030. To accelerate towards this goal, it is essential for the National Malaria Program to detect and treat all infections. However, current approaches limit the Program's the ability to respond effectively. The country is trialling novel diagnostic and transmission mapping techniques to improve surveillance for malaria elimination. This study evaluates the feasibility of integrating such techniques into the existing surveillance system. A case study approach was used, involving document review and semi-structured qualitative interviews of key informants purposefully selected from stakeholders working at all levels of the health system. Community- and provincial-level informants were selected from a project site in Occidental Mindoro. Interviews collected data on informants' beliefs on their organisation's readiness to integrate such novel techniques, and the various challenges and opportunities that they present, which were analysed thematically. A total of 32 interviews were conducted and 27 relevant documents reviewed. Most informants believed integration would require considerable financial resources for equipment and training, which local and national authorities may not prioritize or be able to afford. Other common barriers to integration included insufficient workforce, facilities' limited internet access, and the low quality and timeliness of data reporting from certain provinces. Some mentioned that strong advocacy in support of malaria elimination could help to create an environment receptive to innovation. Many of the barriers to integrating novel diagnostic techniques into the existing surveillance system are general health systems issues found in other pre-elimination countries. This suggests that adopting new measures for malaria control may not proceed if such systemic barriers are left unresolved. However where affordable, opting to invest in these new techniques could catalyse strengthening of the national surveillance system, and the health system more broadly. However, this will require advocacy to renew political support for elimination.

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FACTORS ASSOCIATED WITH MALARIA PARASITEMIA AND ANEMIA IN UGANDA: CROSS-SECTIONAL SURVEY OF 48 DISTRICTS

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A baseline cross-sectional community survey was conducted to assess the prevalence of malaria parasitemia and anemia in Uganda, for an ongoing trial of the impact of long-lasting insecticidal nets (LLINs) distributed in 2017-18. The survey included 104 clusters across 5 administrative regions. Households were randomly selected using two-staged cluster sampling; 50 households were enrolled per cluster. Eligible children aged 2-10 years had blood obtained for thick blood smear and to measure hemoglobin (aged 2-4 years). In March-June 2017, 5200 households were enrolled; 8852 children aged 2-10 years had blood smear results, and 3753 aged 2-4 years had hemoglobin results. Regional differences were seen in household wealth, house type, and adequate LLIN coverage (1 LLIN per 2 residents). Overall, parasite prevalence was 26.0% with wide geographic variation ranging from 8.0% in the southwest to 53.2% in eastcentral. In an adjusted analysis controlling for clustering within households, factors associated with parasitemia included increasing age (adjusted prevalence ratio (aPR) 1.07 per 1 year increase, 95% CI 1.06-1.08, p<0.001), living in a house with a wealth index in the lowest two tertiles (aPR 2.12, 95% CI 1.88-2.38, p<0.001), living in a house made from traditional materials (aPR 1.16, 95% CI 1.06-1.27, p=0.001), and living in a house without adequate LLIN coverage (aPR 1.31, 95% CI 1.15-1.49, p<0.001). Overall, the prevalence of anemia (hemoglobin < 10 g/dL) was 15.1% and also varied by region, ranging from 9.6% in the southwest to 20.2% in eastcentral. In an adjusted analysis, factors associated with anemia included region, decreasing age, living in a traditional house, and being parasitemic. Parasitemia was the strongest predictor of anemia (aPR 2.50, 95% CI: 2.12-2.95, p<0.001). These results indicate that the prevalence of parasitemia and anemia vary widely across Uganda, and that older children and those living in poorer households, made with traditional materials, and inadequate numbers of LLINs, are at highest risk of parasitemia; while the youngest children and those with parasitemia are at highest risk of anemia.

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CONTRIBUTION OF RAPID DIAGNOSIS TEST IN MALARIA CASE MANAGEMENT STRATEGY IN SENEGAL

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According to WHO recommendations issued in 2004 for RDT use to improve quality and accuracy of the diagnosis, Senegal had introduced RDT (based on the detection of the antigen Histidin Rich Protein-2/HRP-2 specific for *Plasmodium falciparum*) in October, 2007. This diagnosis tool was supposed to: allow rational use of CTA, reduce the risk of CTA resistance development in the parasite, increase accuracy and reliability of morbidity data and avoid useless care-costs. Between 2001 and 2007, in the pre-RDT period, the proportion of all consultations due to malaria decreased from 39.7% in 2001 to 26.9% with confirmation cases rate by microscopy just around 10%.; after RDT introduction and change in definition from clinical to laboratory-confirmed, the proportion of all consultations due to malaria decreased from 9.1% in 2008 to 3.26% in 2017 and RDT realization who was around 15% at the beginning jumps to 99.55% in 2017. This was possible as RDT contributed to increase access to biological diagnostic to end users within the community by the mean of community health workers, due to easy use and rapidity in result delivery. In 2017, Senegal recorded 1,637,316 suspected cases (tested negative), all these cases should have been treated with a antimalarial if RDT was not conducted. Thus, by considering the cost of a CTA treatment at one US dollar, NMCP has realized almost 1,637,316 USD savings. RDT introduction into malaria case management was a realistic alternative to no functionality of the laboratories in the peripheral structures and to operational issues in the realization of the microscopic diagnosis Moreover, RDT was accepted by caregivers at any level as its allow rational use of time, credibility for the health system and increased confidence of the populations. RDT had largely contributed to the decrease of morbidity and mortality due to malaria, since it made possible detecting earlier and accurately malaria cases, treating it's with rapidity and by reducing the risks of transmission and evolution of cases to severe ones.

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THE BURDEN OF ANTIMALARIAL TREATMENT FAILURE IN AFRICA: EVIDENCE FROM HOUSEHOLD SURVEYS

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Despite recent scale-up of malaria control interventions such as provision of nets, access to artemisinin combination therapy (ACT) in Africa remains low. Antimalarial treatment failure can result in progression to severe disease or death and is due to several reasons including poor drug absorption, use of substandard or counterfeit drugs, non-adherence, or drug resistance. The latter is a particular problem when older drugs such as sulphadoxine-pyrimethamine (SP) are still used. Data on use of ACT, SP, chloroquine and quinine in 37,826 febrile children under five were obtained from Malaria Indicator Surveys (MIS) and Demographic and Health Surveys (DHS) from 22 African countries in which both rapid diagnostic tests (RDT) and microscopy had been performed. We assumed that a positive RDT indicated a recent clinical infection with *P. falciparum*. An efficacy estimate was calculated for each drug, defined as parasite clearance by microscopy. The overall crude weighted clearance rate was 36.8% for chloroquine, 43.2% for SP, 42.1% for quinine and 61.8% for ACT, but country-specific rates varied significantly. The estimate for ACT was significantly lower than that observed in clinical trials. In the DHS/MIS, 15.1% of those taking ACT did not adhere to treatment guidelines. Country-specific estimates of the proportion of poor quality drugs in private sectors were applied, which accounted for 8.3% of failures. A further 5% of failures is expected to stem from incorrect treatment recall. This study relies on self-reported data and it is difficult to estimate the extent to which incorrect recall influences efficacy estimates. Ongoing work aims to further quantify the proportion of treatment failure which is due to drug resistance, re-infection and other causes, and to obtain a burden estimate across Africa. Our analysis highlights that a large proportion of children continue to take non-ACT antimalarials for malaria (11.0% and 5.5% of infected febrile children taking chloroquine and SP respectively) and the associated failure rate is approximately 1.5-2 times higher than ACTs. These results highlight the urgency of improving ACT access across Africa.

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REAL-TIME PCR ASSAYS FOR THE DETECTION, SPECIATION AND QUANTIFICATION OF *PLASMODIUM FALCIPARUM, P. VIVAX, P. OVALE,* AND *P. MALARIAE*

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The accurate diagnosis of malaria and the precise identification of the *Plasmodium* spp. is necessary for its successful treatment and eventual eradication. While microscopy or morphologic analysis continues to be the "gold standard" for malaria diagnosis, PCR assays are rapidly becoming the new standard alternative to microscopy. This molecular methodology has superior sensitivity and specificity and is becoming more common in the developing world or in otherwise resource limited environments. The *Plasmodium* species known to cause human malaria have varied and often overlapping geographical distributions that have yet to be fully characterized. The four predominant species, *P. falciparum*, *P. vivax, P. ovale,* and *P. malariae* are capable of mixed infections in both symptomatic and asymptomatic patients, with important ramifications in both public health and individual patient treatment. A mixed infection that includes *P. vivax* or *P. ovale* may be incompletely treated if blood-stage therapy is not immediately followed with primaquine. Incompletely treated patients could still serve as reservoirs of the disease, hindering local eradication efforts. qPCR has been used as a method of choice for detecting mixed infections. However, detection methods using sensitive primers and probes based on species-specific 18S rRNA often lack the specificity needed for accurate detection of low parasitemias. Herein, we describe a TaqMan-based qPCR assay using optimized primer and probe sets that lack homology among species and are specific for *P. falciparum (mls), P. vivax (ecpr), P. ovale (rbp2),* and *P. malariae (csp).* Validation experiments of the panel show a <10 copies/ul to 100,000 copies/ul detection range, even in the presence of high copy numbers of every other species detectible by the panel. The specificity of these assays is complete against the other species and contaminating human DNA. We characterize the limit of detection, limit of quantification, and specificity of each assay for use in a current study investigating *Plasmodium* species prevalence and mixed *Plasmodium* infections in HIV positive and negative populations in West Africa.

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FACTORS POTENTIALLY RESPONSIBLE FOR THE DECLINE OF MALARIA IN THE AYEYARWADY REGION OF MYANMAR

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Although Myanmar has the highest burden of malaria in the Greater Mekong Subregion (GMS), the country has achieved an impressive 72% reduction in cases between 2012 and 2016 with a total of 110,146 reported cases in 2016. Myanmar is committed to eliminating falciparum malaria by 2025 and continued efforts are needed because of the threat of artemisinin resistance in the GMS. Understanding the factors that have led to a reduction in malaria burden can inform effective measures for future interventions in Myanmar, which can accelerate elimination efforts. The Ayeyarwady region of Myanmar provides a useful context for examining the reduction in cases; between 2012 and 2016, confirmed cases dropped from 21,782 to 5,525 (a 75% decrease). Trends in potential factors associated with malaria transmission including case management, vector control, surveillance, and ecological factors (e.g. deforestation) were examined in Ayeyarwady in order to assess the main drivers of decline in the region. Data from 2011-2016 were collected at the health facility level on the number of malaria tests performed, the number of confirmed cases reported, and health facility catchment populations. 2011-2016 data on bed net distributions and malaria specific Village Health Volunteer (VHV) placements were collected at the township level and two years at the village level. The results showed that between 2011 and 2016, the total number of tests conducted in Ayeyarwady increased 84% to a total of 105,661 tests in 2016. Also between 2011 and 2016, the number of VHVs increased from 50 to 467, and the tests performed by VHVs increased from 2,044 to 18,923. In townships with VHVs, the ABER increased from 16.7% in 2013 to 31.3% in 2016. LLINs have been distributed in Ayeyarwady since 2012, and between 2012 and 2016, an estimated 276,000 LLINs were distributed in the region. These trends in case management and vector control may have led to the reduction of malaria cases in Ayeyarwady; however, further analysis is required to assess these relationships and disentangle the roles of each potential factor.

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THE EMERGANCE OF *PLASMODIUM OVALE* IN KWANZA NORTE PROVINCE, ANGOLA

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Malaria in Angola is still the leading cause of mortality, morbidity and absenteeism at work and school, being endemic in the country's 18 provinces, with the highest transmission recorded in the northern provinces (Cabinda, Uige, Malange, Kwanza Norte, Lunda Norte and Lunda Sul)[1]. Moreover, it reflects about 35% of the demand for curative care, 20% of hospital admissions, 40% of perinatal deaths and 25% of maternal mortality. Malaria has not only a negative impact on the health of populations, but also on the economic and social development of them[1]. The fight against malaria is focused on the elimination of *Plasmodium falciparum* infections, which continues to be the main cause of malaria infection; whilst cases of *P. o*vale show an evolution from year to year. However, little is known about the degree of growth or decrease of *P. o*vale endemicity, with Kwanza Norte having a percentage of the affected population in the order of 28%, with a parasitemia index of 63% (Hiperendemic). Thus, 10 Municipalities were randomly selected and the population was randomly assigned to the Malaria Diagnostic Test, PRH2 / pLDH (pf/Pan) COMB Malaria, and afterwards the positive results were examined by microscopy, (Standard), obtaining the following results: *P. falciparum* (45%), *P. vivax* (0%), *P. ovale* (0.7%) and *P. malariae* (0.03%). Although the results show a rate (0.7%) within the National average[3], there is a gradual increase in the number of cases of infection with *P. o*vale, and in some municipalities rates are very high (eg. Golungo Alto, from 0% in year 1 to 24% in year 2). This result could be probably at least partly attributed to laboratory technicians lacking experience in reading the slides (for the correct identification of the strain) in the first year; for better understanding trends, it is important that RDTs are able to identify different antigens; the country must prepare itself with stocks of primaquine and equip the technicians with more training in order to prevent and when necessary, to handle cases that may arise. Finally, in species differentiation, including PCR Based Assay should be considered, in order to give greater robustness in the reliability of the results.

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EFFECTIVE COMMUNITY SURVEILLANCE REDUCES SEVERE MALARIA IN RURAL MADAGASCAR

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Malaria remains among the top four causes of death among children under five or CU5 in Madagascar. To reduce malaria infections among CU5, the USAID Community Capacity for Health Program supports community-based activities through community health volunteers or CHV in hard-to-reach communities in 7 of 22 regions of the country, reaching 6.1 million persons (23% of the population). These CHV were trained and equipped to provide integrated community case management services and disease surveillance activities including clinical management of febrile illness among CU5, active case finding in the community to ensure early diagnosis and treatment of malaria, and prompt, complete monthly reporting of data. CHVs also participated in investigation when analysis of

monthly reports revealed increases in malaria cases. We analyzed routine, community surveillance reports from 6,410 CHVs and key informant interviews during January 2017-February 2018. We explored the link between active action of CHV and deaths occurring of malaria. We found complete, timely reporting for 4707 CHVs. They correctly identified and treated 65,184 cases of malaria among CU5 in the community, and their reports contributed to detecting increases in malaria case reports in five districts, ranging from 2 to 30 cases per month per Fokontany. A total of 34 malaria-related deaths including 68% among CU5 were reported in these districts. Of these deaths, 94% had not been detected within 24 hours due to delayed communications and actions. We found that when CHV's contribution occurred within 24 hours, increases in malaria cases were mastered rapidly, avoiding deaths. Fewer cases of malaria related deaths (0-2 deaths) were then observed in the three districts of Antalaha, Manja, Soalala compared to the 2 districts of Besalampy, Boriziny where responses did not happen within 24 hours. We observed also a reduction of 44% malaria cases during the period. Community-based malaria intervention and surveillance may reduce treatment delays, improve outcomes and identify increases in malaria case reports more promptly, and should remain a key component of national malaria control strategy.

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COMMUNITY PERCEPTIONS ABOUT MALARIA RISK AND INFECTIONS IN A REGION OF HIGH MALARIA TRANSMISSION, WESTERN KENYA

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WHO recommends test - based management of malaria infections. Perceptions of the community on febrile illnesses and malaria test results determine the actual success of such guidelines. We conducted a prospective cohort study where an existing cohort of participants in three villages was followed up longitudinally. We report a subset of participants who underwent RDT testing. Requests for RDT testing were initiated by the participants if they felt unwell. During the visit, participants were asked behavioral questions and perceptions towards malaria. Participants who were found positive on RDT were treated with AL. A total of 330 visits were made in a period of 8 months for a total of 162 participants. We recorded 151(45.7%) episodes of malaria. Majority of the participants (130/162, 80.25%) believe that the presence of fever indicates the likelihood of malaria infection even if the RDT results are negative. Most of the participants rated their illness as moderate (79/162, 48.8%). Most of the participants (144/162, 89.4%) had a personal conviction that their illness was actually malaria. RDT test results, individual rating of severity of the illness and having a strong personal conviction that the illness is malaria (X^2 = 145, p<0.001, X^2= 54.5, P>0.001, X^2= 80.8, p <0.001 respectively) were strongly associated with perceptions on the likelihood of the illness being malaria. The community strongly perceives febrile illnesses as likely to be malaria infection despite a negative RDT test. However, overall perceptions on malaria infections are strongly associated with the test results. Successful efforts to reduce malaria in this region need to incorporate health education to correct misinformation.

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PLASMODIUM FALCIPARUM HAPLOTYPE INFERENCE FROM AMPLICON DEEP SEQUENCING TO IDENTIFY MICRO-SCALE PARASITE POPULATION MIXING

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Consistently high *Plasmodium falciparum* malaria prevalence in Western Kenya enhances the need to understand how parasite spatial patterns influence disease transmission and targeted intervention effectiveness. Using a cross-sectional sample of 514 parasites from a 15km by 28km region in Bungoma East sub-county, Kenya, we assessed parasite population mixing across households using polymorphic *P. falciparum* genetic markers. We hypothesized that parasites separated by smaller geographic and temporal distance would be more closely related genetically; therefore, that we would observe increased parasite genetic divergence with larger spatial and temporal distance. To investigate these relationships, we used multiplex sequencing of three polymorphic *P. falciparum* gene segments encoding: 1) apical membrane antigen 1 (*ama1*), 2) circumsporozoite protein (*csp*), and a *Plasmodium* helical interspersed protein B (*PhistB*). Each gene target was amplified from field-collected polygenomic parasitemias and sequenced on an Illumina MiSeq; from these polymorphic reads, polyclonal parasite haplotypes were identified using the haplotype inference-calling program DADA2, as implemented in R, and population genetic analytic approaches were employed to quantify haplotype sharing and clustering on temporal and geographic scales. We found that there was variability in the participant-level *P. falciparum* haplotypes across the three gene targets, suggesting a spatial relation between participant household and parasite genetic divergence. Our results illustrated the feasibility of haplotype inference and the influence of parasite population mixing on a micro-scale.

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EXPANSION OF INSECTICIDE TREATED NETS OWNERSHIP ASSOCIATED WITH REDUCTION OF ALL-CAUSE CHILDHOOD MORTALITY IN KENYA, 2012 - 2014: EVIDENCE FROM INDIVIDUAL LEVEL ANALYSIS

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In the past decade, Kenya has expanded coverage of key malaria interventions, particularly insecticide treated nets (ITNs). There is evidence from ecological and trends analyses showing an association between the expansion of malaria interventions and the decline in all-cause child mortality (ACCM). Further evidence of association at the individual level will better inform the policies, strategies, and activities of Kenya's National Malaria Control Program and its partners. We assessed change in ACCM risk associated with ownership of ITN between 2012 and 2014 using the Kenya Demographic and Health Survey (DHS) 2014. We transformed women's full birth history data into 10-year retrospective longitudinal data reflecting individual child observations from birth until the date of the survey or loss to follow up (death). For each malaria endemic zone, we used a Cox proportional hazards model that included children ages 1 to 59 months for 24-month period of exposure to household ITN ownership (2012-2014). The model controls for several potential confounders. Nationally, household ownership of at least one ITN significantly reduced the risk of mortality among children aged 1 to 59 months by 56% (hazard ratio [HR]=0.44, 95% CI: 0.30-0.63) during the 24-month period before the survey. The protective effect was strongest in the Lake endemic zone, with mortality risk for children aged 1 to 59 months reduced by 72% (HR=0.28, 95% CI: 0.14-0.55). The Lake endemic zone had the highest

burden of malaria and highest ACCM rate throughout the evaluation period and thus the greatest potential to benefit from increased ITN coverage. This individual level analysis provides further evidence of the contribution of expanding ITN coverage to reducing ACCM among highest risk populations in Kenya.

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IRRIGATION AND MALARIA IN MALAWI: MALARIA INFECTION INTERACTS WITH POVERTY AT BWANJE VALLEY IRRIGATION SCHEME

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Irrigation agriculture can increase productivity and household income in rural Africa. However, despite these socio-economic benefits, land transformation for irrigated agriculture may increase mosquito production, enhance malaria transmission, and undermine household productivity. To analyse the association between irrigation scheme participation, malaria and poverty, the following study was conducted in villages around Bwanje Valley Irrigation Scheme, a rice-growing cooperative of ca. 800 ha in Dedza District, Malawi. Three cross-sectional surveys were conducted at the end of the rainy seasons in April 2016, 2017, and during the dry season in November 2017. Socioeconomic, demographic, health and scheme participation data were collected. Blood samples were obtained for malaria rapid test and microscopic identification of *Plasmodium falciparum*. Assets quantified during surveys were used to categorize households into socioeconomic status (SES) with principal components analysis. 29% of households were active participants of the irrigation scheme. Among 5,131 individuals older than six months, prevalence of infection by microscopy was 30.7 % and was significantly higher in residents of households within a 3 km radius (33.2%) compared to 6 km from the scheme (25.9%). Prevalence declined with increasing household wealth quintile, showing an inverse association between malaria prevalence and SES, and declined with formal education. Analysis of 156 households visited during the first (baseline) and third surveys showed that 43.7% of households classified as poor at baseline transitioned into a higher SES by survey 3. Scheme participants were more likely than non-participants to progress to a higher SES (46.1% vs 42.8%). Among households classified into the highest SES at baseline, 44.8% regressed to a lower SES. Compared to scheme participants, non-scheme participants were more likely to regress into a lower SES: 47.2% vs 38.4%. Proximity of human residence to irrigation scheme and relative poverty increased malaria risk. Scheme participation may increase SES and increase likelihood of participants escaping poverty.

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UNDERSTANDING THE EPIDEMIOLOGY OF IMPORTED MALARIA CASES IN VIETNAM AMONG RETURNING INTERNATIONAL LABORERS

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Vietnam has seen a 97% decline in malaria cases between 1991 and 2014, leading to a national goal of elimination by 2030. As local malaria transmission declines a greater focus on the importation and potential reintroduction of transmission is essential to support malaria elimination objectives. Challenges to elimination in Vietnam include an increasing risk of malaria importation through a growing labor force traveling to African and Southeast Asian countries, as well as the continued threat of multi-drug resistant malaria. Currently, little understanding exists on the epidemiology of imported malaria in Vietnam from returning international laborers. A study was conducted at the National Hospital of Tropical Diseases (NHTD) in Ha Noi in 2017 to 2018 to describe the clinical and epidemiologic characteristics of suspected malaria patients recently returning from Africa or Southeast Asia countries. Patients admitted to NHTD with a recent history of living or travelling abroad and exhibiting clinical symptoms of malaria were eligible participants. Travel histories, blood samples and clinical data were collected and analyzed. 31 participants were enrolled in the study. 87% (n=27) were males with a mean age of 36.5 years. 77% (n=24) reported to have had malaria at least once, with 16% (n=5) reporting to have had malaria more than 10 times. Angola (48%, n=15) and Cameroon (16%, n=5) were the most common countries visited. Work (68%, n=21) and business (16%, n=5) were the most common reasons for travel. 92% (n=29) tested positive for malaria by blood film examination, with 62% (n=18) diagnosed with *Plasmodium falciparum*. Day 3 parasite clearance rates were 50% (n=9) and 60% (n=3) for *P. falciparum* and *P. vivax* respectively. Data from these studies indicate that imported malaria from returning international laborers is a potential threat to elimination efforts in Vietnam that requires further attention. Additional scaled research and detailed investigation into appropriate malaria prevention and intervention strategies targeted towards international laborers, both at pre-departure and upon return is required.

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MALARIOMETRIC SURVEY IN GUINEA-BISSAU

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In 2015, the estimated *Plasmodium falciparum* mortality in Africa was 395 000 deaths. The World Health Organisation has called for a 90% reduction in malaria deaths by 2030. The Government of Guinea-Bissau is committed to eliminate malaria from the Bijagos Archipelago, a remote group of islands with limited access to medical care. The primary objective of this study was to map the *Pf* prevalence on Bubaque, the most populated island. A cross-sectional survey was performed during the peak transmission season of 2017. Participants were recruited using systematic random sampling in a stratified cluster design. *Pf* parasitaemia was detected using rapid diagnostic tests. Data on housing, education, larval source management, socio-economic status, anaemia and malaria preventive measures was collected from individuals and households via questionnaires. Multivariate logistic regression models were constructed to establish associations between these indicators and parasitaemia. 404 persons were included in the study. Prevalence of *Pf* parasitaemia was estimated to be 22.2% (95% CI: 18.7-26.0%). Persons aged 6 to 15 years had the highest odds of infection of all age groups compared to children of less than 1 year (OR 5.05, 95% CI: 1.01 – 25.12; p=0.04). Estimated prevalence of anaemia was 74.25% (95% CI: 69.04-78.85). All sampled houses were found to have open eaves. Bed net use was excellent with 99.5% of the population estimated to have slept under a net the night before the survey (95% CI: 97.8-99.9). Current malaria control programmes, such as bed net distribution and intermittent preventive treatment in pregnancy, are likely to be contributing to preventing *Pf* transmission on the island. It is recommended that the benefits of scaling up of malaria control programmes in conjunction with an intervention of mass drug administration using ivermectin and dihydroartemisinin-piperaquine be studied to reduce *Pf* transmission.

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PREVALENCE OF ASYMPTOMATIC AND SYMPTOMATIC MALARIA IN A REMOTE TRIBAL POPULATION OF ASSAM, NE INDIA

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In India, an estimated 1.24 billion people are at risk for malaria infection with over 160 million living in areas of high transmission. The northeast region of India is of particular interest due to higher prevalence rates, the predominance of *Plasmodium falciparum*, and its close proximity and ecological similarity to southeast Asia. A remote 7-village cluster in the Karbi-anglong district of Assam, India, was selected for a community-wide cross-sectional survey during the monsoon season (May-Sept) of 2015. 455 households received study visits, during which each resident was clinically assessed, tested for malaria by microscopy and RDT, and given a questionnaire about demographics and malaria knowledge and prevention practices. Identified malaria cases were referred for treatment per Indian National Drug Policy. Of the 2331 individuals surveyed, 152 (6.5%) were malaria positive; 143 (6.1%) with *P. falciparum*, 6 (0.3%) with *P. vivax* and 3 (0.1%) with mixed infection. The demographics of the villages were similar, though differences in prevalence of malaria were observed. The highest prevalence was in the village of Bhaktegaon (66/415; 15.9%). Among the population, there were slightly higher numbers of symptomatic (87, 3.6%) than asymptomatic (67, 2.9%) malaria. It was noted that while most (2233; 95.8%) individuals reported using a bed net, few (190; 8.5%) reported treated bed net usage. Treatment effectively cleared malaria parasites by day 7 in all but 3 individuals. Though the Government of India has committed to eliminating malaria by 2030, the low usage of treated bed nets (8.5%) and prevalence of symptomatic malaria (3.6%) indicate that routine prevention, diagnostic and treatment options may not be available or accessible to this population. The prevalence of asymptomatic malaria (2.9%) indicates a reservoir of malaria that may not be addressed by elimination efforts relying solely on identification and treatment of symptomatic individuals. Future work will focus on modeling the impact of targeting both symptomatic and asymptomatic infections and characterization of temporal changes in malaria prevalence within this population.

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MALARIA SURVEILLANCE FOR ELIMINATION: A PHASED ROLL OUT OF A CASE-BASED INFORMATION SYSTEM USING DHIS2 IN HONDURAS

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Honduras, characterized by highly focalized malaria transmission in its Northeastern region, reported 1,284 cases in 2017 and aims to eliminate malaria nationally by 2020. A previous surveillance system relied on paper forms to report malaria cases from the health facility to the department where they were electronically entered which has led to problems with completeness of data and reporting delays of up to one month. To ensure complete and timely reporting of case data by health facilities, the Ministry of Health implemented an electronic, centralized, case-based information system using District Health Information System 2 (DHIS2). In an initial phase starting in June 2017, information collected on paper during case notification and investigation was entered using Android tablets with DHIS2 Tracker Capture in 18 health facilities of two departments, Islas de la Bahia and Gracias a Dios. The feasibility of using electronic tools in health facilities with limited infrastructure and unreliable connectivity was evaluated through focus group discussion. Reporting rate (percent of paper forms entered in DHIS2) and timeliness (time between diagnosis and data entry into DHIS2) were estimated by reviewing paper versus electronic reports and information system logs. Between June and November 2017, 33 cases were reported into DHIS2 from Islas de la Bahia and 130 cases from Gracias a Dios. Ninety-five percent of paper reports were entered into DHIS2. The average time from malaria diagnosis to data entry into DHIS2 was 3 days in Islas de la Bahia and 11 days in Gracias a Dios. Findings suggest that there is high user acceptability and satisfaction with the new information system. However limited data entry staff and high staff turnover, especially in Gracias a Dios, remain a challenge for reporting rates and data timeliness. In 2018, Honduras will scale up health facility use of DHIS2 nationally. Simultaneously, Honduras will integrate additional data from laboratory, case management, entomological surveillance and vector control interventions, which will facilitate integrated analysis and decision making for malaria elimination.

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RISK FACTORS FOR MALARIA POSITIVITY AMONG FEBRILE CHILDREN AT FOUR HETEROGENEOUS KENYAN CLINICS

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Clinic-based surveillance of febrile illness is an important metric for estimating malaria morbidity in Kenya where there is wide heterogeneity in malaria endemicity. To describe febrile illness in Kenyan children and risk factors for malaria, we collected comprehensive clinical, environmental, and sociodemographic data on a cohort of acutely ill febrile children less than 18 years of age at four heterogeneous outpatient clinical locations: two coastal clinics (Msambweni District Hospital and Ukunda Health Center) and two western clinics (Chulaimbo Health Center and Obama Children's Hospital in Kisumu). Among 4,608 febrile visits across the four clinical sites from 2014-2017, 50% of febrile children had malaria parasitemia by microscopy with significant differences by site (Chulaimbo 81%, Ukunda 52%, Msambweni 44%, Kisumu 43%, $p<0.001$). In univariate analyses, children with malaria were more likely to report mosquito bites in the last four weeks, were older (6.2 years versus 5.6 years) and reported lower socio-economic status compared to children without malaria. In multivariate logistic analysis controlling for age, sex, site, socioeconomic status, rainfall, and mosquito exposure, odds of malaria infection decreased by 30% when mean daily environmental temperature increased above 26.5°C compared to 22-26.5°C. In the same model, the odds of malaria parasitemia decreased by 11% among those reporting cough at acute infection and increased by 16% if nausea or vomiting was reported. In site specific multivariate models, children reporting mosquito bites in the 4 weeks prior had a 1.69 increased odds of malaria in Chulaimbo; odds of malaria increased by 19% among children

10-15 years of age compared to children 0-5 years of age in Msambweni. By characterizing risk factors for malaria positivity, this study will facilitate improved care of febrile Kenyan children and targeted interventions by clinic type.

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ASSESSING COMMUNITY HEALTH WORKER PERFORMANCE FROM ROUTINE DATA

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Routine data collected in the health system forms the backbone of surveillance in malaria control and elimination programs. In many areas, programs are turning to community-based case management to improve access by decreasing the distance to a point of care. When case investigation or reactive case detection (RCD) is implemented, community health workers (CHWs) are often tasked with these responsibilities in addition to their regular caseload. To ensure high-quality implementation, it is important to assess whether CHWs are carrying out RCD to protocol and to flag individual CHWs who may be overburdened with treating index cases or otherwise underperforming so that program managers can rectify these issues. We present a simple methodology for using routine data collected by CHWs as part of their activities to assess CHW performance. This method is applied to health facility catchment areas in Southern Province, Zambia, where RCD activities began in 2014. We identify catchments where CHW performance clearly improved over the last few years and other areas where performance is still an issue, particularly during the wet season. This framework is flexible and easily extendable to any RCD implementation where a few basic health care metrics are collected.

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TRENDS IN ITN, IPTP-SP USAGE AND MALARIA PREVALENCE AND ANEMIA IN PREGNANT WOMEN ON BIOKO ISLAND, EQUATORIAL GUINEA

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The World Health Organization (WHO) recommends and emphasizes the use of insecticide treated bednets (ITNs) and intermittent preventive treatment (IPT) for all pregnant women in areas of stable transmission of *Plasmodium falciparum* malaria. The Bioko Island Malaria Control Project (BIMCP) in partnership with the Ministry of Health and Social Welfare of Equatorial Guinea aims at increasing the proportion of pregnant women sleeping under ITNs and receiving IPT with Sulphadoxine-pyramethamine (SP). This study examined the coverage of these interventions among pregnant women on Bioko Island and the optimal protection they confer against malaria and anaemia. Long-lasting insecticidal nets (LLINs) are distributed to pregnant women at public health facilities during antenatal care visits. The first dose of IPT-SP is administered through directly observed therapy (DOT) during the second trimester of gestation. Malaria education is also provided to women during antenatal care visits. The BIMCP conducts annual cross-sectional Malaria Indicator Surveys (MIS) on Bioko Island to determine malaria prevalence and anaemia. In 2014, the number of pregnant women who slept under ITNs was 19.3%. This increased to 53.5% in 2015 following a mass distribution campaign, but later dropped to 36.8% in 2016. The percentage of pregnant women who received at least one dose of IPT-SP during their pregnancy remained stable from 2014 to 2016 (79.8% in 2014, 73.6% in 2015 and 73.4% in 2016). However, 22.7% took three or more doses in 2014. This increased to 37.2% in 2015 and more than doubled to 64.7% in 2016. *P. falciparum* parasitemia among pregnant women was 11.5% in 2014, which dropped to 10.5% in 2015 and further to 5.2% in 2016. Moderate/severe anaemia among women was 3.2% in 2014, 3.4% in 2015 and 1.5% in 2016. The substantial increases in the consumption of three or more doses of IPT-SP during pregnancy could have contributed to the reduction of *P. falciparum* parasitemia and moderate/severe anaemia among pregnant women on Bioko Island.

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EARLY FOCAL MALARIA UPSURGE DETECTION AND RESPONSE IN KISORO, A LOW TRANSMISSION, UNSTABLE MALARIA SETTING OF UGANDA

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Areas with unstable malaria transmission are prone to epidemics and these upsurges in malaria cases should be dealt with promptly. The use of information derived from a quality surveillance system to inform targeted delivery of effective malaria interventions, has provided tools for malaria upsurge detection Kisoro Uganda. We reviewed lessons from the Kisoro district led response to a malaria epidemic. Between January and March 2018, the data was collected and analysed from Health information records, DHIS2, reviewed supervision reports from the district health Team, partners and National Malaria Control Program. The review and analysis was part of the comprehensive response to the malaria upsurge detected in Kisoro district. Analysis of DHIS2 data by Kisoro district showed a 3-fold increase in Malaria cases from week 46 to 51, 2017. The Malaria Test positivity rate increased from 20% to 50. The upsurge was localized in the sub-counties of Murora and Kanaba, bordering the Sereri and Mpundu swamp. Entomological assessment showed increased density of *Anopheles gambiae sensu lato* mosquitoes, breeding in the swamp. The district activated the taskforce on detection of the epidemic and responded within one week. The response measures included community sensitisation, restocking medical supplies, outreach screen and treatment, surveillance, tracking and weekly reporting. The Malaria cases reduced by 50% in a period of 12 weeks district led response. Capacity to detect and respond to epidemics exists at district level but this needs to be enhanced to ensure timely and adequate supplies including training and provision of as antimalarial buffer stock. Focal larval source management or Indoor residual spraying would be a suitable intervention for this setting.

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EARLY DETECTION OF MALARIA UPSURGES AT SUB-NATIONAL LEVELS IN UGANDA

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The World Health Organization (WHO) has set specific targets for early detection and control of malaria epidemics as part of a wider strategy to cut the global extent of malaria in half by 2030 (1). In mid-2015, a malaria upsurge was detected in the northern part of Uganda following withdrawal of indoor residual spraying (IRS). As part of the response, ministry of health developed a weekly malaria case monitoring system as a surveillance tool in Uganda. We performed a document review of weekly malaria status reports produced by the Uganda national malaria control programme (NMCP) between January 2016 and December 2017 to assess their effectiveness as a malaria upsurge detection tool. During the period under review, 60/96 (63%) weekly malaria updates were generated. In this same period, a total of 51/122(42%) districts reported surge in malaria cases. Of these, 8 were high, 41 moderate and 2 low transmission,

compared no such report in 2013 and 2014. One of the upsurge was detected in Kisoro, a district with unstable malaria with parasite prevalence < 5%. The status updates also presented details of testing rates, malaria supply status and mortality surveillance. Though limited response to recommendations made in the weekly reports were observed, the reports provided clear and concise recommendation and some of them reported status of implementation of recommendations. Continuous review of district based data is an appropriate tool for early detection of malaria upsurges. An observed limitation was the detection of surges at district albeit too late rather than at sub-district level where actual early detection and response is possible.

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IMPROVING THE USE OF MALARIA SURVEILLANCE DATA FOR COUNTY LEVEL DECISION-MAKING BY ADDRESSING GAPS IN DATA SUPPLY AND BARRIERS TO DATA DEMAND IN KENYA

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While there have been significant reductions in the malaria burden in the past decade, malaria transmission in Kenya remains endemic and heterogeneous across the country. Gaps in the national surveillance system, specifically with regard to data-informed programmatic decision making at sub-national levels, hinders further progress towards malaria elimination. To comprehensively address gaps in data supply (data collection, reporting and analysis) and data demand (data use for decisions) Clinton Health Access Initiative engaged with health management teams in Narok and Kitui counties to implement a set of targeted interventions between 2016 and 2018. Data supply interventions included ensuring all health facilities had reporting registers stocked, training of health care workers, and a review of surveillance practices during supervision visits from sub-county health management teams (SCHMTs). A dashboard was developed to ensure availability and accessibility of relevant analyses of key indicators tailored to SCHMTs needs. To improve data demand and use, a quarterly series of data review meetings were held to build capacity for and a culture of using surveillance data for evidence-based decision making among SCHMTs. Improvements in data supply included increased health facility reporting rates, from 70% in Q1 2016 to 89% in Q1 2018, improved feedback to facilities on data collection and reporting, and closer monitoring of surveillance data quality by sub-county health records officers. Data demand was improved through data review meetings by building capacity and skills among SCHMTs while facilitating dialogue around interpretation and use of data to target and plan programmatic activities. Anticipated long-term outcomes include sustained improvements in data collection and reporting among health facilities and in use of data among SCHMTs. Use of data to guide programmatic decision-making is expected to lead to more efficient and effective allocation of resources and overall improvement in malaria case management and prevention. A more detailed impact evaluation of this process will inform potential national scale-up.

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HUMORAL IMMUNE RESPONSES AGAINST THE MALARIA VACCINE CANDIDATE ANTIGEN *PLASMODIUM VIVAX* CSP AND *IL-1* PLUS *IL-2* GENE POLYMORPHISMS IN INDIVIDUALS LIVING IN AN ENDEMIC AREA OF THE BRAZILIAN AMAZON

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It is well established that infection by *Plasmodium vivax* is a result of host-parasite interactions. This study evaluated polymorphisms in the *IL1* and *IL2* genes and of the central portion of the circumsporozoite protein (CSP) and correlated them with the cytokine profiles, anti-CSP antibody levels and parasitic loads of 138 individuals naturally infected with *P. vivax* in an endemic area of the Brazilian Amazon. *Methods: IL1B -511C>T*, *IL2 -330T>G* and *IL2 +114T>G* polymorphisms were identified using PCR-RFLP and allele-specific PCR. IL-1β and IL-2 cytokine levels were detected by flow cytometry. CSP antibodies were measured by ELISA. The prevalence and magnitude of IgG antibodies were higher for the VK210 variant. Significant differences were observed between SNP -511T>C in the *IL1B* gene and levels of antibodies to the VK247 and *P. vivax*-like variants, but there were no associations between SNPs in genes *IL1* and *IL2* and their plasma products. Studies of associations of cytokine gene SNPs in the humoral immune response to malaria are still incomplete, and the results have been contradictory. Given that the evolution of malaria results from the interaction of factors inherent to both parasite and host, this study helped to identify genetic variants that may influence the humoral immune response against malaria, particularly *P. vivax*.

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TAKING MALARIA GENOMIC TECHNOLOGY TO THE FIELD: A TWO-STEP PROTOCOL OF SELECTIVE WHOLE GENOME AMPLIFICATION AND TARGETED AMPLICON DEEP SEQUENCING (SWGA-TADS) FOR CHARACTERIZING LOW PARASITAEMIA AND LOW QUALITY *P. FALCIPARUM* INFECTIONS

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It is difficult to obtain sufficient malaria DNA for sequencing from field samples that are poor quality or low parasitemia (<50 parasites/microliter). Selective whole genome amplification (sWGA) is an effective tool to enrich the target DNA relative to high levels of contaminating host DNA for sequencing microorganisms. However, it remains limited in its ability to allow for whole genome sequencing from infections of low parasitaemia (<50 parasites per microliter) and in field samples whose quality and suitability for genomic studies are questionable. In order to allow for targeted genotyping of these poor-quality malaria field samples, we investigated a two-step protocol of sWGA coupled with amplicon

deep sequencing. Amplicon deep sequencing was performed used the highly-variable apical membrane antigen 1 (AMA1) as the target, a widely used *P. falciparum* genotyping marker. We validated the approach using mock mixtures of laboratory *P. falciparum* strains with the following characteristics: ***i)*** low starting DNA concentration commonly seen in low-parasitaemia infections (0.01 ng/mL = ~3.5x10^2 genome copies/mL and 0.001 ng/mL = ~3.5x10 genome copies/mL); ***ii)*** varying complexities of infection (three mock mixtures of MOI=1, 3 and 5) and ***iii)*** with uneven within-sample frequencies (MOI=1: 3D7 100%; MOI=3: FCR3 85.5% + DD2 10.5% + 3D7 4.0%; MOI=5: 7G8 50.0% +FCR3 25.0% +3D7 15.0% +DD2 5.0% +HB3 5.0%). In addition, to test for the role contaminating human DNA has on protocol efficacy, three other mixtures were also made by spiking the MOI=3 *P. falciparum* mixture above with human DNA to obtain 10% parasite DNA and 90% human DNA. Finally, the protocol was then tested on clinical DNA samples extracted from *P. falciparum*-positive rapid diagnostic tests (RDTs) collected from Zanzibar, a low transmission setting where low density infections are common. Our findings suggest that a two-step protocol comprising sWGA and amplicon deep sequencing is a reliable and scalable technology which overcomes issues of low parasitaemia and low sample quality issues and allows accurate deep sequencing characterization of otherwise unsuitable field samples.

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GENETIC EVIDENCE OF CLONAL SPREAD, PARASITE EXCHANGE, AND UNIQUE SIGNATURES OF RELATEDNESS AMONG *PLASMODIUM FALCIPARUM* SAMPLES FROM HAITI

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Haiti is one of the last countries in the Caribbean with continued malaria transmission; thus, there is great interest in malaria elimination in this region. While much of the population of Haiti is at risk for malaria, there are a limited number of cases (17,622 in 2016), making conventional epidemiological measures such as case incidence and prevalence challenging. In this context, genetic signals are useful for the identification and characterization of the *Plasmodium falciparum* parasite population to identify *Foci* of transmission, detect outbreaks, and track parasite movement to inform elimination efforts. We evaluated 470 single-genome *P. falciparum* DNA samples extracted from dried blood spots from malaria-positive patients reporting to health facilities from primarily three Haitian departments (Nippes, Grand'Anse, and Sud). Initial genetic assessment using a 21-single-nucleotide polymorphism molecular barcode revealed evidence of clonal expansion within Nippes, in which 158/170 samples were an identical barcode type, as well as the divergence of a single clonal type in Grand'Anse. Furthermore, we detected the exchange of nine parasite lineages between Nippes, Sud, and Grand'Anse. In addition, 460/470 samples shared high levels of genetic similarity with at least one other sample in the dataset within 44 barcode-identical clusters. Based on this initial identification of a highly-related parasite population, we sought to further assess the relatedness of the parasites in these clusters. We performed whole-genome next-generation sequencing to characterize parasite relatedness as the percent of the genome shared between parasites as well as the length of stretches of genomes that were considered identical-by-descent. The results revealed patterns of relatedness suggestive of repeated recombination of a limited number of founding parasite types without significant outcrossing. These genetic signals offer glimpses of the underlying relatedness of parasite populations and may be used to identify *Foci* of transmission against which targeted control efforts may be applied towards the elimination of malaria in Haiti.

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PROBABILISTIC CLASSIFICATION OF *PLASMODIUM VIVAX* RELAPSE AND REINFECTION USING MICROSATELLITE GENOTYPING DATA FOR IMPROVED ESTIMATION OF RADICAL CURATIVE EFFICACY

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Plasmodium vivax is a major cause of malaria outside Sub-Saharan Africa. Vivax malaria is characterized by repeated relapses which arise when formerly dormant liver hypnozoites are reactivated, leading to blood-stage infections. Relapse causes significant morbidity. The inability to distinguish relapse from reinfection hinders the assessment of antimalarial drug efficacy. Accurate estimation of radical curative efficacy therefore requires a probabilistic distinction of relapse from reinfection. We have a developed a Bayesian framework for the probabilistic classification of recurrent vivax infections using polymorphic microsatellite markers. This method was applied to data from a randomized control trial comparing two primaquine radical cure regimens, and jointly models time-to-recurrence and *P. vivax* genetic data on nine microsatellite markers. A model of time-to-recurrence provides the prior probability. The genetic likelihood component integrates over all possible parasite relatedness networks within and across infections. We use a simplified model of identity by descent (IBD), whereby relapsing parasites can be clones (IBD of 1), meiotic siblings (IBD of 0.5), or strangers (IBD of 0), whereas parasites derived directly from reinfection are assumed to be strangers (IBD of 0). Preliminary results suggest that the efficacy of primaquine radical cure is greater than suggested from current standard clinical trial reports, with over 80% of recurrent infections following radical cure having a high probability of being reinfections rather than relapses. The day 7 carboxy-primaquine concentration (a slowly eliminated inactive metabolite) was a weak predictor of treatment failure (relapse). Probabilistic classification of relapse and reinfection significantly improves the estimation of the efficacy of radical curative regimens for vivax malaria. This method can be applied to radical cure efficacy trial data and, importantly, to other applications where the inability to distinguish relapse from reinfection presents a major challenge (e.g. to estimate incidence for the evaluation of intervention measures).

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POPULATION GENOMICS OF VIVAX MALARIA IN THE GREATER MEKONG SUBREGION

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Quick evolution and notable diversity in the genus *Plasmodium* has made effective drug, vaccine and diagnostic design a major hurdle to Malaria eradication. Further, it necessitates regional eradication programs tailored to specific Malaria populations. *Plasmodium vivax (Pv)* is the most common malarial pathogen outside of Africa, including in the Greater Mekong Subregion (GMS). Out of the 6 GMS countries, Myanmar has the highest documented Malaria burden, but very little is known about specific malaria populations in Myanmar, or the spread of *Pv* to and from nearby countries. In the past, population analyses for *Pv* in Myanmar and on its Chinese border have been limited by sample availability, among other factors. Using next generation sequencing data from *Pv* field isolates, we've addressed *Pv* variation around the China-Myanmar border both at

the whole genome level and within individual genes of functional interest. Continuing analysis will expand these results to other parts of the GMS and guide local Malaria eradication efforts.

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EUPATHDB: FREE, ONLINE OMICS RESOURCES FOR EUKARYOTIC PATHOGENS

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The Eukaryotic Pathogen Database (EuPathDB, http://eupathdb.org) is a free, online data mining resource supporting over 190 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. EuPathDB facilitates the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. The intuitive graphic interface allows users to take full advantage of the data without the need for computational training. EuPathDB integrates a wide range of data including genome sequence and annotation, transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, clinical data, and host-pathogen interactions. Data are analyzed using standard bioinformatics workflows and an in-house analysis pipeline generates data including domain predictions and orthology profiles across all genomes. EuPathDB offers several perspectives for data mining - record pages which compile all data for genes, pathways, study subjects, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system for querying pre-analyzed data to find genes or features that share biological characteristics, and a private workspace for analyzing primary data (via a Galaxy interface) and viewing the results in context with public data already integrated into EuPathDB. This free, comprehensive data mining resource easily merges evidence from diverse data types and across organisms to place the power of bioinformatics with the entire scientific community. EuPathDB's active user support offers an email help desk, social media, a You Tube channel and a worldwide program of workshops. Please stop by our booth in the exhibit hall or our poster for a demonstration, to suggest a data set or see our newest addition, ClinEpiDB (http://clinepidb.org) which facilitates the exploration and analysis of epidemiologic studies.

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REPEATED INDOOR RESIDUAL SPRAYING REDUCES *PLASMODIUM FALCIPARUM* EFFECTIVE POPULATION SIZE AND INCREASES INBREEDING IN A VERY HIGH TRANSMISSION AREA OF UGANDA

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The recent reduction in malaria mortality and morbidity in Africa has been primarily attributed to vector control interventions, including long-lasting insecticidal nets (LLINs) and indoor residual spraying of insecticide (IRS). These control programs, if successful, reduce the size of circulating *plasmodium* populations. Measuring population genetic parameters may provide insight into the effect of these interventions on transmission and the parasite reservoir. In this study, we used samples from a longitudinal study conducted in Nagongera, Uganda where universal LLINs distributed in 2013 did not reduce the incidence of malaria but IRS starting in late 2014 had a dramatic reduction (3.25 cases per person year pre-IRS versus 0.63 post-IRS). Samples from 393 *Plasmodium falciparum* infections in 196 children, collected in four distinct time periods were genotyped using 25 microsatellite markers. The time periods considered were: pre-ITN and –IRS; post-ITN and pre-IRS; post-2nd round IRS and post-3rd round IRS. No significant changes were observed in any population genetic parameters after LLINs. However, the frequency of infections containing multiple parasite genotypes decreased following IRS (87% pre-IRS to 67% post-IRS, $p < 0.0001$). The most striking change was the significant decline in the genetic diversity (H_E) of parasites (mean H_E, 0.72 ± 0.01 pre-IRS to 0.66 ± 0.02 post-IRS, $p = 0.004$). Furthermore, the effective parasite population size (*Ne*, 16872 pre-IRS vs 6656 post-IRS, $p < 0.001$) was significantly reduced. The reduction in population size was associated with a 2.6-fold increase in linkage disequilibrium and a 10-fold increase in the proportion of sampled infections which were closely related to each other. These findings suggest that, even in areas of extremely high transmission, effective IRS can significantly reduce the parasite reservoir and decrease the opportunity for outbreeding. This study also illustrates how genetic data can complement epidemiological surveillance, providing support for the utility of genetic parameters to measure changes in transmission intensity and asses the efficacy of interventions.

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EVALUATION OF AMPLICON SEQUENCING ON MOCK COMPLEX *PLASMODIUM FALCIPARUM* INFECTIONS

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Accurate genotyping of complex (polyclonal) malaria infections is important for assessing population-level effects of interventions over geography and time. Amplicon sequencing is a cost-effective, high-throughput genotyping technology, but its data can be subject to technical artifacts, especially when applied to difficult-to-sequence genomes like *P. falciparum*. In addition, traditional tools for amplicon data quality control, which utilize low-resolution classification against known sequences (e.g. 16S databases), are inadequate when single-base haplotypic differentiation is desirable. We evaluated the performance of several existing amplicon sequencing analysis pipelines on data generated for amplicons in *CSP* and *SERA2* from a set of known samples with differing genotype compositions at low parasite densities (1-200 parasites per µl). Using a simple clustering approach that ignores variants in pre-identified homopolymeric regions, our analysis pipeline achieved high overall sensitivity and positive predictive value (0.80 ± 0.098 ROC area-under-curve), meeting or exceeding the performance of comparable pipelines (DADA2, SeekDeep and HaplotypeR). As expected, we saw significant performance gains at high versus low parasite densities (0.88 vs. 0.75 at > 20 parasites/µl and < 20 parasites/µl, respectively). We also observed high concordance between genotype frequency predictions of the two most performant tools (r^2: 0.92, $P < 1.0e-100$), despite the fact that these predictions agreed less precisely with expectation (r^2: 0.55, $P = 3.3e-52$). This suggests that inaccuracies when assessing sample genotype ratios may result from disproportional amplification during PCR and sequencing, particularly at low parasite densities, rather than the analytical methods themselves. We present a well-evaluated, highly-scalable amplicon sequencing analysis pipeline and make it freely available for use on FireCloud, a cloud computing service for genomic analysis workflows.

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TARGETED DEEP SEQUENCING OF MALARIA INFECTIONS IMMEDIATELY POOLED AT COLLECTION TIME EFFICIENTLY PROVIDES ACCURATE FREQUENCIES OF DRUG RESISTANCE MUTATIONS IN GHANA

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Some major challenges to surveillance of antimalarial resistance in sub-Saharan Africa are logistical and financial challenges, limiting its breadth and frequency. Rapid inexpensive methods are therefore needed to monitor antimalarial drug resistance. We present a streamlined process of creating a singular sample immediately pooled (ipool) at collection time followed by high-throughput targeted sequencing using molecular inversion probes. Ten malaria control sentinel sites have been targeted across Ghana for this study. 50ul of each RDT or microscopy malaria positive sample obtained from patients of all ages during routine malaria testing were ejected into a growing pool in a 15ml falcon tube containing 4 ml of DNA/RNA shield to preserve the sample. In all, a pool of a mixture of 100 sequential *P. falciparum* infections was prepared by the laboratory technician at each site and stored at 4°C until ready for DNA extraction. Individual dried blood spots (DBSs) were also prepared on filter papers. Genomic DNA was extracted from ipools and DBSs. Molecular inversion oligonucleotide probes designed to capture known and candidate drug resistance mutations in p*fcrt*, p*fmdr1*, p*fdhfr*, p*fdhps*, as well as span entire length of *pfK13* were used in capture reactions and subsequent sequencing on the MiSeq platform. Our initial results from 2 sites, Cape Coast and Begoro indicate a 99% correlation between prevalence of drug resistance markers estimated from individual patients and the frequency of mutations measured from the ipool samples. Our study validates the ipool method as a cost-efficient, accurate and highly scalable approach for drug resistance monitoring. Our approach to resistance monitoring minimizes the burden on the clinical and public health systems by collecting and processing pools of individuals rather than each individual patient. For *P. falciparum* malaria this will allow us to quantitatively determine mutation frequencies within the pooled mixture of individual infections at numerous locations and multiple times per year moving towards providing fine resolution mapping and tracking of drug resistance across Ghana near real-time.

1775

QUANTIFYING POPULATION STRUCTURE OF MALARIA PARASITES USING EPIDEMIOLOGICAL AND GENOMIC DATA

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Regular circulation of individuals to and from malaria endemic areas undermines local control by reintroducing infections and sustaining local transmission. Quantifying the movement of malaria parasites has become a priority for national control programs, but remains methodologically challenging due to the unique evolutionary and epidemiological features of malaria parasite transmission. In particular, high rates of superinfection with multiple strains and a predominance of asymptomatic infections contribute to difficulty in identifying transmission chains and tracking routes of parasite importation. Here, we assessed the utility of genetic data in combination with epidemiological modeling and detailed travel surveys to measure the spread of malaria parasites in Bangladesh. We collected genetic barcodes of 101 SNPs and epidemiological data from 2,137 patients residing in 184 separate unions in Bangladesh. We found that, at this geographic scale, the proportion of parasites with nearly identical barcodes was highly associated with geographic distance, while standard genetic methods, such as average pairwise difference or F_{ST}, were not. We developed a genetic mixing index that quantifies the likelihood of samples from one location having higher-than-expected relatedness to samples from distant locations. We then inferred the direction and intensity of parasite flow between locations as well as estimated the proportion of imported cases using epidemiological models. Our results show distinct regional mixing in the north and south of the malaria-endemic region in Bangladesh, and that the level of parasite mixing is highest in the northwestern region.

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UTILIZING *IN SILICO* TOOLS TO PERFORM B CELL EPITOPE PREDICTION IN *PLASMODIUM FALCIPARUM*

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In silico epitope prediction is a promising alternative to conventional epitope identification methods, which are normally time consuming and can potentially fail to identify epitopes that are not easily captured using *in vitro* assays. However, the utility of this *in silico* approach depends in the accuracy of the predictions. B cell epitopes can be predicted based on sequence or structure and prediction software use a variety of protein properties to determine the likelihood that a peptide sequence is an epitope. We evaluated four different prediction algorithms that utilize different criteria to predict B cell epitopes for their accuracy in predicting B cell epitopes in the parasite *Plasmodium falciparum*, using as controls known antigen sequences for which we have epitope data. The circumsporozoite protein (CSP) is a well characterized malaria antigen for which epitopes are known and have been experimentally validated. CSP is also the basis of the most advanced malaria vaccine, RTS,S. We also plan to make predictions for other antigenic genes such as AMA1, a leading vaccine candidate that is shown to block parasite red blood cell invasion in *in vitro* assays and VAR2CSA, a candidate vaccine target against placental malaria. We evaluated the following prediction software: ABCPred, SVMTriP, BCEPred and BepiPred, which predict linear epitopes using machine learning and propensity scales. Preliminary data indicates that these software tools predict different epitope sets, which differ in the degree of overlap with experimentally validated epitopes, in addition to predicting new unique epitopes. We will present the sensitivity and specificity of each program and attempt to determine the sequence context that contribute to accuracy.

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IDENTIFYING A MINIMAL SET OF SNPS FROM MALARIA PARASITE GENOME DATA THAT CHARACTERIZES THE SPATIO-TEMPORAL ORIGIN OF A SAMPLE

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Molecular data collected from malaria parasites across a range of transmission settings is beginning to inform operationally relevant analyses and modeling for elimination campaigns. The ongoing poliomyelitis eradication campaign is a modern exemplar to malaria control programs for utilizing genetic data to inform intervention efforts. A virus genome sample is collected from each paralytic case and phylogenetically linked to previous samples to inform outbreak responses or identify circulating vaccine derived polio strains. Despite the success in the polio program with virus genomes, linking malaria *parasite* genomes to transmission characteristics and intervention design encounters serious challenges. Difficulties arise due to the multiplicity of infection, the parasite evolutionary clock, and clonal propagation. Here, we discuss our recent

progress analyzing malaria genomic data for elimination planning/ programs. In this study, we analyze the spatio-temporal similarity of over 3,000 sequenced samples of plasmodium falciparum collected from the major endemic countries by the Malaria Genomic Epidemiology Network. Specifically, we characterize the spatial resolution at which the origin of a sample can be geographically predicted while concurrently identifying the minimal required number of single nucleotide polymorphism (SNPs) for the task. Our results are broadly consistent with previous analyses of these malaria genomes; we highlight connections to known, high-value SNP positions. We have also broadened the scope of previous analyses by adapting a recently developed algorithm from machine-learning to automatically identify a minimal number of SNP positions, perform the origin classification task, and evaluate metrics for parasite genomes. We discuss how this novel framework can contribute to elimination planning by helping identify epidemiologically connected regions and constructing region specific haplotypes.

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SUPPRESSION OF B CELL IMMUNE RESPONSES SUBSEQUENT TO LOW DOSES OF CHLOROQUINE AND PYRIMETHAMINE; IMPLICATIONS FOR STUDYING B CELL IMMUNITY IN MALARIA MURINE MODELS

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Immunomodulation by *Plasmodium sp.* contributes to the pathogenesis of malaria. When studying this phenomenon using mice, researchers may administer the anti-malarial drugs chloroquine and pyrimethamine to prevent early fatalities. It's assumed that low doses have little effect on immune responses so drug treated controls are omitted. We investigated B cell activation to carbohydrate and protein with or without subsequent anti-malarial drug treatment using flow cytometry for phenotyping and ELISPOTs to measure antibody secreting cells. Responses were measured at day 14 and 28 after an immunisation boost. The carbohydrate immunisation included synthetic glycophosphatidylinositol (GPI) conjugated to keyhole limpet haemocyanin (KLH), because GPI is expressed by all *Plasmodium sp*. The model protein nitrophenol (NP) conjugated to KLH was used for protein immunisations since responses are already well characterised. As expected, immunisation in the absence of anti-malarial treatment resulted in significantly higher antigen-specific B cell activation compared to naïve mice. Interestingly, when these mice were administered anti-malarial drugs following immunisation, early B cell responses (day 14) reduced to a level no longer significant. Furthermore, for protein immunised mice, the frequency of memory B cells was significantly lesser when directly comparing drug treatment versus no treatment. By day 28, B cells from protein immunised anti-malarial drug treated mice had recovered from suppression. On the contrary, for the carbohydrate immunisations, most B cell responses were significantly higher than naïve mice exclusive of memory B cells. The latter were significantly higher in carbohydrate immunised mice, but not when these mice were administered anti-malarial drugs. In conclusion, when using the malaria murine model, it is imperative that relevant drug-treated controls are included. This will ensure the exclusion of potential anti-malarial drug effects when measuring parasite immunomodulation.

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LOW ANTIBODY TITERS AGAINST MALARIA VACCINE CANDIDATES RH5, RIPR AND CYRPA IN NATURALLY INFECTED PEOPLE IN AN ENDEMIC AREA OF MALI

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An effective malaria vaccine will likely be one necessary component of a multi-faceted effort to eradicate the disease. The reticulocyte-binding protein homologue 5 (RH5), RH5-interacting protein (Ripr), and cysteine-rich protective antigen (CyRPA) are *P. falciparum* blood-stage vaccine candidates that form a complex involved in merozoite invasion of host erythrocytes. RH5 vaccine development is the most advanced, and an RH5-based vaccine was shown to confer protection in an *Aotus* challenge model. Anti-RH5 antibody titers in malaria-endemic areas are reported to be low; however, immune responses against Ripr and CyRPA in natural infections have not been well-documented. Here we use full-length RH5, Ripr and CyRPA proteins in ELISA to measure antibody titers in sera collected at the peak of the transmission season from 405 individuals of all ages living in a malaria-endemic area of Mali. Most individuals had no or low antibody titers against the three proteins: 45% (183/405), 57% (229/405), and 80% (325/405) of subjects had anti-RH5, -Ripr, and -CyRPA titers under the detectable limit, respectively. Titer increases with age until around age 10, after which there is no significant increase, and males had slightly higher titers against RH5 ($p=0.04$). These patterns are similar to trends in overall malaria infection throughout the year in the test population. Anti-RH5 ($p=0.003$) and anti-Ripr ($p=0.004$) titers were higher in individuals with *P. falciparum* infection at the time of serum collection. Within the subset of positive individuals, antigen-specific antibody titers correlate between the three antigens ($p<0.0001$ for RH5 vs. Ripr and CyRPA vs. Ripr; $p=0.03$ for RH5 vs. CyRPA). Overall, these results show that these three antigens are not highly immunogenic in natural infection and suggest that antibody titers are likely correlated with malaria exposure, which will be confirmed with additional ELISAs. Further experiments will be conducted to test the functional activity of these antibodies using the growth inhibition assay (GIA). Our results will support further development of RH5-, Ripr- and/or CyRPA-based vaccines.

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INDUCTION OF IL-10 PRODUCING REGULATORY MACROPHAGES EXPRESSING PDL-1 AND ILT3 BY *PLASMODIUM* SPOROZOITES

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Professional antigen presenting cells (APCs) like macrophages (Mϕs) and dendritic cells (DCs) can orchestrate immune responses to pathogens. As such, they are central players in the induction of natural and vaccine-induced immunity to malaria. Blood stage malaria parasites are known to suppress APC activation, but very little is known about the effects of sporozoites (SPZ) on APC phenotype and function. Since the first APCs to encounter malaria parasites are located in the human skin, we set out to investigate the response of human skin APCs to SPZ and SPZ antigens. We generated human monocyte derived DCs and Mϕs and stimulated these with *P. falciparum (Pf)* SPZ or recombinant *Pf* circumsporozoite protein (CSP). Both DCs and Mϕs readily take up recombinant CSP, but do not change their phenotype upon doing so. Whole SPZ are preferentially phagocytised by Mϕs (2-10%) instead of DCs (1%). Mϕ uptake of SPZ is strongly increased (up to 30%) by parasite opsonisation with anti-CSP antibodies. Upon stimulation with SPZ, Mϕs increase activation markers CD80 and CD25, but also show enhanced expression of tolerogenic surface markers Programmed death-ligand 1 (PDL-1) and Immunoglobulin-like transcript (ILT)3. Additionally, SPZ-stimulated Mϕs show decreased migration capacity and produce Interleukin 10, a hallmark for regulatory

responses. To confirm that dermal APCs respond to SPZ similar to monocyte derived APCs, we stimulated cell suspensions obtained from human skin explants with mCherry expressing rodent malaria *P. berghei* or recombinant *Pf* CSP. Similar to monocyte derived APCs, dermal APCs readily took up recombinant CSP as well as whole SPZ. SPZ uptake could be enhanced by parasite opsonisation, confirming the macrophage-like phenotype of these dermal APCs. Phenotyping of these cells is ongoing. In conclusion, we show that SPZ induce a regulatory phenotype in human Mϕs. These findings are a first step in enhancing our understanding of the pitfalls of pre-erythrocytic natural and vaccine-induced immunity.

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SERORECOGNITION AND SEROREACTIVITY TO PEPTIDES WITHIN THE SEMI-CONSERVED AND VARIABLE REGIONS OF STEVORS REFLECT CUMULATIVE AND SEASONAL MALARIA EXPOSURE IN MALIAN ADULTS AND CHILDREN

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Subtelomeric variable open reading frame (STEVOR) proteins comprise one of three *Plasmodium falciparum* variant surface antigen (VSA) families involved in malaria pathogenesis and immune evasion. Although individuals in malaria-endemic regions likely acquire natural immunity to clinical disease with the development of antibodies against certain VSAs, there is limited knowledge about antibodies against STEVORs following malaria infection. STEVOR proteins are roughly 300 amino acids in length, consisting of a semi-conserved (SC) region flanked by two variable (V1, V2) domains. We hypothesized that antibodies against STEVORs primarily target epitopes located within the SC region given its relative sequence homology. We aimed to identify STEVOR epitopes associated with malaria exposure using six STEVORs from the 3D7 reference genome expressed as either an entire antigen on a protein microarray or 16-mer peptides with 12 amino acid overlaps on a peptide microarray. We determined serorecognition and seroreactivity for these six STEVORs using Malian sera from children ages 1-6 before and at the peak of a malaria season and adults before a malaria season. Sera from Malian adults (N=18) recognized and had significantly greater reactivity to all six STEVORs compared to sera from Malian children (N=75). For each of the six STEVORs, adult sera (N=10) recognized more peptides in the SC and the V2 regions than pediatric sera (N=10). Adult sera also had increased reactivity to subsets of peptides within the V1, V2, and SC domains. Pediatric sera collected at peak-season (N=10) recognized significantly more peptides in total for each of the six STEVORs than matched pediatric sera collected during pre-season; however, peptide recognition between these two groups did not differ for STEVOR domains individually. Pediatric sera collected at peak-season compared to pre-season did not react more intensely to any group of peptides within a particular STEVOR domain or even spanning each of the six STEVORs. These findings will help elucidate STEVOR epitopes and domains associated with malaria exposure and potentially protective immunity to malaria.

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E3 UBIQUITIN LIGASE MARCH1 IS MASTER REGULATOR OF IMMUNE RESPONSES TO MALARIA INFECTION

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A well-tuned immune response is critical for the host to fight against infections. Regulators of molecular signaling pathways play an important role in regulating host immune responses. Previously, we performed a trans-species eQTL analysis of host-malaria interaction and identified many Type-I interferon (IFN-I) related/induced genes, including a gene called Membrane-Associated RING Finger Protein 1 (MARCH1 or RNF171). MARCH1 is an E3 ubiquitin ligase known to ubiquitinate CD86 and MHC class II proteins in immature dendritic cells. Here we showed that the MARCH1 is a master regulator of host immune response during malaria infection. Overexpression of MARCH1 suppresses STING and MAVS mediated IFN-I responses, whereas March1 deficient cells exhibit elevated IFN-I levels. However, March1 KO mice produced significantly lower levels of IFN-I *in vivo* and were better protected than wild type mice after malaria infection, suggesting additional malaria-stimulated signaling pathways. Further analyses revealed that MARCH1 not only regulates STING and/or MAVS activities, but also interacts with many other regulators to fine-tune host immune responses. This study demonstrates new roles for MARCH1 in innate and adaptive immunity during malaria infection.

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MALARIA INFECTION-INDUCED SYSTEMIC INFLAMMATION IN PREGNANT WOMEN LIVING WITH HIV RECEIVING CART AND DAILY PROPHYLACTIC TRIMETHOPRIM-SULFAMETHOXAZOLE

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In sub-Saharan Africa the risk of HIV and malaria co-infection poses an important health risk to pregnant women. Women living with HIV (WLHIV) have an increased incidence and density of peripheral and placental malaria infection, and severe clinical complications associated with malaria in pregnancy, including maternal anemia and low birth weight. Here we examined longitudinal changes in inflammatory markers in a cohort of pregnant women in Tororo, Uganda living with HIV who were receiving CART as well as daily prophylactic treatment with trimethoprim-sulfamethoxazole. Plasma samples (n = 1115) from 326 women with singleton pregnancy were processed by ELISA to quantify concentrations of CHI3L1, CRP, IL-18BP, IL-6, sICAM-1, and sTNFR2. Linear mixed effect (LME) modelling was used to examine longitudinal changes in analyte concentrations across gestation. 41% of women had evidence of malaria infection at delivery by PCR, placental histology, placental blood smear or placental rapid diagnostic test. The majority of malaria cases were identified by placental histology (33%). Malaria infection in pregnancy was associated with elevated sTNFR2 ($p < 0.001$), sICAM1 ($p < 0.01$) and IL18BP ($p < 0.001$) across pregnancy. Based on the role of inflammation in preterm delivery we examined longitudinal changes in inflammatory proteins in preterm and term deliveries. Women who delivered preterm had elevated concentrations of sTNFR2 ($P < 0.05$) and elevated levels of IL-6 ($P < 0.05$). When we examined protein concentration in the sample collected prior to delivery, we observed an increased relative risk of preterm in women with plasma concentrations of sICAM-1 ($P < 0.05$) and sTNFR2 ($P < 0.05$) in the highest quartile. Our results suggest that malaria infection in WLHIV, despite daily treatment with trimethoprim-

sulfamethoxazole, can induce a systemic inflammatory response that may increase the risk of preterm birth. These findings highlight the need for additional strategies to protect WLHIV from malaria infection in pregnancy to promote healthy outcomes for mother and child.

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T-FOLLICULAR HELPER CELLS IN THE INDUCTION OF FUNCTIONAL ANTIBODIES IN CHILDREN IN HIGH MALARIA TRANSMISSION AREAS

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T-follicular helper CD4 T cells (TfH) are the key T cell subset responsible for mediating B cell activation and antibody induction during infection. The induction of different TfH subsets appears to be pathogen-specific. However, in human malaria infection, the specific TfH subsets required for the induction of protective antibodies has not been identified, and the impact of age on TfH subsets activation during *P. falciparum* infection is unknown. Here, we assessed TfH responses children aged 6 months to 10 years residing in settings of high malaria transmission in Uganda via flow-cytometry. Concurrently, the level and function of naturally acquired *P. falciparum* antibodies responses to blood stage parasites were measured. We detected high levels of IgM and cytophillic subclasses, IgG1 and IgG3 in the majority of serum samples tested. We found that these serum antibodies promoted complement deposition on whole merozoites and merozoite antigens, MSP2 and AMA1. Furthermore, serum antibodies had the potential to cross-link Fc-receptors to promote opsonic phagocytosis. The acquisition of functional antibodies increased with host age and was/not associated with protection against clinical malaria. Preliminary results suggest that higher circulating TfH frequencies are associated with a greater number of functional antibody responses to merozoites and merozoite antigens. Associations of TfH with age, prior malaria exposure and antibody induction are currently being assessed and TfH subsets associated with functional antibodies will be reported at the conference. These findings have major implications to further understand the appropriate TfH responses required to increase malaria vaccine efficacy.

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INNATE IMMUNE ACTIVATION IN HEALTHY MALARIA-IMMUNE ADULTS FROM WESTERN KENYA

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Frequent exposure to malaria and other infectious diseases endemic in western Kenya may lead to chronic inflammation and altered innate immune homeostasis. We assessed monocyte activation in healthy malaria-immune adults from western Kenya compared to malaria-naïve adults from the United States. All Kenyan adults were Pf PCR negative at time of sample collection. Proportions of monocyte subsets were measured by flow cytometry. Isolated monocytes were cultured 18 hrs with media alone, a TLR2/1 agonist (Pam3CSK4), or a TLR7/8 agonist (CL075); supernatant cytokines were measured with a magnetic bead-based immunoassay. Monocyte gene expression profiles were analyzed with a targeted digital RNA sequencing panel. Monocyte genomic DNA was isolated for DNA methylation analysis using the MethylationEPIC array. We found that Kenyan adults had higher proportions of the inflammatory $CD14^{+}CD16^{++}$ non-classical monocyte subset compared to US adults (10.2 vs. 2.9%, p=0.005). Monocytes from Kenyan adults were hyper-responsive to TLR agonists: production of IL-6 was 4-fold higher with TLR2/1 and TLR7/8 stimulation, and TNF production was 5-fold higher with TLR7/8, in Kenyan vs. US monocytes (p<0.0001). Ex vivo monocyte gene expression profiles showed significant over-expression of inflammatory genes in Kenyan vs. US adults, including *IL1A, IL1B, IL6, TNF, PTGS2, STAT4, NLRP3*, *IFITM1*, and *C1QB*. We found differential DNA methylation patterns clearly separating Kenyan and US groups by principal components analysis; the main drivers of methylation values were sex and geographic location. In addition, gene set enrichment analysis revealed decreased DNA methylation in regions of genes in the Allograft Rejection, Oxidative Phosphorylation, and PI3K/AKT/MTOR pathways in Kenyan vs. US adults. These data are consistent with chronic innate immune activation and inflammation in healthy malaria-immune Kenyan adults compared to malaria-naïve US adults. These differences in immune homeostasis may contribute to differences in vaccine immunogenicity and, more broadly, the long-term health consequences of inflammaging in healthy Kenyan and US adults.

1786

DYSREGULATION OF PRO-AND ANTI-INFLAMMATORY CYTOKINES AND CHEMOKINES AND ANGIOGENIC FACTORS IN INDIVIDUALS IN AREAS OF STABLE AS COMPARED TO UNSTABLE *PLASMODIUM FALCIPARUM* TRANSMISSION

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The effect on malaria transmission on baseline levels of pro- and anti-inflammatory cytokines and chemokines and growth factors in children and adults is not well characterized. We measured the levels of 19 pro- and anti-inflammatory cytokines, chemokines, and growth factors in plasma in children <5 years of age and individuals ≥5 years of age in areas of western Kenya with stable (Ajigo, Siaya County) and unstable (Kipsamoite and Kapsisiywa, Nandi County) transmission. Children in the stable transmission area <5 years (n = 178) had higher levels of IL-1b, IL-12, IL-17A, IFN-γ, MIP-1b, RANTES, IP-10, MCP-1 (MCAF), IL-4, IL-10, and FGF-basic but lower levels of IL-6, IL-8, MIP-1a, IL-1ra, G-CSF, PDGF-bb, and VEGF (all $p < 0.0001$) than children in the unstable transmission area (n = 94). TNF-α levels did not differ between the sites. Findings were similar in *P. falciparum* PCR negative children <5 years (stable, n=144, unstable, n=94). Individuals ≥5 years of age in the stable transmission area (n=96) had higher/lower levels of the same markers compared to those in the unstable transmission area (n=42) as those <5 years with the addition of elevated TNF-α ($p = 0.0007$) in stable transmission, and without differences in MIP-1a. Findings were similar in *P. falciparum* PCR negative individuals ≥5 years (stable, n=79; unstable, n=42). Children and adults in areas of stable malaria transmission, whether actively infected or not, have higher levels of both pro- and anti-inflammatory cytokines than individuals in unstable malaria transmission areas, but lower levels of factors that affect angiogenesis than individuals in unstable transmission. The study findings suggest that stable malaria transmission leads to upregulation of pro- and anti-inflammatory cytokines and downregulation of angiogenic factors even in the absence of active infection. The long-term clinical consequences of this dysregulation of inflammation and angiogenesis require further study.

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THE IMPACT OF SEX AND HOST VARIABILITY IN THE OUTCOMES OF IMMUNIZATIONS WITH *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN DBP II IN MICE

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Vaccines seek to induce long-lasting protective immunity to microbial pathogens by focusing immune responses to epitope targets, sensitive to broadly neutralizing immune inhibition. Murine studies with some parasite antigens have produced high-titer, parasite-specific inhibition *in vitro*; however, the clinical outcome of these candidates have not generated a strong anti-parasitic response upon parasite challenge. To date, there is no subunit vaccine clinically available that is capable of eliciting cellular responses or cellular memory and this has been a major limitation in the progress of malaria vaccines. In this pilot study, we evaluated the variation in the immune responses elicited by male and female mice immunized with Duffy-binding protein II (DBPII), a leading pre-clinical vaccine candidate against *Plasmodium vivax* malaria. The goal is replicate induction of high-titer, broadly neutralizing antibodies that occur in 'elite responder' individuals living in endemic countries. Important aspects of our studies are to evaluate the impact of sex and the variation in the HLA class II genes on the development and persistence of the antibody responses to DBPII. Inbred (BALB/c) and outbred (Swiss ND4) mice were immunized with DBPII formulated in CpG and alum, which both produced high antibody titers and a strong Th1 and Th2 response. Phenotypic analysis was performed by flow cytometry using different cell surface markers specific for immune cells. The kinetics of B cell memory responses were characterized using murine markers such as CD73 and CD80. The central and memory T cells were defined by murine markers CD44 and CD62l. The activation of MHC class I and class II were studied. We observed high antibody titers and strong memory B cell responses after four immunizations. These data provide further insight to designing the immunization schedules in ethnicity-specific males and females for DBPII and its related allele variants.

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MALARIA CONTROL IN GHANA: PREVALENCE OF PARASITE AND HOST FACTOR

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The transmission of malaria parasites depends on the presence of the sexual stages (gametocytes) of *Plasmodium falciparum* in the blood. The probability of establishing an infection in the mosquito depends on several factors including the densities of male & female gemotcytes as well as their sex ratio. These factors are therefore important determinants of the fitness of *P. falciparum.* The availability of functional antibodies against gametocyte antigens including Pfs48/45 and complement dependent Pfs230 & other sexual stage antigens can neutralize & / or prevent the further development of these sexual stage parasites into infectious sporozoites. We intend to compare the prevalence of gametocytes, transmission blocking & merozoite antibodies against Pfs48/45, Pfs230 & MSP3 in two areas: Obom, with high transmission intensity & Asutsuare, with low transmission intensity. We also try to determine the diversity of factors such as cytokines which also may influence malaria gametocyte development. Blood samples were collected from *P. falciparum* infected & uninfected patients living in two different malaria transmission intensity. Total IgG (and subclasses IgG1 & IgG3) and IgM levels are determined by ELISA. The levels of cytokines in the plasma are estimated by Multiplex. Gametocyte prevalence is measured by microscopy & qRT-PCR. We noted overall that our work confirmed the natural acquisition of antibody against sexual as well as asexual stage parasite antigen. Our results are not sufficient to explain the contribution of cytokine on gametocyte carriage and thus its contribution to malaria transmission. These results confirmed the decrease of malaria showed by a low prevalence of gametocyte in the endemic area.

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CONTROLLED HUMAN MALARIA INFECTION INDUCES TRAINED INNATE IMMUNE RESPONSES IN HEALTHY VOLUNTEERS

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Controlled human malaria infection (CHMI) is an infection of healthy volunteers through bites of laboratory reared and *Plasmodium* infected *Anopheles* mosquitoes. This model has been effectively used to unravel the induction and duration of adaptive immunity and evaluate the protective efficacy of vaccines. However, the induction of innate immunity and a possible lasting effect of *Plasmodium* parasites on innate immune cells (trained innate immunity) has never been explored. In vitro monocyte cytokine responses to stimulation with *E. coli* lipopolysaccaride (LPS), *Candida albicans*, *Staphylococcus aureus*, *Salmonella* and *Mycoplasma tuberculosis* were analysed in five healthy volunteers undergoing a CHMI. During infection, monocyte responses to all pathogens were severely diminished compared to baseline. However, four weeks after infection, cytokine production was consistently higher than at baseline. This is the first evidence indicating that *in vivo* infection with *P. falciparum* leads to trained innate immunity in humans.

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ROLE OF THE KUPFFER CELL CD68, A *PLASMODIUM* SPOROZOITE RECEPTOR, IN MODULATION OF EXPERIMENTAL CEREBRAL MALARIA (ECM)

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Role of the Kupffer cell CD68, a *Plasmodium* sporozoite receptor, in modulation of experimental cerebral malaria (ECM). Sung-Jae Cha and Marcelo Jacobs-Lorena *Johns Hopkins Bloomberg School of Public Health, Department of Molecular Microbiology and Immunology and Malaria Research Institute, 615 N. Wolfe St., Baltimore, MD, 21205, USA* ABSTRACT Malaria infection of a vertebrate host starts with liver infection by *Plasmodium* sporozoites. Sporozoites move from the mosquito bite site to the liver via the blood circulation and leave the circulation by traversing Kupffer cells that line the liver blood vessels. Traversal requires interaction between the CD68 Kupffer cell receptor and the sporozoite surface-GAPDH ligand. We previously reported that a strong (~70 %) reduction occurs in the efficiency of sporozoite liver invasion in CD68 knockout (KO) mice compared to wild type controls. More recently, we made the unexpected observation that development of experimental cerebral malaria (ECM) in these CD68 KO mice is strongly inhibited. This inhibition only occurs when the mice are infected with sporozoites, not when infected with blood-stage parasites. Importantly, transfer of plasma from a sporozoite-infected CD68 KO mouse into a wild type mouse induces the ECM-inhibitory phenotype in the recipient mouse suggesting that ECM inhibition is mediated by soluble factors. In support of this hypothesis, we found that the plasma from sporozoite-infected CD68 KO mice has a dramatically different biomarker activation profile compared to wild type (WT) mice. We hypothesize that in the absence of a CD68 receptor, sporozoites traverse Kupffer cells or endothelial cells by breaching them, causing cellular injury and release of protective factors. We identified several candidate cytokines, chemokines, and growth factors that are likely correlated with the ECM-suppression phenotype. This line of investigation may lead to novel approaches for prevention of ECM development and malaria death.

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USING MACHINE LEARNING TO IDENTIFY DISTINCT IMMUNE SIGNATURES INDUCED BY NOVEL ADJUVANT FORMULATIONS

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The success of recombinant vaccines often depends on the selection of adjuvants that drive the magnitude, type, and quality of the vaccine-induced immune response. In this study, we combined broad immunoprofiling of antibody, cellular, and cytokine responses with multivariate analysis and machine learning methods to provide quantitative insight into exactly how different adjuvant formulations alter the resulting vaccine-induced immune response. The current study uses a self-assembling nanoparticle (SAPN) presenting the malarial circumsporozoite protein as model vaccine to describe the impact of different three different liposomal adjuvant formulations, alum (ALFA), liposomal adjuvant QS21 (ALFQ), and both (ALFQA), delivered at two different antigen doses. Initial univariate analysis revealed that the adjuvant formulation did not only impact the type of immune response (Th1 vs. Th2), but also the fine specificity of the antibody response. Using a computational approach that integrated a wide range of immune response data, we identified vaccine-induced immune responses and developed a multivariate model that was capable of identifying the vaccine adjuvant condition from the resulting immune response data alone with 92% accuracy ($p = 0.003$). Furthermore, using linear regression modeling, we were able to determine the relative contribution of ALFA and ALFQ components in the combined ALFQA adjuvant formulation. This approach enabled us to define unique immune signatures for the ALFA and ALFQ adjuvant formulations and quantitatively describe how those adjuvant combinations interact with each other in the mixed formulation. Furthermore, we found that by using multivariate analysis and machine learning, we were able to define clear, reliable, immune response characteristics across different adjuvant formulations and antigen doses (six conditions in total), with a relatively small sample size of three animals per vaccine condition - demonstrating the potential of using machine learning methods to integrate data collected from a broad sampling of vaccine and adjuvant formulations.

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MODELING THE IMPACT OF DIFFERENT LARVICIDING DEPLOYMENT REGIMENS TO INFORM STRATEGIC PLANNING

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By targeting outdoor malaria vectors, larviciding may complement insecticide treated nets and indoor residual spraying. Given the possibilities that longer lasting agents or novel deployment strategies might become available, large scale implementation is being reconsidered. However, not much is known about the effects of different deployment regimens on vector abundance and malaria burden. In this modelling study we explored a wide range of deployment variables to define their relative impact in different transmission settings. We used a population-based mathematical model to simulate the impact of larviciding on vector density, entomological inoculation rate and human malaria prevalence during and after the intervention. Deployment variables were: coverage, start and duration of intervention, effectiveness decay (for microbial larvicides or insect growth regulators), frequency, and seasonal targeting. Transmission seasonality was defined using data from Kilombero Valley in Tanzania. We found that larviciding had the largest impact at low transmission intensity, but this was strongly dependent on coverage and timing. At higher transmission intensity, only high coverage during the wet season had substantial effects. At high transmission, frequent deployment during one wet season had more impact on prevalence than covering consecutive wet seasons with fewer deployments. Overall, larviciding during two wet seasons, with a break during the dry season, was more effective in reducing the prevalence than larviciding during wet and dry season in the same year. Continuous larviciding for at least one year achieved a higher and longer lasting impact when starting at the peak of the rainy season than starting at the end of the dry season. Finally, a similar impact could be achieved with different deployment regimens. Hence, the results could support efficient planning of larviciding strategies adapted to local settings. Our modelling framework could help guide National Malaria Control Programs in adopting and coordinating the deployment of larviciding at large scale.

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WARMER TEMPERATURES AND SPATIALLY COUPLED TRANSMISSION DYNAMICS IN HIGHLAND MALARIA

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Highland malaria remains a major global problem despite several decades of control efforts. The development and survival of the *Plasmodium* parasite and its mosquito vectors are associated with climatic factors, raising concern over the consequences of global climate change. It has been demonstrated that the inter-annual temperature variability modulates the altitudinal distribution of malaria incidence, expanding the disease into higher elevations during warmer years. While the analysis of retrospective data for such epidemic-prone areas has suggested interactions between regions of contrasting elevation and epidemiology, the resulting transmission dynamics have remained unexplored. To examine the dynamics, including in response to future climate, we identified two main regions: a 'spatial-reservoir' region comprising of localities with relatively stable transmission and cases reported year-round; and an 'expansion' region whose localities are more prone to epidemic outbreaks and report cases only during these events. We then formulated a process-based model, coupling transmission in humans and vectors, and between these two adjacent regions, with key parameters a function of climatic factors. Our results show that a spatial model coupled through the movement of infectious and susceptible humans fits the data best when we account for the different climatic conditions of these two regions. Assuming the seasonality of temperature and rainfall in the two regions remains unchanged, a 1ºC increase in local temperature would result in increases of 18% and 112% in annual cases for the reservoir and expansion regions respectively. These changes would also be accompanied by differential effects on the seasonality of transmission, where the reservoir region becomes more stable with smaller peaks, and the expansion region develops larger peaks. These results should apply more broadly to other highland regions in Africa and South America, both in terms of the increases in number of people at risk of malaria under unmitigated conditions, and the type of interventions that would be most appropriate given the changing seasonal patterns.

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MODELING THE ADDED BENEFITS OF SUPPLEMENTAL INTERVENTION TOOLS ON MALARIA TRANSMISSION IN ENDEMIC SETTINGS IN WESTERN KENYA HIGHLAND

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In the past decade, mosquito control interventions such as massive scale-up of insecticide-treated nets (ITN) and indoor residual spraying (IRS), together with the introduction of artemisinin-combination treatments (ACT), have led to substantial reductions in malaria prevalence and incidence in African highlands. However, rising insecticide resistance and increased outdoor transmission have greatly hampered the effectiveness of ITN and IRS, and most highland sites maintain sustained low-level transmission while some others have recently experienced resurgence in malaria rates. Therefore, to determine the efficacy of the supplemental intervention tools that can tackle outdoor transmission and pyrethroid insecticide resistance are urgently needed. Major objective of this research is to evaluate those supplemental intervention tools abilities with special focused on Long-lasting Microbial Larviciding (LLML) in western Kenya Highland. The results show that without supplemental interventions, the impact of LLINs and IRS on malaria transmission and prevalence gradually decline due to increasing insecticide resistance and outdoor transmission. The results indicate that supplementing a LLIN-only intervention with LLML in an area with high insecticide resistance and increased outdoor transmission could reduce the prevalence by 35.2%. Adding LLML to LLIN interventions in areas with little to medium levels of insecticide resistance and increased outdoor transmission could reduce prevalence to 28.5% and 32.5% respectively within 3 years of the initial LLML application. The optimal application time for the LLML intervention is at the start of the dry season when habitats are at their lowest capacity.

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ESTIMATING THE HEALTH IMPACT OF A SEASONAL MALARIA CHEMOPREVENTION INTERVENTION IN MALI IN 2017: MODELING DEATHS AVERTED, CASES AVERTED AND DISABILITY ADJUSTED LIFE YEARS (DALYS) AVERTED

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Using epidemiological models, Population Services International (PSI) retrospectively estimated the health impact of a seasonal malaria chemoprevention (SMC) intervention, as deaths averted, cases averted and DALYs averted. SMC prevents malaria illness by maintaining a therapeutic antimalarial drug concentration in children ages 3-59 months in areas of highly seasonal malaria transmission through intermittent administration of full courses of antimalarials. Over four months in 2017, PSI and partners procured and distributed over 2.4 million full treatment courses of sulfadoxine-pyrimethamine and amodiaquine (SP-AQ) as SMC to 25 health districts across Mali. Malaria deaths averted, cases averted and DALYs averted were calculated in three steps. Avenir Health's Spectrum-Malaria model was used to estimate the number of under-five (U5) deaths and U5 cases that would be averted in 2018 by increasing SMC coverage levels from 0% to 90% in 2017. The number of U5 deaths averted and U5 cases averted per child receiving a full SMC course were calculated by dividing the output from step one by the number of children that would need to receive SMC to reach 90% coverage. Years of life lost and years of life disabled per malaria death from Global Burden of Disease was overlaid to estimate potential health impact per child receiving SMC. An estimated 0.003 malaria deaths, 0.616 cases and 0.258 DALYs could be averted per child receiving SMC in 2018 in Mali. Across the intervention area, an estimated 7,210 deaths, 1,485,104 cases and 621,892 DALYs could be averted. SMC contributed approximately 25% of PSI's malaria health impact in 2017 in Mali. Long lasting insecticide treated net and artemisinin-based combination therapy distribution comprised the rest. The model can help to set and optimize SMC targets in the context of a national Malaria Strategic Plan. This modeling methodology can be used to proactively estimate the potential health impact of SMC interventions across the Sahel. Spectrum-Malaria can also be applied at the provincial-level and is an increasingly important tool as countries move from control to elimination.

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MALARIA PROPHYLAXIS EVALUATION OF LONG-TERM ANTIMALARIAL AGENTS USING PHARMACOKINETICS AND PHARMACODYNAMICS CONSIDERATIONS

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Several FDA-approved antimalarial agents, including Malarone (atovaquone-proguanil), doxycycline, and mefloquine, are commonly recommended for preventing malaria in travelers going to malaria-endemic areas using a daily or weekly regimen. Recently, a safety evaluation of mefloquine and doxycycline versus Malarone participants demonstrated that those receiving mefloquine were more likely to discontinue their medication due to adverse effects compared to Malarone and doxycycline users. Although Malarone showed a better safety profile than mefloquine, the absolute risk of malaria during short-term travel appears low with all three agents. Adherence to malaria prophylaxis regimens needs improvement. Evidence from clinical and pharmacokinetics-pharmacodynamics (PK/PD) studies have confirmed that a shortened oral Malarone regimen may convey full protection to the gold-standard dosage regimen. There is another relevant aspect of Malarone PK/PD to consider, namely the very long blood schizonticidal effect which makes this combination attractive for consideration as the first line malaria prophylaxis. So far, there is no perfect option among available antimalarial regimens for long-term travelers and expatriates. Most prophylactic regimens provide about 75% to 95% protection, even if taken correctly, and no prophylactic regimen is 100% effective. To optimize the current regimens, the long-term prophylactic agents with monthly dosage as well as a yearly implantation design are being developed at the Walter Reed Army Institute of Research (WRAIR). In this topical review, we will explore the advantages of our long-term prophylaxis approach in comparison with current drug regimens. We will also examine the evidence behind current indications for the use of Malarone and will summarize the current body of literature surrounding its safety and efficacy. Additionally, this review demonstrates that Malarone may offer a statistically significant prevention benefit with a much longer and safer prophylactic efficacy.

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ANTIBODY DENSITY MODEL OF MALARIA TRANSMISSION: PRACTICAL IMPLEMENTATION IN HAITI

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In comparison to traditional measures of malaria transmission intensity, serological data is typically less expensive to obtain and has particular utility in areas of low transmission. The commonly used method of analyzing serological data is to estimate seroconversion rate using a catalytic model. This model is limited by the requirement to dichotomize samples into seropositive and seronegative groups, ignoring the differences within groups. While complex mathematical models are frequently used for epidemiological forecasting, they are less often used for data analysis. Methods to estimate malaria transmission intensity via partial differential equation model of malaria antibody density make use of the full continuous nature of the data and typically lead to improved precision in estimates of transmission intensity, but are difficult to

implement. The purpose of this study was to (1) apply such a method to estimate malaria transmission intensity in Haiti and (2) improve the usability of this method. Preliminary results of applying such a method to estimate transmission intensity from samples collected in 2013 from four different sites in the Ouest and Sud-Est departments of Haiti show quantitative differences between the traditional (catalytic model) and novel (partial differential equation model) methods. A total of 831 serum samples were assayed for apical membrane antigen (AMA-1) and merozoite surface protein-1 ($MSP\text{-}1_{19}$). We report and compare results of both traditional and novel analyses of both assays. The usability issues in transitioning a complex model from research to an operational role are significant; overcoming these is a prerequisite to any model gaining wide acceptance. We discuss these usability issues within a general framework for the deployment of complex mathematical models for the analysis of epidemiological data.

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DEVELOPING AN INVESTMENT CASE FOR MALARIA ELIMINATION IN KWAZULU-NATAL, LIMPOPO, AND MPUMALANGA, SOUTH AFRICA USING MATHEMATICAL MODELLING

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South Africa (SA) aims to achieve the target of national malaria elimination by 2020. Malaria transmission is restricted to three of the nine provinces in SA: KwaZulu-Natal, Limpopo, and Mpumalanga. From 2012 to 2016 sustained malaria transmission levels were experienced: 1828 local cases and 5249 total cases in 2012; 1100 local cases and 4252 total cases in 2016. In 2017 SA experienced a malaria outbreak in which local and total cases increased to 21882 and 28529 respectively. Transmission has been largely sustained due to population movement across the northeastern border with Mozambique and the northern borders with Zimbabwe, where transmission intensity is significantly higher. SA funds its own malaria programme primarily for IRS and is a member of the Elimination 8 and MOSASWA, regional entities that receive regional funding from the Global Fund (GFATM). However, SA is ineligible to receive a national allocation from the GFATM despite the funding gap to address the additional burden of imported cases. To support malaria elimination efforts in SA, the South African Department of Health and key partners are developing an investment case for elimination. The investment case seeks to determine the micro and macroeconomic costs and benefits of malaria elimination using a mathematical model that projects rates of decline to elimination by 2020. Empirical cost data are incorporated into the model to estimate the cost of elimination and the return on investment of selected interventions against transmission by using estimates of the number of malaria mortality and morbidity cases averted. Numerous scenarios are explored to determine the most cost-effective mix of interventions to achieve elimination. The investment case findings can be used by national and provincial malaria elimination programmes to identify financing gaps, support programme budgeting and strategic planning, and to advocate for sufficient financial resources and political commitment to achieve elimination by 2020 and to maintain elimination status in South Africa. The investment case approach can be adapted for other malaria eliminating countries in Southern Africa.

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MODELING THE AGREEMENT AND COST OF INDOOR RESIDUAL SPRAY IMPLEMENTATION STRATEGIES TO CONTROL MALARIA TRANSMISSION

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Indoor residual spraying (IRS) is an effective method to control malaria-transmitting *Anopheles* mosquitoes, and often complements insecticide-treated mosquito nets, the predominant malaria vector control intervention. With insufficient funds to cover every household, malaria control programs must balance the malaria risk of a particular human settlement against the financial cost of spraying that settlement. This study creates a framework for modeling the cost of IRS implementation, and applies it to potential targeting strategies in four provinces (Luapula, Muchinga, Eastern, and Northern) in Zambia. We used network models to assess the travel cost between operational bases and target households in settlements identified through remote sensing. We tested the cost of spray strategies assuming 50% of structures were sprayed based on a targeted approach using various risk maps: a) predicted probability of the presence of each of three main anopheline vectors (*An. arabiensis, An. funestus, An. gambiae*), or b) predicted *Plasmodium falciparum* parasite rate in 2-10 year olds (*Pf*PR). The estimated cost of reaching settlements to deliver IRS ranged from $0.01 to $37.89, with 75% of settlements costing $5.80 or less. Costs were no different between targeted and untargeted settlements for *An. arabiensis* and *An. funestus* targeting. Mean spray costs were lowest for settlements when targeting *An. gambiae*, and highest for *Pf*PR, and both of these were significantly cheaper than the mean cost of spraying untargeted settlements with lower values of *Pf*PR and *Anopheles* vector capacity. The *An. funestus* targeting strategy had the highest cost, but the difference was negligible. Differences were observed in the cost of reaching settlements with higher estimates of *Pf*PR and *Anopheles* vector capacity, both in cases of applying blanket IRS and in cases of applying IRS based on risk maps. These findings confirmed the idea that reaching areas with higher malaria burden, which often are more rural and challenging to access, is more expensive than reaching areas with lower malaria burden. More research is needed in how best to apply risk maps for control programs.

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ASSESSING THE COURSE OF INFECTION OF DIFFERENT RODENT MALARIA PARASITES IN *GRAMMOMYS SURDASTER* (*GRAMMOMYS DOLICHURUS*)

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Investigations of malaria are often conducted by examining infections of rodent malaria parasite species in inbred mouse models, which raises the question whether these lab-adapted, artificial hosts accurately recapitulate features of malaria infections in humans. For example, rodent vaccine efficacy results often do not predict protection in humans. African woodland thicket rats (*Grammomys surdaster*) are the natural host for some rodent malaria parasites such as *Plasmodium berghei* and are suspected to be the natural host for *P. vinckei vinckei* parasites. *Thamnomys rutilans* are the natural host for *P. yoelii*, *P. chabaudi chabaudi* and other *P. vinckei*. In published findings, we demonstrated that thicket rats are highly susceptible to various strains of rodent malaria, including *P. berghei, P. yoelii,* and *P. chabaudi chabaudi.* We also confirmed that

thicket rats better reproduce the efficacy of whole organism vaccines reported in human studies compared to laboratory mice. Here, we expand our studies of the thicket rat model by assessing the course of infection with additional malaria species, including various strains of *P. berghei, P. yoelii, P. chabaudi chabaudi*, and *P. vinckei* (*Pv*). We compared the virulence (growth characteristics and lethality) of each parasite species or strain between thicket rats (TR) and laboratory mice (BALB/c and C57Bl6), and we assessed the relative virulence of sporozoite (SPZ)-induced versus blood stage (BS)-induced infection. The relative virulence of parasites in TR versus laboratory mice varied depending on the parasite isolate, and in most but not all cases, early lethality corresponded to accelerated growth. Similarly, the relative virulence of SPZ-induced versus BS-induced infections depended on the parasite isolate and the host. For nearly all parasites, growth characteristics and/or lethality differed between TR and the laboratory mice. These results confirm that the course of rodent parasite infections differ substantially between their natural and artificial rodent hosts, although the nature of the difference is not predictable and requires assessment for each parasite-host combination.

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INFERRING THE PARASITE RESERVOIR: UNDERSTANDING THE FORCES THAT SHAPE INDIVIDUAL MALARIA INFECTIONS

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Predicting the efficacy of interventions used to interrupt malaria transmission in an endemic population depends on knowing how parasite density is distributed: who is at risk for infection, who is infectious, and what is the appropriate scale for response. Active and passive surveillance measures, including sampling of parasite densities, in malaria-endemic settings provide critical but limited information on the infection and immune status of individuals. Mathematical modeling can bring together disparate data sources into a single explanatory framework that fully describes individual-level infection and immune status. However, an explicit model for the accumulation of acquired immunity to major and minor epitopes arising from multiple strains can be too computationally intensive for large-scale practical applications. Here we combine a statistical model that infers an individual's cumulative exposure and history of infection (malaria age) with a stochastic individual-based model to simulate individual infections across a range of transmission intensities. We use malaria therapy data to draw the shape of an individual infection by deriving transition probabilities between density states for each antigenic wave. Malaria age modulates the transition probabilities and ensuing shape of the malaria infection, which we model through a set of functions parameterized by longitudinal data from the Garki Project in Nigeria and the PRISM study in Uganda. This flexible model framework of infection trajectories and acquisition of immunity can be used to estimate the fraction of the population at any given time with asymptomatic infection and accurately model the intervention impact on populations with dynamic immunity to malaria.

1802

ASSESSING AND PROJECTING THE ROLE OF DISTANCE FROM LOCAL HEALTH FACILITIES ON EARLY CHILDHOOD MALARIA PREVALENCE: A CASE EXAMPLE FROM NORTHERN GHANA

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Distance to the nearest health facility presents a significant impediment to access to malaria treatment in rural areas throughout sub-Saharan Africa, which can sustain high transmission and prevalence rates. In Ghana, the community-based health planning and services (CHPS) initiative was designed to increase access to quality treatment for malaria and other diseases. In this project, we characterize the role of distance to nearest health facility on early childhood malaria in Bunkpurugu-Yunyoo, a rural district in the Northern region of Ghana. From 2010 to 2013, six biannual surveys collected the malaria status of 10,029 individuals under the age of five years old from 428 communities. Environmental variables, including distance to health facility, were collected from remote-sensed and GIS-derived sources. We constructed a Bayesian regression model which was used to compare distance to health facility to other environmental risk factors, compare the effect of CHPS to traditional health centers, interpolate prevalence across the district, and estimate the optimal location for a new health facility. In both the rainy and dry season surveys, only distance to urban center had a stronger effect than distance to nearest health facility. We did not observe a significant difference between the effects of CHPS compared to traditional health centers, which indicates that the type of facilities does not significantly influence the protective buffer provided by the health facility. The model optimization exercise was used to generate proposed locations for new CHPS compounds. Finally, in order to extend the utilization of our model we created an interactive web application for projecting the impact of proposed locations for a new health facility. We believe that this framework provides a useful tool for supporting data-driven policy decisions to improve access to quality treatment and reduce malaria burden.

1803

THE USE OF REMOTE SENSING FOR ESTIMATING THE RISK OF TRANSMISSION AND PREDICTING CASES OF MALARIA IN ARGENTINA

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Malaria is a parasitic disease which affects millions of people all over the world. Early warning systems which provide information about spatial and temporal predictions of epidemics might help control and prevent malaria outbreaks. One of the most used tools for the development of such systems are statistic prediction models based on historical reports of cases and indicators of environmental risks. Remote sensing constitutes an essential source of environmental information for the development of these predictive models. The present work is focused on the use of remote sensing for the estimation of the risk of transmission and the prediction of malaria cases in the northwest of Argentina. The study was carried out in San Ramón de la Nueva Orán city, where cases of the disease have been

reported from 1986 to 2005. The existent relationship between reported malaria cases and climatic/environmental variables (Normalized Difference Vegetation Index (NDVI) Normalized Difference Water Index (NDWI) and Land Surface Temperature (LST)) obtained from Landsat 5 and 7 satellite images was analyzed through multilevel Poisson regression analyses. A higher abundance of reported cases of malaria in summer was observed. A model of ARIMA temporal series which included the environmental variables was generated to forecast malaria cases in the year 2000. In turn, the relationship between malaria cases and environmental/climatic factors showed that malaria cases were associated to an increase in LST and in mean temperature and to a decrease in NDVI. This study is expected to be used for the development of future prevention and control actions by the health officials.

1804

ESTIMATING MOBILE FOREST WORKERS' CONTRIBUTION TO MALARIA TRANSMISSION IN SOUTHEAST ASIA: A MATHEMATICAL MODELING APPROACH

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Recent successes in reducing the malaria burden globally could fail dramatically if resistance to artemisinin-combination therapies continues to expand in Southeast Asia and spreads to high-endemic areas of Africa. Rapid identification and elimination of remaining *Foci* of infection is critical for preventing this. However, these last infections are often the most difficult to identify and eliminate, as they are most prevalent in specific high-risk populations (HRP). Preliminary studies in Champasak Province, Laos, and Aceh Province, Indonesia suggest that HRPs such as forest workers act as mobile reservoirs of infection and transport infections between forest work-sites and other locations. We will use a mathematical modeling approach to assess the relative importance of these HRP groups in sustaining malaria transmission. The Ross-Macdonald model will be extended to include a high-risk human population exposed to a forest-based mosquito population. We plan to inform these models using data from ongoing research sites in Laos and Indonesia where we are collecting data on 1) incidence from passive surveillance records; 2) geo-referenced prevalence data; and 3) gps tracking data of forest workers. Outcomes of interest will include prevalence in high-risk group, proportion of high and low-risk groups in the population and proportion of exposure to forest vs village mosquitoes. The model will enable us to 1) explore the necessity of risk-based population stratification to explain observed malaria infection dynamics in low-endemic settings of Southeast Asia; 2) characterize the HRP groups' key features, in particular in terms of their mobility patterns and interaction with the lower-risk village-based population and; 3) model the effectiveness of HRP targeted interventions on malaria outcomes, evaluating in particular the HRP treatment coverage parameters needed in reactive case detection strategies, respondent driven samplings and adaptive survey designs interventions.

1805

MGDRIVE: A SIMULATION FRAMEWORK FOR GENE DRIVE IN SPATIALLY-EXPLICIT MOSQUITO POPULATIONS AND ITS APPLICATION TO THRESHOLD-DEPENDENT SYSTEMS

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Malaria, dengue, Zika, and other mosquito-borne diseases continue to pose a major global health burden through much of the world. The advent of CRISPR/Cas9-based gene editing and its demonstrated ability to streamline the development of gene drive systems has reignited interest in the application of this technology to the control of mosquitoes and the diseases they transmit. The versatility of CRISPR technology has also enabled a wide range of gene drive architectures to be realized, creating a need for their population-level and spatial dynamics to be explored. To this end, we present MGDrivE (Mosquito Gene Drive Explorer): a simulation framework designed to investigate the population dynamics of a variety of gene drive architectures and their spread through spatially-explicit mosquito populations. A key strength of the MGDrivE modeling framework is its modularity: a) a genetic inheritance module accommodates the dynamics of gene drive systems displaying user-defined inheritance patterns, b) a population dynamic module accommodates the life history of a variety of mosquito disease vectors, and c) a landscape module accommodates the distribution of mosquito metapopulations connected by migration in space. To demonstrate the functionality of the software package, we present example MGDrivE simulations for threshold-dependent drive systems: a) reciprocal chromosomal translocations, and b) toxin-antidote-based underdominant systems. These systems are particularly suited to field trials and other local releases as they are expected to: a) spread at their release site following an intentional release, b) only spread to low levels in neighboring populations, and c) be eliminated through dilution with wild-type organisms. Using the MGDrivE framework and metrics from network theory, we describe design criteria for these systems. In closing, we describe other systems to which the MGDrivE framework applies, and future directions for its development.

1806

VALIDATING FUNCTIONAL HEALTH FACILITIES AND CAPACITY FOR IMPROVED DISEASE SURVEILLANCE THROUGH DHIS2: EXPERIENCE FROM KANO AND ZAMFARA STATES, NIGERIA

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In 2013, the Nigeria Federal Ministry of Health established a Master Health Facility List (MFL) which has not been updated since. Some health facilities (HF) have ceased functioning and new facilities were built in different areas. These affect calculating indicators in District Health Information System (DHIS) which lacks unique identifiers for HF. We conducted HF service delivery assessment in Malaria Frontline Project areas in Kano and Zamfara states to document HF geo-location, functionality, human resource (HR), DHIS reporting status and reasons for non-reporting. A census of all government HF in each Local Government Area (LGA) was conducted from May to August 2017 using semi-structured questionnaire. The National Primary Health Care Development Agency (NPHCDA)'s classification was used to define functional HF, primary health care (PHCs) facilities and their minimum human resources (HR) requirement. In Kano 20 LGAs, 726 HFs were geo-located, 31 (4.3%) facilities were previously not on MFL, 608 (83.7 %) were PHCs, 118 were secondary or tertiary facilities, 710 (97.8%) facilities were functional and 644/710 (90.7%) reported to DHIS. The 14 LGAs in Zamfara had 739 HFs, 8 (1.1%) were previously not on MFL, 694 (93.9%) were PHCs, 45 were secondary or tertiary facilities, 695 (94.0%) were functional and 656/695 (94.4%) reported to DHIS. Reasons for non-reporting included lack of training and low supportive supervision. Per NPHCDA definition, only 1 PHC in Kano State met the minimum HR requirement for PHC. The results have

improved calculation of indicators by providing accurate numerator and denominator from DHIS. Most HFs were functional but did not meet minimum HR required staff number. Most functional facilities reported to DHIS. MFL should have unique identifiers in DHIS to determine numerator and denominator for rates. Health authorities should address reasons for non-reporting. Regular review of HR strength is essential to provide quality primary health care services. We recommend other States conduct similar assessments to improve DHIS reporting.

1807

PLASMODIUM VIVAX MONO-INFECTION AS MAIN CAUSE OF SEVERE MALARIA IN A CO-ENDEMIC REGION FOR *P. VIVAX* AND *P. FALCIPARUM* IN THE PERUVIAN AMAZON

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Loreto is the department most affected by malaria in Peru. In 2017, it reported 53163 malaria cases (about 90% of total cases in the country), of which 75% and 25% were caused by *P. vivax and P. falciparum*, respectively. Recent reports in South-American co-endemic countries for both species indicated that severe malaria (SM) caused by *P. vivax* is not rare. This observational study describes the characteristics of hospitalized individuals with malaria at the referral hospital in Loreto, Peru. Data were prospectively collected from medical records of hospitalized patients with malaria at the Medicine Unit of the Regional Hospital of Loreto from January 1st to December 30th 2017. Severity was classified based on the criteria published by the World Health Organization (WHO) in 2014. Forty patients with malaria were hospitalized during the study period. The mean age was 39.4±19.5 years (range: 13-89) and the female/male ratio was 1.7/1. SM was identified in 31 patients (31/40, 77.5%), including one woman in the third trimester of pregnancy and one individual with admission diagnosis of ophidism. One third of these patients had confirmed malaria in the past 12 months. *P. vivax* mono-infection (23/31, 74.2%) confirmed by expert microscopy predominated over *P. falciparum* mono-infection (6/31, 19.4%) and mixed infection (2/31, 6.5%) as cause of SM. Among the criteria of severity, prostration was the most common (19/31, 61.3%), followed by shock (12/31, 38.7%), severe anaemia (5/31, 16.1%), jaundice (4/31, 12.9%) and significant bleeding (4/31, 12.9%). About 39% (12/31) of patients with SM had comorbidities, such as diabetes mellitus-2, obesity, chronic kidney disease, immunodeficiency virus infection, hepatitis B, respiratory tract disease, and short bowel syndrome. The pregnant woman with SM had mixed *Plasmodium infection*, and her newborn presented low birth weight (2440 gr.). All SM cases were managed with intravenous artesunate according with WHO treatment guidelines, and evolved favourably without lethal outcomes. *P. vivax* was the main cause of SM in the Amazonian department of Loreto, and it should not be longer considered as "benign" species.

1808

CLOSING THE LOOP: IMPROVING MALARIA TREATMENT CARE AT THE COMMUNITY LEVEL IN MADAGASCAR

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Community health volunteers (CHV) provide integrated management of childhood illnesses services in remote communities in Madagascar, including diagnosing and treating cases of uncomplicated malaria among children less than five years of age (CU5). However, over 50% of CU5 with fever do not seek care. To improve care, the USAID Mikolo Project trained and supervised CHVs in malaria case management in six high-transmission districts and taught them to educate parents about malaria. The project worked alongside CHVs to improve the process of malaria treatment, including educating parents on seeking care from a CHV within 24 hours of the onset of illness; administering a rapid diagnostic test (RDT); treating RDT-confirmed, uncomplicated cases of malaria with artemisinin-based combination therapy (ACT); ensuring that parents have their child adhere to the treatment regimen and follow-up with the CHV; and referring suspected severe malaria cases to the nearest health center. Reports from 1,157 CHVs in 679 Fokontany were included in the analysis. 52,922 febrile children were seen for consultation in 2016; 43% of the cases came within 24 hours of the onset of fever. In 2017, there were 61,114 cases, which is an increase of 15%; likewise 49% of cases of febrile children came within 24 hours of fever onset. In 2016, 99% of the cases were tested with the RDT. This performance decreases slightly to 92% in 2017 due to indivisible RDT kits (a box of 25 units in a hospital kit, which cannot be easily distributed amongst community-level actors). However, in 2017, 34,167 (or 90%) of the cases of malaria were treated with ACT, which is an increase of 12% compared to 2016. Finally, there were 4,253 cases of severe malaria in 2017, which is a reduction of nearly 38% compared to 2016. These results may reflect parents' increased satisfaction with and acceptability of the services provided by CHVs, as they feel more confident in taking their child to the CHV for health care. Parental education on early care seeking, coupled with treatment adherence follow-up by CHVs, can improve the malaria treatment outcome.

1809

MALARIA AND THE MICROBIOME: A SYSTEMATIC REVIEW

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The microbiome influences malaria parasite fitness and transmission efficiency in mosquitoes, and appears to affect malaria dynamics in mammalian hosts as well. Nascent research examining the interrelationship of malaria and the mammalian host microbiome has yielded interesting insights inviting further study. We conducted a systematic review of the literature examining associations between the microbiome and malaria in mammalian hosts. An electronic search algorithm was adapted to PubMed, MEDLINE, Scopus, Embase, and Web of Science, and reference lists of relevant sources were manually searched. Identified studies (n=4,177) were screened and assessed independently by two authors, and results were compiled in a qualitative synthesis of the evidence. Ten relevant studies were identified. They demonstrate associations between certain intestinal communities and protection against *Plasmodium* infection, and modulation of disease severity. *Plasmodium* infection acutely and reversibly reshapes gut microbial composition in mice. The makeup of

human skin microbial communities may influence mosquito attraction and thus disease transmission. Early research supports a relationship between malaria and the microbiome. The evidence is incomplete, but the observed associations are evocative and signal a promising avenue of inquiry.

1810

HOW DO THE REPORTS TRANSLATE INTO FIELD PERFORMANCE? CROSS-VALIDATION OF DATA FROM ROUTINE REPORTING VS MYSTERY CLIENT VISITS AMONG PRIVATE SECTOR MALARIA PROVIDERS IN MYANMAR

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In 2015, Population Services International (PSI) started the roll out of malaria RDTs among informal private sector providers in Myanmar, to improve the quality and coverage of malaria testing and treatment services in remote areas where formal health care sector coverage is most stretched. These providers were trained to conduct RDTs for all suspected malaria cases and to report the results. Since then, PSI received hundreds of thousands of RDT reports from these providers, yet it was largely unknown whether the providers were testing all suspected fever cases nor how the reports related to actual field performance. Up to October 2017, PSI trained 8,673 providers across 5,331 villages in 15 regions of Myanmar. In November 2017, PSI conducted mystery client (MC) visits to a randomly selected sample of those providers. A local person, "MC", was trained to approach the provider with symptoms suggestive of malaria fever. PSI staff pretended to be a friend of MC and observed provider-client interaction. MCs visited 457 providers across 422 villages within 34 days. The results from the visits were compared against data from routine reports, and possible relationships were explored. From routine data, those 8,673 providers conducted 482,197 RDTs, and detected 6142 malaria cases (1.27% positivity) in 2017. Yet, the performance was not uniform: 1,899 (21.9%) did not report at all, and top-performing 36% among 6,774 reporting providers contributed to 80% of all RDT tests, and just 20% detected all malaria positive cases. Among 457 MC visits, only 79 (17.2%) got tested with RDT. The likelihood of performing RDT was found to be related to routine reporting. Those who reported any RDT testing were 3 times more likely to perform RDT on MC, compared to those who reported none (20.3% vs 6.2%). And those whose RDTs produced zero positives in routine reports were half as likely to perform RDT, compared to those who had detected any positives (15.2% vs. 29.4%). This comparison between routine data and MC visits showed that providers may not test all suspected fever cases, and furthermore, the lack of seeing any malaria positive cases over time could affect their field practice.

1811

IMPACT OF CODON USAGE ON SEQUENCE VARIANT COMPOSITIONS OF *PLASMODIUM FALCIPARUM* CELTOS

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Numerous proteins are difficult to express in heterologous expression systems. Codon usage frequency is a factor that has been shown to influence final protein structure and function. The frequency with which codons are used imparts vital information on the formation of secondary and tertiary protein structure. Inconsistencies in codon usage can lead to poor levels of overall expression, expression of non-functional, insoluble or truncated proteins. Generally, protein translation and folding in *E.coli* occurs co-translationally. The approach of codon harmonization replaces native codons aligning them with the expression host usage frequency while also identifying putative inter-domain segments to allow for slowing down of translation rates. To address challenges of heterologous expression of *Plasmodium falciparum* target-genes in *E. coli*, a "codon harmonization" approach was applied to the malaria antigen CelTOS (Cell traversal protein for ookinetes and sporozoites). CelTOS plays an important role in traversal of cells in the mosquito and vertebrate hosts and is highly conserved in *Plasmodium* species, suggesting a conserved functional role and making it a desirable candidate for a malaria vaccine. Direct comparison of a codon harmonized-protein and a wild type codon usage-protein at the level of protein expression, solubility, yield, stability, and immunoreactivity against CelTOS-specific mAbs did not show significant differences between the expressed products. Though the difference in expression and yield of the codon-harmonized and native proteins was negligible, we found the proteins to be different in their molecular masses and secondary structure. LC MS/MS detection of low frequency misincorporations in polypeptides against a background of wild-type molecules revealed low level of variant amino acid misincorporation, and a single likely misincorporation event between the two proteins, reflecting a potential error in translation. The implication of amino acid misincorporation on product homogeneity and as a product quality attribute must be considered during preclinical investigations and process development.

1812

HEALTH WORKER'S POST-TRAINING PRACTICES FOR CASE MANAGEMENT OF SEVERE MALARIA IN SOUTHERN NIGERIA

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Patients with severe malaria require prompt and appropriate treatment to prevent death. Training of health workers on severe malaria case management, provision of commodities for definitive management and ongoing supportive supervision were provided for hospitals in three states in Nigeria. This study was a cross-sectional assessment of severe malaria case management practices of 677 health workers and 562 anonymized records of severe malaria patients in 75 hospitals in the three states using WHO's service availability and readiness assessment (SARA) tools. Data analysis was done using SPSS software. Nurses/midwives (50.4%), doctors (13.1%), community health workers (10%) and pharmacists and laboratory scientists were interviewed. Most hospitals (96.6%) profiled had either rapid diagnostic test kits or microscopy for malaria diagnosis, stocks of injectable artesunate (Inj AS) (89.3%) and artemisinin-based combination therapy (82.7%) during the assessment. Case records of severe malaria patients indicated that diagnosis of severe malaria was documented in 363 (64.6%) out of which parasite-based microscopy was 231 (41.1%). Most of the patients (525, 93.4%) were reported to have received Inj AS for severe malaria within 24 hours of admission. Twenty percent of the patients with negative blood smear were still prescribed an antimalarial. Biological diagnosis of severe malaria was significantly associated with having a functional laboratory (p=0.008), Inj As availability (0.025) and availability of malaria treatment policy chart (p=0.005). Availability of policy chart also significantly influenced appropriateness of antimalarial prescription practices and promptness of antimalarial treatment (p=0.003, and 0.007) with Inj AS. Though availability of Inj AS and diagnostic services were high with prompt treatment of severe malaria cases, there was inadequate microscopy to monitor patient progress. Provision of functional microscopy, continuous supply of injectable artesunate and availability of malaria treatment charts will help to ensure appropriateness manage.

1813

HEMATOLOGICAL DETERMINANTS FOR A G6PD DEFICIENCY AND DRUG-RELATED HEMOLYTIC RISK REGRESSION-BASED MODEL

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Radical cure for *Plasmodium vivax* with 8-aminoquinoline drugs, such as primaquine, can clear *P. vivax* parasites by killing malaria hypnozoites. However, 8-aminoquinoline drugs can cause severe hemolysis in patients with reduced activity of the glucose-6-phosphate dehydrogenase (G6PD) enzyme. The X-linked G6PD gene mutations resulting in reduced G6PD activity levels are prevalent in malaria-endemic regions, reaching more than 15% prevalence in some Southeast Asian populations. The severity of hemolysis is dependent on the level of G6PD activity, the G6PD allele variant, drug dosing, patient status, and disease factors. The 2D6 isoform of the cytochrome cyp450 (CYP2D6) is the key metabolizer of primaquine and may be associated with both poor response to primaquine treatment and the hemolytic toxicity of the drug. This uncertain risk among those with reduced G6PD activity levels, particularly intermediate levels, represents a major barrier to wide-scale use of radical cure. Using data generated in a clinical trial conducted in Thailand, we aim to develop a regression-based model that will quantify the associated hemolytic risk in females heterozygous for G6PD deficiency upon exposure to 8-aminoquinoline drugs. We will present the proof of concept of a risk model that could be used to predict the severity of hemolysis based on the most significant of the following determinants: age, gender, complete blood count data, G6PD activity levels, distribution of G6PD intracellular activity measured using flow cytometry, primaquine dosage levels, and CYP2D6 phenotype. A model for hemolytic risk based on detailed profiling of intracellular red blood cell G6PD activity levels, combined with novel quantitative point-of-care G6PD diagnostic tests, has the potential to improve clinical treatment guidelines for the radical cure of *P. vivax* using primaquine within the context of G6PD deficiency and other physiological factors.

1814

IDENTIFICATION OF IMMUNE-PROTECTIVE ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM*

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For many of the world's military forces, malaria continues to be a major complicating factor in conflict zones around the world. It drastically, and dangerously reduces troop readiness during times of warfare, and frequently compromising mission readiness in malaria endemic regions. Antibodies against sporozoites are the first line of defense against malaria infection but despite decades of research it is unclear what the fine specificities and/or functional activities of these antibodies are. Currently it is understood that not all immune antibodies specific for sporozoite-expressed antigens are able to block the infection of hepatocytes, in fact only a small subset seem to slow down or block invasion. The identification of invasion slowing or stopping factors would prove invaluable for malaria drug and vaccine development. In working towards this goal proteomic methods were developed to establish the fingerprint of protective antibodies and identify key targets of the humoral immune response utilizing mass spectrometry. With further interrogation of the proteome through bioinformatics we are able to establish profiles of antibodies in the protective and un-protective sera from the IMRAS sera (irradiated sporozoite, NF54, Phase 1 CHMI trial). Further testing allowed for the isolation of schizonts in HC04 human liver cells exposed to pooled protective/un-protective IMRAS sera which were processed and analyzed for infectivity rates. Tables will exhibit pathways defined by the KEGG database, and the PathNet algorithm that is used to identify which protein pathways show statistical enrichment between the infected and non-infected conditions. Compilation of this information allows for bioinformatics analysis finding unique and pertinent proteins (including antibodies and antigens) of interest. With this information drug and vaccine development may become targeted to select candidates that will cure or confer the highest level of protection allowing the warfighter to focus on the mission.

1815

TRANSFORMING THE MARKET FOR QUALITY-ASSURED ACT (QAACT) IN KINSHASA: AVAILABILITY, PRICE AND MARKET SHARE RESULTS FROM A REPRESENTATIVE OUTLET SURVEY IN SUPPORT OF LARGE-SCALE MARKET DEVELOPMENT ACTIVITIES

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The government of the DRC has set 2020 targets of 80% suspected malaria cases tested and 100% confirmed cases appropriately treated, in line with WHO recommendations. The private health sector in Kinshasa is a critical partner in delivering quality malaria care to achieve these targets as it serves 70% of childhood fevers and has 86% antimalarial market share in the city. However, in 2015 QAACT availability was only 19% in drug shops, the most popular antimalarial source. The median private sector adult-equivalent (AE) QAACT course cost $5.04 and QAACT market share was just 2.2%. Beginning in December 2015 PSI, in partnership with the NMCP, negotiated QAACT price reductions with manufacturers, introduced co-payments for importers, conducted large-scale promotion of a Green Leaf quality logo, and supported medical detailing. Additional supporting interventions included the provision of free RDTs to qualified pharmacists and MOH-led supervision. Between Feb 2016 and Feb 2017 manufacturers sold 1.4 million QAACTs to local importers. A city-wide representative outlet survey in Feb 2017 assessed the availability, affordability and market share of QAACTs. 841 private and public outlets were screened for availability of malaria testing and treatment, 831 outlets were interviewed, and 4,929 antimalarial products audited. Standard ACTwatch sampling, fieldwork and analysis methods were used. Availability of QAACT in 2017 increased to 39.2% in private facilities, 58.8% in drug shops and was 93.8% among registered pharmacies. Median retail price for AE QAACT was $1.80 and private sector QAACT market share increased to 14.4%, gaining share from non-artemisinin therapies. These data suggest that subsidized QAACT have been embraced by private providers. After 1 year, this intervention has met the AMFm benchmarks for success for availability and market share, and greatly reduced the price differential between QAACTs and common non-artemisinin therapies. As the co-payment is reduced, ongoing M&E activities will provide valuable lessons on how to maintain private sector market improvements while reducing the reliance on external subsidies.

1816

EXPERIENCES AND PERCEPTIONS OF CARE SEEKING FOR FEBRILE ILLNESS AMONG CAREGIVERS AND PROVIDERS IN EIGHT DISTRICTS OF MADAGASCAR

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Delayed care seeking for febrile illness in Madagascar may contribute to poor outcomes for diseases including malaria, particularly among children and pregnant women. We conducted an assessment with caregivers of children, pregnant women, and clinicians to identify gaps, attitudes and practices that hinder timely care seeking and adherence to national malaria treatment guidelines.In November 2017, we conducted in-depth interviews with 32 pregnant women and 16 caregivers of children < age15 years in eight malaria transmission zones to elicit care seeking behavior information. We conducted 16 focus group discussions (FGD) of approximately 128 participants among this demographic. Thirty-two private and public health care providers were interviewed and completed knowledge tests to describe barriers to and facilitators of health provider adherence to treatment guidelines. Availability of commodities at facilities was tracked. We used descriptive in-depth analyses of recurring themes and quantitative scoring of provider knowledge. Pregnant women and caregivers of children cited poor quality of services, lack of malaria rapid diagnostic tests (RDT) and medications, unwelcoming attitudes of providers, cost, and distance from health centers as reasons for delayed care seeking. Additional influencing behaviors and conditions reported by providers as reasons for delayed care seeking included self-medication, use of traditional healers, fear of hospitalization and not perceiving fever as a severe condition. All providers reported consistently testing febrile patients with RDTs before treatment or transfer, which was confirmed through knowledge tests. While the majority of providers (90%) correctly answered most (80% or more) of the knowledge questions, gaps in knowledge were still apparent. Stockouts of key malaria diagnostic and treatment products was recorded in several facilities. Improving stock management, providing very low-cost care, training clinicians to create a welcoming environment, and raising awareness of the importance of evaluating fevers could improve timely care seeking.

1817

MALARIA AND KAPOSI SARCOMA HERPES VIRUS (KSHV) SEROPREVALENCE IN A COHORT OF CHILDREN IN WESTERN KENYA

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Malaria may be an important factor in the transmission of Kaposi sarcoma herpesvirus (KSHV), the etiological agent of Kaposi sarcoma. Both malaria and KSHV are endemic to Sub-Saharan Africa and cause substantial public health burden. We tested samples from a cohort of Kenyan infants, followed through age three, to explore whether children with *Plasmodium* infection compared to no infection are more likely to become KSHV seropositive. Blood plasma from 167 Kenyan children were tested for KSHV and *Plasmodium* sero-response over a three year period. Children were *Plasmodium* seropositive if antibodies to MSP-1 proteins from any of three *Plasmodium* strains (kd42, kda42, and fup) were detected using a bead-based multiplex Luminex assay (Plos Pathogen, 2014 10(3) e1004046). Seropositivity for KSHV was determined by presence of antibodies to either (1) ORF73 or K8.1 or (2) ORF73, ORF61, ORF38, ORF65, ORF59, K8.1, K8.1b, or K5; as detected by Luminex. To determine if *Plasmodium* infection in children led to earlier KSHV seroconversion, we utilized a cox proportional hazards regression model including *Plasmodium* seropositivity as a time varying covariate and KSHV status at 12, 18, 24, 30 and 36 months as outcome. By age three, 157(94%) children were *Plasmodium* seropositive at least once. When measured by K8.1 and ORF73 only, 16(10%) children were KSHV seropositive by age three; compared to 52(31%) when using a broader array of KSHV proteins. We saw a significant increase in the hazard of being KSHV seropositive among children with *Plasmodium* infection versus not, when KSHV was measured using antibodies to eight KSHV proteins (HR=2.3, 95%CI: 1.1-4.9); but no significant difference when measured by K8.1 and ORF73 only (HR=1.7, 95%CI: 0.5-6.1). In conclusion, our study shows that most children will have a *Plasmodium* infection in Kenya by age three, confirming the importance of malaria in the region. In addition, children with a *Plasmodium* infection have a greater risk for infection with KSHV at an earlier age. Malaria may have a potential role in enhancing KSHV transmission, which requires further exploration.

1818

IMPROVING ADHERENCE TO NATIONAL MALARIA TREATMENT GUIDELINES BY VILLAGE MALARIA WORKERS IN SELECTED TOWNSHIPS THROUGH A LOW-DOSE, HIGH-FREQUENCY TRAINING APPROACH

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Myanmar, until recently with the highest malaria burden among Greater Mekong Sub-region countries, has seen decreasing morbidity and mortality through scaling up of vector control and case management interventions. The PMI-funded Defeat Malaria project aims to reduce malaria burden and contribute to the national goal of malaria elimination. One of the key strategies is to provide quality diagnosis and early treatment through a network of community-based village malaria workers (VMW) covering 1.5 million people in 33 targeted Townships. This review assessed capacity development of VMWs to adhere to National Malaria Treatment Guidelines (NTGs) through a competency-based low-dose, high frequency (LDHF) training approach. Review of progress through records and reports was conducted before and after LDHF sessions. The approach was used for 87 VMWs from Gwa (Rakhine State) and Palaw (Tanintharyi Region) Townships by 36 township-level general trainers previously trained by 11 State/Region level master mentors. VMWs received on-site trainings on NTGs (in three "doses") in June, July and August, 2017. Post-training follow-up was conducted by clinical audit during supervision visits, routine data quality assessment and verification of monthly reports. Monthly clinical audits revealed decreasing need for verification as most VMWs were using job aids properly and responded correctly to case scenarios during supervision. These results were consistent with beneficiary interviews during on-site data verification. Improvement in adherence to NTGs was assessed as percent of uncomplicated malaria cases that received correct antimalarial treatment. Findings show adherence to NTGs increased from 72% to 100% in Gwa and remained high but unchanged (from 91% to 92%) in Palaw before and after the three-month training period. The LDHF approach was appropriate for capacity building of VMWs on protocol adherence in Rakhine State, but in some circumstances as in Palaw, VMWs will require materials in local languages with culturally appropriate illustrations. These will be formulated and assessed in future reviews.

1819

IMPROVING MALARIA CASE MANAGEMENT (MCM) INCLUDING MALARIA IN PREGNANCY (MIP) THROUGH NATIONAL ROLL-OUT OF MALARIA SERVICE AND DATA QUALITY IMPROVEMENT (MSDQI): A CASE STUDY FROM TANZANIA

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As a high malaria burden country facing an increased demand of health services, quality of care remains a major concern for the Ministry of Health (MoHCDGEC) in Tanzania. In response, the MoHCDGEC and partners developed the Malaria Service and Data Quality Improvement (MSDQI) checklist for guiding supportive supervision teams to evaluate the quality of malaria case management (MCM) and malaria in pregnancy (MIP) services at facility level. The USAID Boresha Afya Project, in collaboration with National Ministry Trainers and Region and Council Health Management Teams, supported cascading of MSDQI mentorship to other providers across 1817 facilities in 7 regions, to encourage the monitoring of quality of MCM and MIP services. A standardized health facility MSDQI package was used to observe providers' diagnosis, treatment and antenatal care (ANC) practices. Facilities were selected as part of supportive supervision, with priority given to low performers identified from DHIS2 data. Modules covered included OPD & ANC services, Microscopy, severe malaria, and data quality assessment. Facility performance of each step was scored, summarized within indicator category, and assigned a percentage performance score. Overall providers did well testing febrile patients for malaria (81%), providing correct diagnosis (92%) and provision of correct treatment (86%), yet a large number of facilities did not meet the minimum performance standard of 75% at the OPD. For OPD service provision, the overall score was 71%, with history taking (61%), physical examination (50%), and patient counselling (51%) as the lowest preforming areas. To address quality of care and improve services, Boresha Afya plans to disseminate malaria and ANC guidelines, job aids and SOPs, and continue with supportive supervision and mentorship through the MSDQI tool to build capacity of providers in microscopy, improved diagnostics, and estimation of gestational age. Effective implementation of MSDQI requires systematic preparation of the supportive supervision team, including orientation of the team to roles, competency-based training, resource allocation and use of tablets.

1820

USING THE COLLABORATIVE QUALITY IMPROVEMENT APPROACH TO INCREASE ADHERENCE TO THE TEST, TREAT AND TRACK MALARIA CASE MANAGEMENT FRAMEWORK: EXPERIENCE FROM 10 HEALTH FACILITIES IN UGANDA

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Malaria is the leading cause of morbidity and mortality in Uganda, contributing 30-50% of outpatient visits and half of inpatient pediatric deaths. The President's Malaria Initiative (PMI), through USAID's Malaria Action Program for Districts (MAPD) project, is supporting the Ministry of Health's National Malaria Control Program to improve malaria services in 47 districts through building the capacity and quality service delivery at health facility and community levels. Data reliability at health facilities is critical to providing evidence-based interventions, tracking epidemiological trends and stratification. Through collaborative quality improvement (CQI), MAPD supported improving the quality of malaria service delivery records at selected health facilities. The focus of the data review was to track and improve the malaria test results and compliance with treatment guidelines and the test, treat and track framework. The CQI approach was introduced in November 2017 starting with ten health facilities in the project regions selected for the CQI pilot. Each health facility was coached to improve its ability to review malaria data, identify gaps and develop solutions. Facility improvement teams were formed and each member assigned a role in improving malaria services data management. The improvement process was tracked by the health facility teams through a national quality improvement tool. From November 2017 to February 2018 completeness of malaria patient information in the outpatient register increased from 0 to 86%, concordance of malaria diagnostic information in the laboratory registers and the outpatient register improved from 47% to 100%, patients diagnosed as positive who were treated for malaria increased from 91% to 99%, patients with a negative malaria test treated decreased from 9% to 1%, and pregnant women receiving 3 or more doses of IPTp-SP increased from 43% to 53%. Data is tracked on a monthly basis using documentation journals. The CQI approach has the potential of improving adherence to the national malaria policy of test, treat and track if implemented to scale.

1821

MALARIA DEATH AUDITS: A TOOL TO HELP IMPROVE SEVERE MALARIA CASE MANAGEMENT AND POTENTIALLY PREVENT MALARIA-RELATED DEATHS IN ZIMBABWE

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Nearly 50% of the Zimbabwean population is at risk for malaria. Total numbers of malaria related deaths have remained almost constant over the past 5 years. The National Malaria Control Program's National Malaria Strategic Plan aims to reduce malaria-related deaths by 90% from 2015 levels (462 deaths) by 2020. To improve severe malaria care and reduce mortality, NMCP documents and investigates all malaria deaths to ascertain the cause of the death and understand if and how it was avoidable. Malaria death audit meetings are held quarterly with health facility staff using a standard death investigation form and case management notes, and form a learning platform to look at qualitative and quantitative data related to the deaths. The audits also examine the quality of care offered as per treatment guidelines, and seek to identify ways to prevent future malaria deaths based on omissions and errors in presented cases. This review examines the findings from death audit meetings facilitated by the PMI-funded Zimbabwe Assistance Program in Malaria project in the Zimbabwean provinces of Mashonaland Central, Mashonaland East and Matabeleland North. Six death audit meetings were conducted over an 18-month period, resulting in a total of 80 deaths audited. The audited deaths were purposely sampled for the potential learning value they offered and to diversify lessons learned. According to audit reports, the main contributing factors to malaria deaths included:

delayed presentation by patients, lack of comprehensive assessment and documentation of cases, inadequate care for patients with reduced level of consciousness and shock, inadequate follow-up of patient progress, lack of supportive investigations, and lack of access to renal replacement therapy/dialysis and blood transfusion. The introduction of malaria death audit meetings has added an active, learning platform to complement the use of the malaria death investigation form, and also served as a useful learning tool within Zimbabwe's clinical mentorship program. Regular malaria death audit meetings are potentially useful in improving malaria care and reducing malaria related deaths.

1822

ENHANCING THE PRIVATE HEALTH SECTOR'S ROLE THROUGH ACCESS TO SUBSIDIZED MALARIA COMMODITIES: GAME CHANGER IN BENIN'S SUPPLY CHAIN

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As much as 60% of Benin's malaria cases are diagnosed in the private health sector, where 70% of all antimalarials are also purchased, yet historically, the private sector has not adhered to national malaria diagnosis and treatment guidelines and has faced challenges with maintaining adequate stock-levels of malaria commodities. To enhance the private sector's role reducing malaria morbidity and mortality, we conducted a study of market preferences and a pilot activity to introduce subsidized malaria commodities at selected private health facilities in Benin. We administered a semi-structured questionnaire to 38 key informants from the public and private sectors, including 20 private clinics (8 medical clinics, 6 medical cabinets, and 6 antenatal care centers in Atlantique/Littoral, Borgou/Alibori and Zou/Collines). We found that the private health sector was amenable to complying with Ministry of Health (MOH) norms and wanted to integrate their malaria commodity needs into the national quantification: 56% of private sector stakeholders interviewed preferred the public supply chain, while only 18.8% preferred direct delivery without a middleman. Most respondents favored the set-up of a formal legal framework to be implemented via an MOU between the National Malaria Control Program and accredited entities. Sixty percent of entities were willing to provide public malaria commodities for free, while 13.3% only agreed to do so if they were compensated by the MOH. In 2017, we implemented a pilot activity introducing subsidized malaria commodities in 4 health zones via 145 private health facilities and pharmacies. Private sector staff were trained on the national guidelines, supervision, the national supply chain management system, and disease surveillance reporting before receiving supplies. To date, 102 of the 145 accredited private entities have complied with national guidelines, reporting and respecting the sale price of subsidized malaria commodities. Challenges remain, however supplying subsidized malaria commodities through private facilities and pharmacies has proven to be viable.

1823

EXPANDING THE HEALTH SYSTEM INTO THE FOREST: ANALYSIS AND RESPONSE TO THE CHALLENGE OF PROVIDING MALARIA SERVICES INSIDE FOREST AREAS WITHIN THE GREATER MEKONG SUB-REGION IN THE CONTEXT OF MALARIA ELIMINATION

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The WHO Strategy for Malaria Elimination in the Greater Mekong Subregion and the Cambodia Malaria Elimination Action Framework note malaria risk is highest in forest or forest fringe areas. From 2006-2016, deforestation increased and the number of confirmed malaria cases decreased 84% from 143,758 to 23,492 (with no change in annual blood examination rates). Simultaneously the percentage of *P. vivax* cases rose from 6 to 50%. Malaria control strategies rely heavily on village-based schemes to provide malaria services. Mobile malaria workers (MMWs) are employed to reach outside villages, but are often located in farms far from forests. Reactive case detection within villages with standard rapid diagnostic tests (the most operationally feasible diagnostic tool) yielded very low test positivity rates (TPR) in Cambodia (<1%), with co-exposed individuals slightly higher at ~3%. Screening data from the USAID Cambodia Malaria Elimination Program (CMEP) of villages with the largest recent case increases (<1%) also suggest most cases are imported from the forest. The Cambodia National Malaria Control Program (NMCP) has pioneered an elimination strategy training those living in forest/forest fringe areas as MMWs. From January-December 2017, the Regional Artemisinin-resistance Initiative empowered local forest residents with MMW training to perform malaria services in Stung Treng Province resulting in 42% TPR with 71% of cases identified as *P. falciparum*. CMEP data shows similarly high TPR rates (36%) from MMWs screening in forest areas of Phnom Kravanh District (70% identified as *P. falciparum*). The Vietnam NMCP piloted a program to identify and treat actual forest transmission *Foci* in Phu Yen Province from 2015-2016. From 2014-2017, this province had the greatest malaria reduction in Central Vietnam (94%). Future efforts should include law enforcement officers (e.g. forest rangers and military) as an epidemiologically important and often neglected populations living in the forest. A rapid scale-up of malaria services into the forest is possible and can improve effectiveness of malaria programs in Cambodia and the Greater Mekong Sub-Region.

1824

THE ROLE OF MOBILE DATA MANAGEMENT SYSTEMS IN MALARIA ELIMINATION IN MYANMAR

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In the Greater Mekong Sub-region (GMS), Myanmar still has a high burden of malaria. However, Myanmar is committed to eliminating malaria by

2030. Surveillance is a core component of malaria control and elimination and the Ministry of Health and Sports (MOHS) is strengthening the existing data management system for malaria surveillance with support of the Asian Development Bank (ADB). University Research Co., LLC (URC), through ADB, is helping to improve malaria data flow and use from village malaria workers (VMWs), health facilities and private providers in Mon State and Sagaing Region. A pilot project in Mawlamyine, Bilin and Khamti townships uses a malaria case-based reporting (MCBR) mobile application, developed by the National Malaria Control Program (NMCP) and partners, for real time malaria data sharing. In early 2018, the WHO developed a District Health Information System II (DHIS2) application for case-based reporting. The two applications will be synchronized. From November 2017 to January 2018, 78 VMWs and 101 basic health staff (BHS) were trained on the MCBR and DHIS 2 applications, respectively. URC, with the NMCP and partners, developed guidelines on use of the MCBR application for BHS staff and VMWs. The project team conducts regular supervision and monitoring visits with Vector Borne Disease Control (VBDC) staff for VMWs and BHS staff to ensure proper use of the application. After training, supervision and monitoring visits were conducted for 46 VMWs in the three townships. Of them, 89% actively used the MCBR application. All 101 BHS staff trained with the DHIS2 application actively used the application at the March 2018 meeting. From December 2017-March 2018, 2,009 tested persons and 25 positive cases were notified via the applications. BHS staff now report positive cases in real time and VBDC staff take quick action. For instance, in Bilin Township, VBDC staff conducted case investigation and response based on 19 notified positive cases via the DHIS2 application. Overall, real-time notification and quality malaria data are crucial to the NMCP and partners for planning and response in the context of malaria elimination.

1825

REVIEWING THE STATUS OF SOUTH AFRICA'S MALARIA ELIMINATION PROGRAM, AND REVISING THE NATIONAL MALARIA ELIMINATION STRATEGY (2019-2023)

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In 2012 South Africa drafted its National Malaria Elimination Strategic Plan, with the goal of eliminating malaria from the country's three endemic provinces (KwaZulu-Natal, Limpopo, Mpumalanga) by 2018. Through this period low levels of malaria transmission were successfully sustained (1828 local cases and 5249 total cases in 2012; 1100 local cases and 4252 total cases in 2016) until 2017 when South Africa experienced a malaria outbreak (21882 local and 28529 total cases). In 2018 South Africa has been scheduled to conduct its malaria program review, and revise its National Malaria Elimination Strategic Plan. In this context, the 2017 outbreak has highlighted the need for introspection, and motivated the country to undertake a rigorously data-driven approach to the malaria program review and revision of the elimination strategy. The foundation of the malaria program review will be to consolidate and analyze all existing data in KwaZulu-Natal, Limpopo, and Mpumalanga using the Elimination Checklist. The Elimination Checklist is a tool that has been customized by South Africa to operationalize the World Health Organization's (WHO) 2016 Framework for Malaria Elimination. The Elimination Checklist assigns forty-nine indicators within the 6 strategies recommended by the WHO's framework to assess performance of the program. It evaluates how close the program is to being malaria free, whether all systems are in place to pursue sub-national verification of elimination, and what else is needed to get to zero local indigenous infections. The Elimination Checklist will be completed for each province in May 2018. Gaps identified through the Elimination Checklist will then be prioritized based upon stakeholder consultations and the outputs of an epidemiological-economic model to identify a cost-effective path to malaria elimination. Recommendations will inform the strategic objectives for South Africa's 2019-2023 Malaria Elimination Strategic Plan, which will be drafted from May through August 2018. The tools developed for the malaria program review can subsequently be used by other countries to inform their approach.

1826

MIGRATION PATTERNS AND CARE-SEEKING BEHAVIOR OF VIETNAMESE INTERNATIONAL LABORERS INFECTED WITH MALARIA: A QUALITATIVE STUDY

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In support of Vietnam's malaria elimination goals, there is interest in the threat of imported malaria from Vietnamese citizens returning from international labor, particularly following service in Africa and other Southeast Asian countries where malaria is endemic. A qualitative study was conducted to gain an understanding of patterns of migration as well as behavior and knowledge of returning international laborers during stages of migration. Thirteen in-depth-interviews were conducted at the National Hospital for Tropical Diseases in Hanoi from October 2017 to March 2018 among confirmed malaria patients who recently returned from Africa or Southeast Asia. Information was recorded, transcribed, and translated into English. Analyses included examining, categorizing, tabulating and recombining data. Thematic analysis was undertaken using Nvivo 9® software. Triangulation and critical case analysis added rigor to the process. Most participants traveled from malaria-free regions in Northern Vietnam for employment mainly in Sub-Saharan Africa countries. Lack of malaria knowledge and misconceptions regarding malaria transmission and protective measures were reported. Either no or incorrect protective measures were adopted while abroad. Most participants engaged in high-risk occupations in highly endemic areas. Difficulties accessing the public health system abroad resulted in patients seeking health care in unregulated private sectors. A significant delay in seeking health care in Vietnam on return was observed in most patients likely due to lack of awareness of malaria symptoms. Results highlight risks to malaria elimination associated with international laborers returning to Vietnam from malaria endemic countries. Given the growing Vietnamese international labor force, consideration should be given to appropriate targeted interventions and malaria prevention strategies for this emerging high-risk population group that covers key stages of migration including pre-departure education and awareness, in-country prevention and prophylaxis, and malaria screening on return.

1827

MALARIA ELIMINATION IN LOW INCOME COUNTRIES: DOES IT MATTER WHEN, WHERE, AND HOW WE SLEEP?

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Long-lasting insecticide treated nets (LLITNs) is the most effectiveness malaria interventions. It has been associated with decline in malaria burden in most sub-Saharan African countries. However, several regions have reported persistence of malaria infection despite high coverage of multiple interventions including LLITNs. Reasons for these observed hotspots are yet to be identified. This study explored the effect of 3-dimension sleeping behaviors namely *when, where,* and *how* on occurrence of malaria in a rural community of Kilosa District, Tanzania. A study assessed occurrence of malaria for members of households as reported by the head. Sleeping behaviors where measured as i) time the person goes under a net (*when*); ii) quality of the house in respect to mosquito entry restriction (*where*); and iii) average sleepers per net (*how*). Principal component analysis was used to construct house quality index using information on building materials; time-to-go-to-net was categorized into 2-hours intervals from 6pm to midnight and average sleepers/net was calculated based on the number of individuals slept in a house by observed nets. Logistic regression models were used to quantify the association. A total of 3,784 individuals were assessed (48.9% males, 58% singles and 60% involved in income generating activity). A quarter (24.3%, n=918) reported to experience malaria during the assessed period (high in females, singles and those with activities). Results indicated a 52% decrease in malaria risk for individuals residing in a good quality house (OR=0.48, 95%CI:0.39-0.59); doubled malaria infection risk when individuals stay inside the house but not under net between 8pm-10pm (OR=2.0, 95%CI: 1.29-3.1); and house quality modified effect of average sleepers, with poor quality houses presented high risk even if few individuals slept in a net (OR=1.4, 95%CI:1.1-1.8, p=0.005). The three sleeping behaviors were highly associated with occurrence of malaria. Results are eye opener to reasons for presence of malaria hotspots in most regions. Communities should be educated on proper utilization of LLITNs to maximize its effect.

1828

IMPLEMENTING MALARIA MASS DRUG ADMINISTRATION: EXPERIENCE FROM A HIGH TRANSMISSION SETTING IN NORTHEASTERN UGANDA

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Mass drug administration (MDA) is one of the suggested means to accelerate efforts towards elimination and attainment of malaria-free status. There is limited evidence of optimum methods of implementing MDA programme such as promoting community participation and compliance with treatment in low income countries. We describe experience of implementing a population-based MDA using dihydroartemisinin piperaquine (DHAPQ) delivered using a fixed distribution strategy in Kapujan sub-county, an area of high malaria transmission in North Eastern Uganda. MDA was implemented in a study to assess the impact of population based MDA in combination with indoor residual spraying (IRS) and IRS alone in a high transmission setting in Uganda. Four rounds of interventions are planned over a period of two years at six months intervals. A baseline housing and population census was conducted in the target area to establish the eligible population. Household members were screened and eligible participants consented for MDA. A database for all eligible persons was created. The population was sensitized at all levels through meetings, and use of mass media. Established village meeting points were used as MDA distribution sites at every village such as, schools, sub-county headquarters and village meeting centers. A team of 19 personnel comprising health workers, data officers and community resource personnel conducted the exercise at each distribution site for 4 days in 18 villages over a total period of 15 days. The first dose of DHAPQ was directly observed on site. Health workers were used to follow up participants to monitor completion of the second and third doses. With a baseline population of 14,468 people living in 2490 households, MDA coverage of 85% was achieved for the first round and 75.5% for the second round. Adherence to all three doses of treatment in the second round of interventions was 76.5% for first dose, 75.02% for second dose and 75.00% for third dose. Using community structures facilitates community participation and adherence to MDA. The best timing of the distribution is when school children were for holidays and during the dry season.

1829

OPERATIONALIZING MALARIA ELIMINATION BY STEPS/ COMPONENTS: THE CASE OF ZAMBIA

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The Zambia National Malaria Elimination Program (NMEP) has recorded a number of successes in reducing malaria transmission across the country in the last decade; Zambia has now set a national target of eliminating malaria by 2021. The call to eliminate malaria requires high coverage and use of a full package of interventions including vector control (long lasting insecticidal nets [LLINs] and indoor residual spraying [IRS] with insecticide rotation) and passive and active case detection and prevention (confirming cases with rapid diagnostic tests [RDTs] or microscopy and treating with artemisinin combination therapy [ACTs] and targeting reactive case detection (RCD) and population-wide mass drug administration [MDA] to specific areas). Building on existing district and health facility teams, the NMEP trained new community health workers (CHWs) and retrained existing CHWs to effectively confront malaria in their communities. Between 2015 and 2018, more than 3,000 CHWs were trained in passive and active case detection in 4 of 10 provinces and more than 2,000 were trained in MDA activities in Southern and Western provinces. CHWs trained in MDA have treated 484,044 people in the past 3 years and, in 2018, approximately 50% of all confirmed malaria cases were reported by trained CHWs; as a result, in Southern Province, malaria cases reported from the health facilities have decreased by 85% and substantially reduced the patient load at facilities. Creating the workforce for malaria elimination has required a consultative and coordinated approach by the NMEP guided by data generated from the health care delivery system. We will review 2018 case data and malaria indicator survey data to assess current progress and needed workforce expansion to operationalize the 2021 elimination plan.

1830

INCREASE IN OUTDOOR BITING IN PRIMARY AND POTENTIAL SECONDARY VECTORS: POSSIBLE IMPLICATIONS FOR MALARIA ELIMINATION

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Since the early 2000s, Zambia's Ministry of Health has successfully scaled up the mass distribution of long lasting insecticide-treated nets (LLINs) and targeted indoor residual spraying (IRS) in an effort to mitigate the burden of the disease. The success of both methods depends on robust entomological surveillance, and, as the country strives to eliminate

malaria by 2021, monitoring the presence of potential vectors and outdoor biting is critical Conducted in the rural areas of Sinazongwe and Siavonga districts in southern Zambia between February-April and September-November 2017, this study evaluated indoor and outdoor mosquito foraging behavior using Centers for Disease Control light traps, indoor resting density using pyrethrum spray catches (PSC), and mosquito feeding behavior using human landing catches (HLC). Of the 2,681 female *Anopheles* mosquitoes caught by indoor and outdoor light traps and PSC, 61% were identified as malaria vectors (*An. gambiae s.l.* and *An. funestus s.l.)*, a slight increase from what has been observed in the two previous years of entomological monitoring. A total of 1,073 mosquitoes, made up mainly of *funestus* (n= 859; 80.1%) and *gambiae* (n= 183, 17%), were captured using HLC. Analysis of biting times showed that most biting took place outdoors for *An. gambiae s.l.* (62%) and *An. funestus s.l.* (65%) with no significant difference in outdoor verses indoor biting rates in either species. The heterogeneity in the biting pattern suggests that malaria transmission may be predominantly taking place outdoors and in the early evenings by both *gambiae* and *funestus* and that malaria elimination programs should consider developing interventions that target both indoor and outdoor vectors. This study also documents the presence and diversity of potential secondary vectors, which may prove to be epidemiologically important as control efforts are focused on the primary vectors.

1831

STRENGTHENING MALARIA SURVEILLANCE IN A HIGH TRANSMISSION REGION OF HAITI: FEASIBILITY OF DHIS2-BASED INDIVIDUAL MALARIA CASE REPORTING AT THE HEALTH FACILITY LEVEL

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Malaria elimination in Haiti is achievable, however improvements in surveillance system structure are required to ensure that individual case data are comprehensively and systematically captured and used to inform elimination activities. In December 2017, Haiti introduced DHIS2 individual case reporting by health facility staff in 11 health facilities in the department of Grand Anse, which has reported at least 40% of the country's annual malaria case burden since 2015. New case notification forms were designed for ease of data collection and entry and included questions to help to classify cases, understand transmission dynamics and parasite mobility, and evaluate social and behavioral risk factors to better inform interventions. Data are recorded on paper forms during patient consultation and later recorded in the DHIS2 Tracker module using an electronic version of the same paper form. Tablets and internet access were provided to health facilities for reporting into DHIS2. Each week, surveillance officers validate data at the health facility by comparing DHIS2 data with paper forms and morbidity and laboratory registries. Indicators such as data completeness (key patient indicators reported) and timeliness (data entered and synced within 24 hours of diagnosis) are used to monitor progress and success of DHIS2 reporting. In the first three months of implementation and across all health facilities, 62% of DHIS2 reports were considered complete, though this varied by week from 30.7% to 92.6%. Timeliness of reporting rates were not as high (28.1% of all cases were reported within 24 hours of diagnosis), due to issues with internet connectivity. Results and interviews with end users indicate that with improvements in connectivity, DHIS2 case-based reporting is feasible and can help to strengthen facility-based surveillance. Recommendations are to provide additional on-site support and evaluate timeliness of reporting using a timeline of one week as opposed to 24 hours. Future plans include expansion to an additional 29 health facilities in the same department, and building capacity for data use and integration with other data systems.

1832

TARGETED REACTIVE INVESTIGATIVE APPROACHES AT REMOTE AREA SLEEPING SITES TO INTERRUPT MALARIA TRANSMISSION IN VIETNAM

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Vector and epidemiological complexities in the Greater Mekong Subregion (GMS) present significant challenges to malaria elimination efforts, particularly in consideration of forest malaria and the increasing threat of drug-resistant malaria. In 2016, a cross-sectional study was conducted using a novel targeted reactive investigative approach at remote area sleeping sites in Phu Yen Province Vietnam. Investigators gathered data on confirmed malaria cases at remote area sleeping sites, characterized common local remote area sleeping site settings and identify malaria prevention and risk behaviors of those frequenting the areas. One-hundred and ten confirmed malaria patients were identified as index cases and interviewed at the suspected site, where transmission was suspected to have occurred at a forest or forest-fringe farm sleeping site. An additional 187 individuals sleeping within 500m of the suspected transmission sites were also interviewed. Logistic regression models were used to calculate Prevalence Odds Ratios (PORs) and 95% Confidence Intervals (CI) for risk factors for being an index case. 82% of index cases were males with a mean age of 36.6 years. Among 93 participants who slept in the forest, index cases frequently did not use nets (POR=2.95; 95% CI 1.26–6.92) and were less likely to use insecticide treated nets (ITNs) (adjusted-POR=0.10; 95% CI 0.02–0.58). Index cases were also more likely to work after dark (adjusted POR=6.33; 95% CI 1.92–20.9). A significantly higher proportion of forest index cases worked in natural resource occupations (hunting, trapping) (POR=11. 7; 95% CI 4.37–31.2). Of 204 respondents who slept on a farm, ITN use was not significantly different between index cases and neighbors. A significantly higher proportion of index cases were involved in planting or logging on farms (POR=2.74; 95% CI 1.27–5.91). Results from this study identify common risk behaviors among forest goers with a history of malaria, and speak towards appropriate targeted interventions and educational strategies.

1833

MULTIPLE APPROACHES FOR MALARIA CASE MANAGEMENT IN THE STRUGGLE TO REACH PRE-ELIMINATION OF MALARIA

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While Tanzania remains a high malaria burden country, recent progress has been made along the pathway to elimination. Malaria Indicator Surveys have shown that prevalence has halved from over 30% to less than 15% in the last 15 years, regions with extremely low prevalence (<1%) increased from 1 in 2008 to 7 in 2016, and the proportion of population living in low transmission areas (<10% prevalence) has increased from 31% in 2000 to 49% in 2015. Since 2016, the 5-year USAID Boresha Afya Project has worked in 1817 facilities in the 7 regions of the Lake/Western Zone, where according to 2016 MIS data, malaria prevalence remains

high. Boresha Afya has collaborated with the NMCP to to support the goal of a reduction in malaria case fatality rate to below 1% by 2020 through promotion of universal access to early diagnosis and prompt treatment, and provision of preventive therapies to vulnerable groups. Strategies have been implemented including training providers on quality testing using mRDT, stratification of malaria burden using GIS mapping, introduction of malaria service and data quality improvement, and outreach programs to remote areas. In project regions since 2016 there has been provision of access to appropriate and timely malaria diagnosis to at least 90% of suspected malaria cases. Additionally, as of February 2018, a decrease in clinical malaria cases have been observed from 9% in 2016 to 2% (national level 3%), mRDT testing has increased from 75% in 2016 to 88%, and use of blood slide microscopy has decreased from 16% in 2016 to 10%. As project activities proceed, we have also observed improved quality (completeness, consistence and timeliness) of malaria indicator data within the routine health information system and improved use of data for decision making by providers. To support progress towards malaria elimination, Boresha Afya will focus on improved testing of suspected cases at facility level and prompt treatment of positive cases to promote rational use of antimalarials, as well as additional community outreach activities to increase access to malaria case management in remote areas, and employment of GIS mapping to rapidly target services.

1834

ELECTROCARDIOGRAPHIC SAFETY EVALUATION OF REPEATED COURSES OF DIHYDROARTEMISININ-PIPERAQUINE FOR USE IN MASS DRUG ADMINISTRATION

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Repeated rounds of Mass Drug Administration (MDA) of antimalarial drugs is a modern approach to eliminate malaria. Dihydroartemisinin-Piperaquine (DHA/PQP) is an effective, well tolerated and long-acting antimalarial suitable for MDA. We assessed the cardiac safety of repeated treatment courses with DHA/PQP. METHODS: We conducted an interventional cohort study in Lihir Island, Papua New Guinea from Sept 21 to Dec 21, 2015. Consenting healthy volunteers were enrolled for repeated treatment with standard 3-day course of DHA/PQP 2.1/17.1 mg/Kg daily given monthly over 3 months. Eligible participants were healthy individuals aged 3 to 60 years. Triplicate twelve-lead ECG readings were conducted with an ELI 150 Cardiograph® at pre-dosing and 4h after the third dose of treatment with each monthly course. Interpretation of the tracings was independent and centralized at a cardiac laboratory. The primary endpoint was QTc prolongation from baseline to the 4h post-dosing ECG; safety was assessed comparing the difference in prolongation of the third course post-dosing ECG and the first course post-dosing ECG. Of 84 enrolled participants, 69 (82%) completed all treatment courses and ECG measurements. In the primary analysis, the mean increase in QTcF was 17.1ms (SD 17.1) in the third course post dosing ECG compared with 19.6ms (SD 17.8) in the first course post dosing ECG (risk difference -2.4 [95%CI - 6.9 to 2.1], p-value=0.285). We recorded QTcF prolongation >60 ms from baseline in 2 (2.9%) participants after the third course, compared to 3 (4.3%, p-value=1.00) after the first course. None of the participants had QTcF intervals >500ms. Three repeated courses of DHA/PQP given monthly are as safe as single course. DHA/PQP could be administered in consecutive monthly courses as part of elimination strategies, with no increased or cumulative risks in comparison to single course.

1835

ASSESSING AND ENHANCING DIGITAL SOLUTIONS FOR MALARIA ELIMINATION

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Surveillance systems are the backbone of malaria elimination programs, providing information on where and how transmission is occurring and how interventions should be targeted. However countries face substantial technical challenges in rolling out integrated case-based information systems that collect timely, high quality data and facilitate appropriate decision making for elimination. To address these technical challenges, a partnership of organizations (Clinton Health Access Initiative, University of Oslo, Vital Wave, and World Health Organization) assessed existing surveillance systems in order to identify gaps that can be addressed with enhancements to DHIS2 (the de-facto malaria information system in many countries) and selected mobile tools. To inform technical requirements for software development, in-depth interviews with malaria programs, end users, and technology experts were conducted globally and in three regions - Greater Mekong Sub-region, Sub-Saharan Africa, and Mesoamerica. Initial findings emphasize the importance of user-friendly mobile interfaces suited for low connectivity contexts, as malaria cases happen infrequently in elimination settings, thus limiting familiarity with technical tools. Users would also like to see basic dashboards on mobile tools for performance tracking; dashboards that overlay information from different sources such as case data, breeding sites, *Foci* boundaries, and response interventions; and alternative mechanisms for accessing online dashboards and reports (i.e. email, WhatsApp). Finally, insufficient resources for surveillance may warrant need for digital tools that support optimal resource allocation and task management. With the consolidation of the findings and requirements for software development, enhanced features will be developed on DHIS2 and mobile applications for case notification, investigation, *Foci* investigation, and response activities, over the coming months. These solutions will be user tested, piloted, and rolled out in ten countries across regions to strengthen existing surveillance systems.

1836

LOW RISK OF SUDDEN UNEXPLAINED DEATH AFTER DIHYDROARTEMISININ-PIPERAQUINE: A SYSTEMATIC REVIEW AND BAYESIAN META-ANALYSIS

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Dihydroartemisinin-piperaquine (DHA-PPQ) is a highly effective and well-tolerated artemisinin-based combination therapy which has been evaluated extensively in the prevention and treatment of malaria. Piperaquine, like several structurally-related antimalarials currently deployed, can prolong cardiac ventricular repolarisation duration and the electrocardiographic QT interval, leading to concerns about its proarrhythmic potential. We conducted a systematic review and meta-analysis to assess the risk of potentially lethal iatrogenic ventricular arrhythmias in subjects receiving DHA-PPQ. We searched multiple databases, last on 24 May 2017, for studies of DHA-PPQ in humans. Further unpublished studies were identified with the WHO Evidence Review Group on the Cardiotoxicity of Antimalarials. Prospective randomised-controlled trials or cohort studies in which subjects received at least one 3-day treatment course of DHA-

PPQ for mass drug administration (MDA), preventive therapy, or case management of uncomplicated malaria, with follow-up over at least three days were eligible. The risk of sudden unexplained death (SUD) after DHA-PPQ with 95% credible intervals generated by Bayesian meta-analysis was compared with the baseline rate of sudden cardiac death (SCD). Among the 197,867 individuals who received DHA-PPQ in 94 studies: 154,505 in MDA, 15,188 as repeated courses in preventive therapies and case management, and 28,174 as single-course treatments of uncomplicated malaria there was one potentially drug-related SUD. The median pooled risk estimate of SUD after DHA-PPQ was 1 in 757,950 (95% CI: 1 in 2,854,490 to 1 in 209,114). This is not higher than the baseline rate of SCD. DHA-PPQ is associated with a very low risk of sudden unexplained death. Concerns about drug-related repolarisation-related cardiotoxicity need not limit its current use.

1837

PUTTING EVOLUTION IN ELIMINATION: WINNING OUR ONGOING BATTLE WITH EVOLVING MALARIA MOSQUITOES AND PARASITES

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Malaria elimination efforts rely heavily on antimalarial drugs and insecticide-based interventions, with artemisinin-based combination therapies, indoor residual spraying and long-lasting insecticidal nets being the cornerstones of malaria treatment and prevention. However, resistance has emerged against nearly every antimalarial drug and insecticide that is available. Here we discuss the evolutionary consequences of the way we currently implement antimalarial interventions, how this may facilitate the evolution of resistance, and how evolutionary principles can be applied to extend the lifespan of current and novel interventions. We argue for a need of greater understanding of general evolutionary principles, for public health in general and for malaria elimination programs in particular, to develop improved resistance management strategies for sustainable malaria control and ultimately elimination.

1838

MALARIA PREVALENCE AMONG ASYMPTOMATIC PARTICIPANTS DURING MALARIA CASE MANAGEMENT PROMOTION CAMPAIGN IN THE PRIVATE SECTOR IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO.

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Considering the high malaria prevalence variability in the prevalence, NMCP took the opportunity to analyze the asymptomatic carriage of this parasite to generate data that could update the estimation of the malaria burden in Kinshasa population. Opportunity to promote "ACTs with Green Leaf" in the private sector. This campaign was organized by ASF / PSI as part of the Defeat Malaria project. A cross-sectional survey was carried out among 2062 volunteers during August 2016. This period corresponded to the end of the dry season and the beginning of the rainy season in Kinshasa. SD BIOLINE Malaria Antigen Pf/Pan Rapid Diagnostic Test was used for testing. Age and sex were collected and analyzedMean age of the participants was 26.2 years. It appears that the majority of volunteers were adults over 26 years and male. The sex ratio (men: women) was therefore 1.37. The total of RDTs positive was 14.7% IC 13-17%. Prevalence in age group was respectively 20,8%; 19,4%; 18,2% and 9,2% for 0-5 years , 6-15 years, 16-25 years and up to 26 years. In conclusion, the prevalence of asymptomatic malaria is high in Kinshasa population. The program should continue prevention activities, in this case the Long Lasting Insecticidal Net (LLIN) campaign, as well as the promotion of control measures.

1839

STRATIFICATION FOR MALARIA ELIMINATION USING LIMITED DATA

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National malaria control programs must decide which interventions to implement where, in a manner that maximizes disease impact within budgetary constraints. This spatial targeting of interventions, or stratification, is often further complicated by a limited availability of data to inform the decisions. Previously, we used a detailed mathematical model of malaria in the Lake Kariba region to identify that population clustering, historical transmission intensity, and importation rates are key quantities for determining the interventions required to achieve elimination. While these metrics are themselves difficult and expensive to measure, here we show how they can be substituted with proxies in the form of settlement maps (from satellite imagery) and routine incidence reports. Both of these data sources are readily available in many endemic countries. We demonstrate the application of our approach to the stratification of all of Southern Province, Zambia, and discuss how the method may be extended to other regions of Zambia and beyond.

1840

VOICES FROM THE FRONT LINE: RESULTS FROM INTERVIEWS WITH EMPLOYEES AT WORKSITES IN HIGH-MALARIA BURDEN DISTRICTS REGARDING MALARIA AWARENESS AND PRACTICES

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In Vietnam, malaria transmission is highest in rural, forest areas in the central/southern regions along the Cambodia and Lao borders. An estimated 4 million people live in high burden districts within the 4 provinces covered by PSI's malaria programming: Gia Lai, Binh Phuoc, Dak Lak and Quang Binh. In 2017, the National Malaria Program reported 4,548 confirmed cases—an increase over 4,161 confirmed cases in 2016. Worksites within/near the forest are presumed transmission hot spots, although data regarding the location/type of worksites and malaria care was lacking in Vietnam. To address this evidence-gap, PSI collaborated with government partners to list 12,633 worksites of all types and sizes, which was 14 times higher than the official number of worksites registered in the same areas (893). PSI then mapped 158 worksites with at least 50 workers and of four priority types, of which 20 were prioritized to receive onsite malaria programming. To better understand the health needs among workers at risk of malaria, PSI interviewed 155 workers at 5 sites in January 2017. Workers at these sites reported seeking care for fever from a district/provincial hospital (60%), commune health station (51%) and private clinic (24%). Only 1 in 3 workers interviewed reported awareness that a rapid diagnostic test (RDT) for malaria exists and only 60% of workers who had a fever reported receiving a malaria test of any type. Less than 1 out of 4 (23%) workers interviewed knew where to find RDT. 81% of workers knew that treated bednets prevent malaria, but only 2% are aware of treated hammocks. Only 24% of bednets used by workers in the previous night were treated with insecticide. The most common health concerns among workers interviewed were fever (84%), malaria

(43%), digestive or respiratory illnesses 20%) and dengue (13%.) While concern about fever was high, only half of interviewees were aware that fever is a common symptom of malaria. Data collected from workers at high-risk worksites indicates a need to improve access to and motivate use of malaria information, products and services to reduce worker risk and achieve the Ministry of Health's goal to eliminate malaria by 2030.

1841

UNDERSTANDING THE DRIVERS AND DETERMINANTS OF AN EFFECTIVE MALARIA RESPONSE IN A CHALLENGING OPERATING ENVIRONMENT: THE BURUNDI CASE STUDY

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Between 2010 and 2017, Burundi has continued to report increases in malaria incidence and related deaths, whilst the African region reported 20% reduction. Retrospective review suggests that malaria incidence has been steadily increasing since 2005. The most significant deterioration occurred between 2014 and 2017, even as the available funding for malaria operations reached its pick in 2015, before dropping dramatically in 2016. Indeed, malaria incidence skyrocketed from 47/1000 in 2014 to 815/1000 at risk population in 2017, a near twenty-fold increase, coinciding with the end of the national malaria strategic plan 2013-2017, and the first year after the endorsement by the country of the Global Technical Strategy 2016-2030 and the African Malaria Strategy as the planning and implementation framework. Malaria related deaths followed same trends, reaching over 4,000 cases in 2017 alone. The malaria outbreak was only officially declared in March 2017. The official declaration paved the way to the initiation and implementation of a multi stakeholder response under the leadership of the Ministry of Public Health and HIV/AIDS, with support from WHO, USAID, the Global Fund, the World Bank and other Partners. The outbreak was brought under control for the first time and declared over in December 2017, when overall incidence crossed the WHO epidemic threshold. Reported incidence has continued to decline, but a closer look at the core areas of focus related to lessons learned from eradication era highlights systemic deficiencies in malaria surveillance, early warning and response, weak community engagement, and outdated malaria policies, as well as lack of collaboration with research institutions. In-depth policy reviews combined with customized approach to surveillance can revolutionize malaria control efforts and may provide the clue to putting the country back on track towards malaria elimination, thus contributing to eradication efforts.

1842

PREPARING FOR THE NEXT GLOBAL THREAT - A CALL FOR TARGETED, IMMEDIATE DECISIVE ACTION IN SOUTHEAST ASIA TO PREVENT THE NEXT PANDEMIC IN AFRICA

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Global investments have had great impact on malaria - these are now at risk of being reversed. Cambodia is where drug resistance historically emerges and spreads globally to drive resulting pandemics - we are currently watching history repeat itself. Despite large investments and recent success in driving down overall rates of malaria, high levels of resistance to nearly all antimalarial drugs are now widespread in Cambodia. Malaria cases are again rising in both Cambodia and Vietnam. Nearly incurable malaria in this region is a real and present threat. Critical actions to prevent further spread of the emerging incurable parasites are: 1) Commitment and real sense of urgency through declaration of a "Public health emergency of international concern" or a similar set of directives; 2) Establish leadership with sufficient authority, respect, expertise and operational funding; 3) Engage affected security forces to stop disease transmission and support elimination operations; 4) Utilize surveillance as a core intervention with result-based funding targeting malaria transmission *Foci* with rapid and effective action. Immediate decisive action is needed in Southeast Asia to prevent the next malaria pandemic. This presentation highlights persistent gaps in the region with methods to address them. Our 2015-6 collaboration with NIMPE pilot tested tools needed to intervene in actual forest transmission *Foci*. Our study district saw a 96% decrease in malaria from 2014-2017, with the entire province seeing the largest decrease in Central Vietnam in this same timeframe. We describe methods to tackle transmission *Foci*, with both an integrated prevention and treatment package and update the audience to the continuously evolving situation in SE Asia. We will call on all stakeholders to make critical changes to current investments to address this critical challenge.

1843

EVALUATING THE FEASIBILITY AND ACCEPTABILITY OF INTEGRATING NOVEL APPROACHES TARGETING HIGH RISK POPULATIONS INTO ROUTINE PROGRAM ACTIVITIES IN ACEH PROVINCE, INDONESIA

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In Aceh Province, Indonesia, where malaria transmission is primarily associated with occupational exposure such as forest work, epidemiological studies are being conducted to identify risk factors and interventions for high-risk populations and to test novel reactive case detection (RACD) approaches in forest-going groups. Operational research can provide national malaria programs with important evidence to improve program policies and activities, yet there may be barriers to implementing these approaches in a programmatic setting. A prospective case-control study and evaluation of routine household RACD compared to socio-behavioral RACD (venue-based and peer-referrals) were carried out between April 2017 and September 2018 in Aceh Besar and Aceh Jaya districts. In order to determine the operational feasibility and acceptability of these approaches to be integrated into routine program activities, an evaluation will be conducted prior to the study end in September 2018. Qualitative methods including semi-structured interviews and focus group discussions will be implemented with health facility and surveillance staff from the four study subdistricts, study personnel, and other key decision-makers within the program. This evaluation will capture information on field operations related to the case-control and socio-behavioral RACD studies including transportation, communication, sample collection, processing and transport, conducting interviews, treatment follow-up, training, and costs. By evaluating the feasibility and acceptability of these approaches, the national malaria program in Indonesia will be better equipped to integrate these evidence-based approaches into routine surveillance to target high-risk populations.

1844

A HEALTH COMMUNICATION PACKAGE TO INCREASE DRUG ADHERENCE AMONG VIVAX MALARIA PATIENTS WITHOUT G6PD DEFICIENCY ON THE INTERNATIONAL BORDERS OF NORTHERN THAILAND

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Thailand's national treatment guideline for treatment of uncomplicated vivax malaria is a 3-day chloroquine (blood schizontocide) and 14 days of primaquine (tissue schizontocide). Previous retrospective study of patients in Mae Sarieng and Muang districts of Mae Hong Son Province, showed up to 76% of 206 vivax patients reported varying degree of non-adherence to the treatment guidelines, as reported previously. More recent clinical trials along the western borders of Thailand showed that *Plasmodium vivax* patients who self-administered treatment showed higher non-adherence rate and e compared to patients who received directly-observed treatment (DOT), as reported previously. We developed a new health communication package aim to improve treatment adherence in vivax patients with normal glucose-6-phosphaye dehydrogenase enzyme (G6PD) in northern Thailand. Information from previous studies on patient's drug adherence behavior and participation from the local malaria clinical staff were incorporated in the development of the health communication package. The format and contents were tailored to target the communities in northern Thailand, including hill tribe communities and cross-border migrants from Myanmar. Thirty-six *vivax* patients diagnosed in malaria clinics in Mae Hong Son province from January to March 2015 provided interviews before and after introduction the communication package. Patients were given a G6PD fluorescence spot test (FST) prior to receiving treatments according to the national guidelines along with health communication package. Exit interview were conducted during the follow-up visit to determine patient's treatment adherence behavior. The majority of the patients 83.3% (30 out of 36) complied with drugs correctly and followed the guideline of treatment program of the clinics. The average scores on knowledge and perception of malaria and malaria treatment were significantly higher than the scores before the implementation ($p< 0.05$). Our results showed targeted health communication package can help improve patient's understanding and adherence to vivax treatment.

1845

ADAPTING REACTIVE CASE DETECTION FOR MALARIA IN FOREST WORKERS IN ACEH, INDONESIA

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Reactive case detection (RACD) is conducted by many malaria elimination programs to detect and treat infected individuals living in close proximity to an index case presenting to a health facility or community health worker. However, in settings where the risk of malaria infection is primarily due to occupational exposures such as forest work, there may be minimal spatial clustering of infections, limiting the utility of RACD approaches that rely on geographic proximity. We evaluated a novel RACD strategy in Aceh Province, Indonesia, whereby an index case that met pre-determined socio-behavioral risk factors triggered follow-up testing at forest work sites (i.e. small mines and plantations) or other agreed venues, and amongst socially-networked groups of individuals reporting recent forest travel. We compared this approach to RACD amongst the household and neighbors of the index case. Between April 2017 and March 2018, 41 confirmed malaria cases in Aceh Jaya and Aceh Besar districts were screened for risk factors related to forest work. Amongst those with risk factors, 107 individuals were tested at work sites and other venues, and 3 infections confirmed by LAMP (2.8%); 38 individuals were identified and tested through their social networks, yielding 3 confirmed infections (7.9%); and 754 individuals were tested during household RACD, with only 2 confirmed positive (0.3%). These preliminary results suggest this "socio-behavioral" RACD approach is more effective in targeting the highest risk individuals in this setting. Cost-effectiveness data, entomological assessments conducted at high-risk work sites, and an additional six months of data will be included in the final analysis. RACD targeted based upon risk factors such as recent forest work can provide a method for Indonesia's national malaria program to treat the highest-risk populations and help achieve its elimination goals, and has the potential to be adapted to other malaria elimination contexts.

1846

MASS DRUG ADMINISTRATION COMBINED WITH INDOOR RESIDUAL SPRAYING FOR ACCELERATED REDUCTION OF MALARIA IN A HIGH TRANSMISSION SETTING IN NORTHEASTERN UGANDA: PRELIMINARY RESULTS

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Strategies to navigate from control to pre-elimination are not well defined in high transmission settings. An ongoing multi-year study in NE Uganda assesses the impact of four rounds of population based indoor residual spraying (IRS) with pirimiphos methyl, alone or in combination with mass drug administration (MDA) with dihydroartemisinin-piperaquine (DP), on malaria transmission, prevalence and incidence. Our hypothesis, supported by *Openmalaria* modeling, is that a temporary synchronous addition of DP to IRS compared to IRS alone in a high burden area will substantially reduce the initial burden of malaria. Three contiguous sub counties with a total population of ~50,000 people experience either: A: IRS + MDA + standard of care (SOC), B: IRS + SOC, or C: SOC, which includes continuous case management and a mass distribution of long-lasting insecticide treated nets (LLINs) in April 2017. Two rounds of IRS and MDA have been conducted, and two more are planned. Four surveys measuring prevalence by rapid diagnostic test (RDT), microscopy, and qPCR of approximately 2500 individuals, 200 households in each study area, were conducted in November 2016, one month before the interventions began, in June 2017, six months after the December 2016 round, in November 2017, three months after round 2, and most recently in March 2018. Baseline prevalence by rapid diagnostic test (RDT) was 49% (A), 49% (B) and 56% (C) in children <1 5. Prevalence after one round in this same population was 24% (A), 27% (B) and 61% (C). Differences between A and B were not significant. Prevalence after two rounds was 8% (A), 16% (B), and 46% (C), with A significantly reduced compared with B. Comparing this latter survey with the baseline, the relative risk of malaria positivity in A relative to B was .42 ($p<.001$), and essentially the same relative risk (OR .42, $p<.01$) was found for microscopy. Similar trends were seen in adults. Preliminary findings support the use of MDA in combination with IRS for dramatic reductions in malaria burden, which should ease subsequent control. This finding is important for policy makers in such settings as a means of acceleration towards elimination.

1847

AFGHANISTAN MOVING TOWARD MALARIA ELIMINATION

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Over the past 16 years, scaled-up key tools (ITN & ACT) contributed to malaria control in Afghanistan. This impact & strong commitment allows move from control to elimination. So, the species-specific & sub-national elimination efforts are based on: Malaria & its epidemic control tactics, technical & operative feasibility of malaria elimination, indigenous transmission break by 2020, strong political & financial commitment & synchronization with neighboring countries for elimination. >90%

PV causes morbidity mainly in east and requires radical cure with primaquine (PQ) to prevent relapse. Recent studies showed significant store of hypnozoites & that low dose (0.25mg/kg/d for 14 days) reduces relapse by 5-fold. G6PD testing is recommended for PQ deployment at scale. Based on *Annual Parasite Incidence (API)*, *Test Positivity Rates (TPR)*, malaria epidemiology, health systems development, the country is divided into three malaria Categories: 1- Transmission-reduction phase & on the path to elimination: Areas with *API* of ≥1 case /1000 population at risk /year; relatively high *TPRs* (≥9%); highly prevalent efficient malaria vector, nearness to endemic areas of Pakistan with uncontrolled bilateral migration; un-sufficient socio-economic & health systems development etc.) 2- Recommended for malaria elimination: Areas with *API* ≤1 case/1000 population at risk/year; relatively low *TPRs* (≤9%); absence of efficient malaria vectors; bordering eliminating countries, 3- Transmission free & recommended for re- transmission prevention: to start implementation of case based surveillance & case investigation from 2019. By 2020: 50% malaria incidence reduction nationally vs. 2016, Pf transmission interrupt & 0 indigenous malaria in Badghis, Farah, Ghor, Hirat, Nimrozl, Baghlan, Kunduz, Takhar & Badakhshan, the re-transmission prevented in eliminated areas. By 2022:70% malaria incidence reduction nationally vs.2016, Pf transmission interrupted & 0 incidence of indigenous malaria in Kabul, Logar, Parwan, Dykundi, Kapisa, Panjsher, Urozgan & all Provinces moved from Category 2 to 3; & re-transmission prevented in malaria eliminated areas.

1848

AN ORIGINAL FRAMEWORK FOR SETTING IMPROVED MALARIA STRATEGIC INTERVENTION PACKAGES AND RELATED TARGETS IN MAINLAND TANZANIA

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Recent efforts in malaria control in Tanzania mainland has led to progressive changes in the epidemiological profile of malaria with prevalence in children aged 6-59 months declining from 18.1% in 2008 to 7.3% in 2017. As prevalence across the country continues to decline and heterogeneity in transmission increases, district level stratification of malaria burden becomes increasingly important for optimizing implementation of interventions to chart the transition from malaria control to elimination stage. In order to identify proper intervention packages, the country was stratified using data from DHIS2 and community based survey. This included school malaria parasitological survey (SMPS), annual parasite incidence, test positivity rate, confirmed malaria incidence and malaria prevalence in pregnant women. The SMPS was used as reference point to determine the cut offs for the other indicators. Four epidemiological strata were proposed; very low, low, moderate and high. The resulting stratification showed that 28 districts with 12% of the population were in the very low strata, 34 districts with 28% of the population in the low strata and 122 districts with 60% of the population residing in the moderate to high strata. Following stratification and selection of most appropriate intervention packages per strata, modelling was used in the strategic planning process to predict the expected impact on malaria prevalence and cost of deploying different interventions packages. This stratification approach with support from modelling provided an improved framework for mainland Tanzania to set differential targets towards elimination.

1849

INTEGRATING ROUTINELY COLLECTED SURVEILLANCE DATA WITH SURVEY INFORMATION TO MAP AREAS OF MALARIA TRANSMISSION AND INFORM INTERVENTIONS TARGETING

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Large scale risk mapping of malaria has, until recently, been largely based on parasite rate (PR) data from cross-sectional surveys. Publicly available national household surveys make this a robust, population representative data source. These data are, however, of limited utility outside of sub-Saharan Africa where national malaria surveys are far less common. PR is also a limited metric in low transmission settings where the sample sizes needed to detect blood stage infections are untenably high. Serological surveys overcome this problem somewhat due to the long-lasting antigens and the ability to detect historical exposure. The geographic scope of such surveys is often limited, though, due to the higher cost of processing such results. In many malaria endemic settings, case reports may therefore be a more reliable measure of the transmission landscape. Digital health information system platforms have improved both the completeness and accuracy of routine surveillance data, and moreover have made these data more readily accessible beyond the point of the health facility or district alone. This data is temporally rich, but may lack the spatial granularity needed to generate high resolution risk maps. Joint modelling methods have been developed to allow PR, serology and routine surveillance case incidence data to be leveraged to generate fine scale spatio-temporal outputs informed by multiple complimentary data sources. Such methods have been illustrated in varying transmission settings such as Haiti and Mozambique. The resulting maps have been applied to inform risk stratification and other control and elimination planning efforts.

1850

EFFICACY OF THE NOVEL *PLASMODIUM FALCIPARUM* BLOOD-STAGE VACCINE RH5.1/AS01B IN A PHASE I/IIA CLINICAL TRIAL

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The development of an effective blood-stage malaria vaccine holds significant promise for reducing the morbidity and mortality associated with clinical malaria. The reticulocyte-binding protein homologue 5 (RH5) is the most promising blood-stage *P. falciparum* candidate antigen to date. It is essential for erythrocyte invasion, and has shown *in vivo* efficacy in non-human primates. Protection was strongly correlated with anti-RH5 serum IgG antibody concentration and *in vitro* functional growth inhibition activity (GIA). We have shown the recombinant protein RH5.1 delivered with GSK's adjuvant AS01B to be safe and immunogenic in

a dose-escalating Phase Ia study in healthy UK adults (NCT02927145, unpublished). Here we report on the promising immunogenicity of the fractional dose group from the Phase Ia trial, where 12 volunteers received 2x 50 µg of RH5.1/ AS01B 4 weeks apart, followed by a delayed 10 µg dose 6 months later. We then present data on vaccine efficacy, assessed using a blood-stage controlled human malaria infection (CHMI) model against both primary and secondary homologous *Plasmodium falciparum* 3D7 clone challenge. 30 healthy malaria-naïve UK volunteers were recruited into this CHMI study. 15 received 3x 10 µg doses of RH5.1/ AS01B (4 weeks apart) and then received an intravenous injection of parasitized red blood cells in parallel with 15 control volunteers. Impact of the vaccine on qPCR-derived parasite multiplication rate (PMR) was the primary efficacy endpoint. 9 of these vaccinees and 8 controls have since undergone a second homologous CHMI to assess durability of immunity, compared to a third group of 6 new malaria-naïve controls. Results of both the first and second *P. falciparum* blood-stage challenges will be presented.

1851

ANTIBODIES AGAINST PYMIGS, A NOVEL TRANSMISSION-BLOCKING VACCINE CANDIDATE, REDUCE THE MOTILITY OF MICROGAMETES

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Malaria transmission-blocking vaccine aims to inhibit the development of malaria parasites in mosquitoes by antibodies targeting surface proteins of sexual stage parasites. We have previously identified PyMiGS, a protein expressed on the surface of microgametes of *Plasmodium yoelii (*Py) to elicit antibodies with potent transmission-blocking activity. In this study, we aimed to investigate the mode of action of anti-PyMiGS antibodies against parasite development. Activated male gametocytes in mosquito midguts first egress from the erythrocytes, followed by exflagellation soon after the blood meal. To mimic the *in vivo*environment, Py gametocytes were mixed with activation medium containing anti-PyMiGS antibodies or anti-GST (control) and subsequently processed for electron microscopy after 15 min incubation. We observed exflagellation in both anti-PyMiGS and control group. However, whereas most microgametes were released from the residual body of activated male gametocytes in the control group, a significantly reduced number of microgametes were released in the presence of anti-PyMiGS antibodies, with most left attached on the residual body. Moreover, anti-PyMiGS antibodies shortened the duration of the active movement of microgametes after the onset of exflagellation to a fifth of the control. Put together, these findings suggest that anti-PyMiGS antibodies bind to microgamete surface immediately after exflagellation, thereby reducing microgamete motility and inhibiting microgamete release from activated gametocytes.

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IN SILICO T CELL EPITOPE PREDICTION IDENTIFIES HIGH VALUE RH5 EPITOPES THAT CORRELATE WITH RESPONSE IN VACCINEES

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In silico epitope prediction and analysis tools EpiMatrix and ClustiMer were used to identify class I and class II epitopes providing broad HLA coverage in RH5, a highly conserved *Plasmodium falciparum* blood-stage antigen that has recently been assessed in a Phase I clinical trial with controlled human malaria infection (CHMI). In addition, sequence similarity to the human proteome regarding the potential for cross-reactive epitopes, which may induce regulatory T cell responses, was also analyzed using the JanusMatrix algorithm. In contrast to other well-studied *P. falciparum* antigens such as TRAP, CSP, and EBA-175, high levels of class I and class II T cell epitope content were found in RH5, including thirteen class II epitope clusters. Predicted epitopes were synthesized as peptides and validated in competition binding assays with soluble class I and class II HLA as well as in Interferon gamma ELISpot assays using PBMC from clinical trial vaccinees administered RH5.1, a full-length recombinant RH5 protein vaccine. Criteria for selection of high value vaccine candidate epitopes as well as data correlating T cell response with in silico and *in vitro* indicators of donor HLA-specific potential for inflammatory, regulatory or lack of response will be shown.

1853

CLINICAL ILLNESSES IN A HEALTHY POPULATION AT A MALARIA TRANSMISSION BLOCKING VACCINE TRIAL SITE IN MALI

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Malaria vaccine trials assess product safety, immunogenicity, and efficacy; these endpoints can be confounded by intercurrent illnesses that may be deemed adverse events or may mimic or aggravate clinical malaria episodes. Furthermore, concurrent illnesses, such as malaria or respiratory infections, can impair vaccine responses. Therefore, defining the prevalence of common diseases and their distribution in different socio-demographic stratifications is helpful to prepare a site for the evaluation of vaccine safety and efficacy. In preparation for malaria community transmission blocking vaccine trials, we initiated a study of Community Dynamics of Malaria Transmission and Mosquito Feeding in Bancoumana, Mali in February 2018. Bancoumana is a village located 60 km southwest of Bamako with a population of about 10,000 people, and malaria transmission is highly seasonal with low incidence during the dry season from approximately January through June. As part of this observational study, we assessed disease frequency at enrollment of all age groups. We enrolled 957 subjects who were screened, including clinical exam, baseline hematological parameters, and malaria parasite density, with diagnostic assays and assessments as appropriate for any ongoing illness.Out of all participants, 32.3% (309/957) presented with at least one clinical abnormality at enrollment. Respiratory infection were by far the most commonly reported disease [11.0% (105/957)], with highest frequency among children under 5 years old [26.1% (47/180)]. The second most common infection was uncomplicated malaria [2.8% (27/957)] followed by gastroenteritis [2.1% (20/957)], both again most commonly in children under five years old [3.9% (7/180); 5.0 % (9/180)]. No difference was observed between sexes regarding the distribution of reported diseases. Less than 25% of volunteers reported a hemoglobin value under 11.7 g/dl. Infectious diseases, which are known to modify human immunity, were prevalent at the Bancoumana clinical trial site but varied widely between age groups. These findings will be considered when designing vaccine trials at this site.

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DOES CHALLENGE ROUTE MATTER? AN INVESTIGATION INTO THE CHALLENGE MODEL ELEMENT IN MOUSE MALARIA PROTECTION STUDIES

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While researchers have nominally identified numerous malaria vaccine candidates, many of these candidates have yet to be translated into successful products for human use. A significant hurdle to malaria vaccine development remains the improvement and adaptation of biological models to approximate the natural condition of human infection and disease. Animal models are routinely used as a means to investigate a vaccine candidate's immunological and protective potential, in particular the *Plasmodium berghei* infection in rodents. Methods of infection within the *P. berghei* model may include artificial injection or inoculation by infected mosquitoes. Each of these routes have their merits and pitfalls. For example, appeals of artificial injection are direct control of sporozoite dosage and simplicity of delivery route. However, needle inoculation often bypasses relevant phases of natural infection while introducing other obstacles, e.g. viability. The natural infection route by mosquito is the most relevant option, but is labor intensive and introduces several uncontrollable factors, e.g. salivary gland sporozoite burden. To investigate how artificial and natural challenges compare in murine studies, our lab performed a series of experiments to assess the *P. berghei* murine model for infectivity and protection using two homologous antigens, PbCSP and PbCelTOS. Our findings suggest that challenge route can have an impact on antigen-specific protection interpretations and vaccine efficacy determinations. PbCSP vaccination conferred increased protection from malaria infection by mosquito bite when compared to intravenous, suggesting that bypassing the skin-stage reduced sensitivity to antibody-mediated inhibition. We will define fine specificity of humoral immune responses in order to establish the role of antibodies in the protection models. Since the malaria scientific community relies on the use of rodent parasite challenges to predict clinical outcomes, investigating vaccine candidates using a range of challenge methods may improve understanding of the relationship between infection and vaccine-induced immunity.

1855

INFORMING TARGET PRODUCT PROFILES FOR A SECOND-GENERATION CHILDHOOD MALARIA VACCINE: A MODELLING STUDY

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Clinical trials of the RTS,S/AS01 vaccine for *P. falciparum* malaria have demonstrated a protective effect in young children and, beginning in 2018, the four-dose vaccine will be evaluated through a pilot implementation program in Ghana, Kenya and Malawi. Work is currently underway to optimise this vaccine. A recent phase 2a human challenge study indicated that varying the timing and dose amount of the fourth dose could further improve vaccine efficacy. In our study we used a dynamic modelling approach to inform target product profiles (TPPs) for a second-generation malaria vaccine for 5-17-month-old children, focussing on the specific characteristics of initial efficacy, duration of protection, dosing schedules and coverage. We also compared the RTS,S/AS01 phase 3 efficacy with an efficacy profile based on data from the phase 2a challenge study. In the first decade of delivery, we found that initial efficacy was more important than duration, due to the efficacy benefit occurring in younger ages. This effect was more pronounced in high transmission settings. However, the low initial efficacy and long duration schedule averted more cases across all ages if a longer time horizon was considered. The modified vaccine profile outperformed the RTS,S/AS01 phase 3 profile, although a profile with higher efficacy at the fourth dose, and a longer delay between doses three and four, conferred the most benefit overall. Our findings indicate that for an imperfect childhood malaria vaccine with suboptimal efficacy, it may be advantageous to prioritise initial efficacy over duration. We predict that next-generation vaccine with a modified dosage and schedule could outperform the current RTS,S/AS01, although fourth dose timing will affect the age group that derives the greatest benefit. This study provides insight into prioritising malaria vaccine characteristics and shows how distinct vaccine properties translate to public health outcomes.

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EVALUATION OF PROTEIN CARRIERS FOR TRANSMISSION BLOCKING VACCINE ANTIGENS

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Immunogenicity of poorly immunogenic antigens can be enhanced by chemical conjugation with carrier proteins or other particulate carriers. We have developed a conjugate nanoparticle system with EPA (Exoprotein A from *Pseudomonas aeruginosa*) as carrier for the delivery of malaria antigens. EPA conjugates of Transmission Blocking Vaccine (TBV) antigens, Pfs25 and Pfs230 are being evaluated currently in clinical studies. With the goal of augmenting the immunogenicity of the conjugates further, we are currently exploring other carriers for the delivery of these antigens. These include carriers such as Tetanus Toxoid and CRM_{197}, used in approved vaccines as well as two variants of these carriers, Tetanus Toxin heavy chain (rTThc) and EcoCRM™ (Fina Biosolutions), both expressed in E. coli. We conjugated Pfs25 to TT, CRM_{197}, rTThc and EcoCRM™ and characterized their physicochemical and immunological properties. Chemical conjugations were carried using thioether chemistry that is currently employed for EPA conjugates. Conjugates were analyzed for their molecular weight and antigen/carrier composition by SEC-MALS and amino acid analysis respectively. Mouse immunogenicity studies of Pfs25 conjugates showed strong immune response against Pfs25 with antibody titer similar to EPA-Pfs25 conjugate. Functional analysis of the immune sera by Standard Membrane Feeding assay demonstrated high inhibition of oocyst formation in the infected mosquitos. Antibody titer and functional activity of rTThc were equivalent to those of TT, despite their structural differences. Similarly, EcoCRM and CRM_{197} showed essentially identical immunogenicity and functional activity. In conclusion, all four carriers appear to be suitable for TBV antigen delivery.

1857

THE FIRST HUMAN MONOCLONAL ANTIBODY AGAINST THE SEXUAL STAGE *PLASMODIUM FALCIPARUM* ANTIGEN PFS230

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Malaria transmission-blocking vaccines (TBVs) induce antibodies against proteins expressed by the parasite in the mosquito midgut and are an innovative approach to reduce parasite transmission and contribute to malaria elimination. Antibodies against Pfs230, a protein on the surface of *Plasmodium falciparum* gametes, can block parasite transmission, and Pfs230 vaccine candidates are currently in clinical trials. Antibody repertoire analyses, performed by B cell receptor (BCR) sequencing, have been employed to evaluate responses to other vaccines and to identify sequences of neutralizing antibodies. Here, we used a Pfs230 tetramer to sort Pfs230-specific single B cells (CD3-, CD14-, CD56-, CD19+, CD20+, CD27+, Pfs230+) generated after the fourth dose of Pfs230-EPA/Alhydrogel® in Malian adults. We sequenced both heavy and light antibody chains from these cells and identified paired BCR from nine vaccinees. The resulting sequences indicated expansion of clonotypes using IGHV1-69 (heavy chain) in 8 subjects and using IGKV4-1 (light chain) in 7 subjects. The IGHV1-69 clonotypes were characterized by high mutational rates with at least ten mutations per sequence in CDR1 and CDR2 regions, suggesting proliferation and selection in response to the vaccine. We developed and applied a method to rapidly generate Fab fragments by cell free expression; individual BCR of interest were identified based on repeated frequency on the plate, again suggesting clonal selection. Five paired heavy, and light chains were PCR amplified from selected wells. Using overlap extension PCR, all necessary elements for in vitro transcription and translation and either the CH1 or C-kappa-domain were added to both the 5' and 3' ends of the single cell VDJ. After in vitro transcription and translation, four out of five tested Fab fragments demonstrated binding through a colorimetric ELISA assay. To assess complement-dependent function, we generated a human IgG1 antibody in HEK cells using the same VDJ sequence. This new antibody, LMIV230-01, bound to Pfs230D1 recombinant protein in ELISA, to extract of female gametocytes in Western blot and to the surface of both female gametes and parasites in early developmental stages isolated from midguts three hours post-feeding; functional assays are ongoing and will be reported. These results will be fundamental for design and improvement of TBV strategies to induce potent antibody responses against mosquito stage parasites.

1858

INVESTIGATION OF TWO VACCINE-DELIVERY PLATFORMS TO ENHANCE IMMUNOGENICITY AND TRANSMISSION-BLOCKING ACTIVITY OF MALARIA VACCINE CANDIDATE PVS25

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Over 2.8 billion people worldwide live at risk of *Plasmodium vivax* infection, in particular in the Americas and South-East Asia, where *P. vivax* account for >60% and >30% of all malaria cases respectively. An indispensable tool for malaria control and eradication are transmission-blocking malaria vaccines (TBV), with Pvs25 being one of the most studied vaccine candidate. Pvs25 is expressed on the parasite prior to fertilization and the expression peaks during the ookinete stage. Antibodies against this protein have demonstrated transmission-blocking activity in animals and humans. However, the high and long-lasting titres needed for functionality in humans are difficult to achieve. To overcome this problem, we investigated the use of two different vaccine delivery platforms: we genetically fused Pvs25 to IMX313, a chimeric version of the oligomerization domain from chicken complement inhibitor C4b-binding protein, and we used the SpyCatcher/SpyTag Plug-and-Display technology to display the antigen on the surface of virus-like particles. Preclinical investigation in mice showed that both platforms were suitable for eliciting high anti-Pvs25 antibody titres which were functional against field isolates of *P. vivax*. Data investigating the immune response and functional activity further to select one of the candidates to Phase 1 clinical trial will be presented.

1859

HETEROLOGOUS ADENOVIRAL IMMUNIZATIONS PROVIDE STERILIZING PROTECTION AGAINST *PLASMODIUM VIVAX* IN A SURROGATE MURINE MODEL

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Several heterologous prime-boost immunization regimens including DNA, MVA or adenoviral vectors have been reported to improve T cell responses to *Plasmodium* antigens as well as protective efficacy compared to homologous regimens. However, two immunizations with the same adenoviral vector are sufficient to induce anti-vector antibodies that reduce efficacy when compared to heterologous immunization regimens. Here we described the immunogenicity and efficacy of a heterologous adenoviral immunization regimen including the recombinant human adenovirus Ad5/3 and the simian adenovirus SAd36, tested in mice. These two vectors were used to deliver a chimeric multi-stage *P. vivax* protein that included T cell and B cell epitopes from two immunodominant antigens, the circumsporozoite protein (CSP) and the merozoite surface protein 1 (MSP1). Adenoviral immunizations were followed by two protein boosts with the corresponding chimeric proteins. Heterologous adenoviral regimens were compared with regimens consisting of only protein immunizations and naïve mice. Heterologous adenoviral regimens induced high titers of antibodies capable of recognizing the native structure of both *P. vivax* CSP and MSP1 by immunofluorescence, a balanced IgG1/IgG2a humoral response, and significantly improved IL-2 production by CD4 and CD8 T cells when the SAd36 was used for priming. When protection was assessed using a transgenic *P. berghei* expressing a *P. vivax* CSP repeat region as a surrogate model of protection, we observed greater than 99% reduction in parasite loads in the liver of immunized mice two days post experimental challenge compared to unvaccinated animals. Sterilizing protection was observed in 8/10 mice immunized with the Ad5/3-SAd36-protein regimen, and 9/10 mice immunized with the SAd36-Ad5/3-protein regimen, while only 50% of the protein immunized mice had sterilizing protection. Overall, our data demonstrate the ability of this vaccine candidate to induce protective and long-lasting humoral and cellular immune responses when delivered via heterologous adenoviral vectors.

1860

THE INFLUENCE OF SOCIO-ECONOMIC STATUS ON PERFORMANCE OF THE RTS,S MALARIA VACCINE AMONG CHILDREN WHO PARTICIPATED IN THE PRE-LICENSURE TRIAL

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Despite a substantial progress made in fighting malaria, it continuous to have a devastating impact on people's health and livelihoods. GlaxoSmithKline's (GSK) RTS,S malaria vaccine is currently the most advanced malaria vaccine in global development which has been found to provide a protective efficacy against clinical malaria among children (5-17 months). The objective of this analysis was to determine the influence of household (HH) socio economic status (SES) on the efficacy of the RTS,S malaria vaccine administered to children aged 5-17 months within Kintampo North Municipality and Kintampo South District of Ghana. The RTS,S malaria vaccine trial was a randomized, controlled, multicenter, participant- observer-blind phase III trial conducted at 11 different centers. Clinical malaria episode was defined as fever accompanied by a temperature greater or equal to 37.5^{o}C and *P. falciparium* asexual parasitemia greater than 5,000parasite/mm^{2}. HH assets such as television,

radio, etc were used in generating HH assets scores. Data of 1002 children ranging from 5 to 17 months who were followed up to 20 months was used for this analysis. A sum of 7,960 (approximately 11 episodes on average) distinct episodes of clinical malaria ranging from 1 to 30 were reported over the 20 months' period of follow-up. Within the RTS,S group, on an average 11 distinct episodes per child compared to 13 distinct episodes per child for children in the control group were reported. For each additional occurrence of clinical malaria, a child at baseline was found to be associated with 39% [0.72 (95% CI: 0.65 - 0.80)] increase in the recurrence risk for the control group compared to RTS,S group. A child within the RTS,S group with a low or medium socio-economic status has a decrease of 28% and 25% in the hazard of experiencing an episode of clinical malaria respectively. However, at a particular survival time, twice as many subjects from the high socio-economic class in the control group experience an episode of clinical malaria compared to the RTS,S group. In conclusion, a child's HH Socio economic status was found to have an influence on the effectiveness of RTS,S malaria vaccine.

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EXPLORATION OF VHH NANOBODIES AS STRUCTURAL MIMICS OF *PLASMODIUM VIVAX* INVASION EPITOPES AS A VACCINE

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Development of successful subunit protein vaccines often fail because they may not precisely mimic the naturally occurring antigen responsible for protective immune responses, or are themselves weakly immunogenic. We propose Camelid single-domain (VHH) antibodies are capable of epitope mimicry by targeting the idiotope region of characterized functional human antibodies. Production of VHH antibodies in *E. coli* could have high yields without aggregation or loss of functionality. Anti-Id Ab vaccines have shown promising results against a number of tumor-associated antigens. Antibodies (humAbs) to rPvDBPII-SalI, which demonstrated blocking of rPvDBPII to erythrocytes, were generated from single-cell antigen-specific sorted B-cells from two Cambodian individuals with immunity to *P. vivax*. huMAbs were digested with papain to remove the Fc region. Protein G purified Fab fragments were coated onto 4HBX plates, and a commercial VHH phage display library was used to pan for anti-Id antibodies. Sequences of phage from round 4 provided determination of panning endpoint. Clones were produced as VHH-phage to test binding to target ligand. Competition ELISAs using the purified VHH antibodies and biotinylated rPvDBPII allowed for testing if the VHHs of block the rPvDBPII-huMAb interaction, and thus determine if the VHHs bind to the paratope. Sequencing results have revealed 5 distinct clonal groups for 2 different blocking humAbs panned against. Initial ELISA results using VHH-phage particles indicate positive binding to the primary antibodies. We await further results using purified VHH antibodies in a competition ELISAs. Phage display of VHH nanobodies offers a reproducible method of producing specific antibodies to antigens. Utilization of camelid VHH antibodies in vaccinations in mice and rabbits may reveal their value as a potential vaccine candidate for humans. The additional benefit of working with a camelid VHH library is our development of VHHs to malaria antigens offering advantages in imaging, flow cytometry, and ELISA assays that are not available with traditional antibodies.

1862

"PRIME-TARGET" IMMUNIZATION WITH VIRAL VECTORS FOR ENHANCED EFFICACY OF LIVER-STAGE MALARIA VACCINES

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Induction of cell-mediated immunity targeting the liver-stage of malaria is a well-established strategy for eliciting sterile protection against human *Plasmodium falciparum* malaria. Intramuscular (im) administration of the liver-stage vaccine antigen ME-TRAP encoded within the viral vectors ChAd63 and MVA induces 21% sterile protection in a controlled human malaria model (CHMI) using *P. falciparum*-infected mosquitoes. Protection was associated with antigen-specific $CD8^+$ T cells expressing the cytokine IFNγ, measured in peripheral blood. Recently, tissue-resident T cells (Trm) have been identified as potential sentinels against invading pathogens in a variety of tissues and intravenous (iv) administration of irradiated, cryopreserved sporozoites has been shown to generate high-frequencies of liver Trm in non-human primates associated with sterile-protection against malaria. We hypothesised that increasing the frequency of TRAP-specific Trm in the liver by iv administration of viral vectors that are known to be liver-tropic would enhance sterile-protection against malaria. Building on positive pre-clinical data in mice, we proceeded to a small Phase I dose-escalation study assessing the safety of increasing doses of ChAd63 ME-TRAP iv. Immunisations were well-tolerated, with a safety profile similar to that observed following im administration of the same vaccine. Immunogenicity, measured by IFNγ ELISPOT using peripheral lymphocytes was associated with vaccine dose. We assessed the expression of two markers of human liver Trm (CD11a and CD69) and showed that the frequency of $CD8^+$ $IFN\gamma^+$ T cells was greater in participants immunised by the iv route than the im route. Further vaccinations are underway to include dose-escalation of MVA ME-TRAP and extended immunology for both vaccines will be presented to determine impact of iv vaccination on activation-induced markers, using the "AIMS assay". We will then progress to a Phase II trial assessing immunogenicity and efficacy of im prime-boost (ChAd63 and MVA ME-TRAP) followed by iv "targeting" with either vector to assess the improvement in efficacy against CHMI.

1863

ACCOUNTING FOR EVERY LLIN: THE CASE OF LLIN ACCOUNTABILITY SYSTEM IN TANZANIA

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Tanzania has adopted Reproductive and Child Health (RCH)-based distribution as a continuous distribution channel for long-lasting insecticide-treated nets (LLINs). The Tanzanian National Voucher Scheme was implemented from 2004-14 to deliver LLINs to pregnant women and infants at antenatal and immunization visits. Vouchers were given to pregnant women and could be redeemed for an LLIN at participating retail points. In 2014, the program was stopped due to fraudulent voucher redemptions. In 2016, a new distribution system for pregnant women and infants, the *Chandarua Kliniki (CK)* program, was launched. LLINs are delivered to recipients directly at health facilities without vouchers. The *CK* program has expanded to all 26 regions in Tanzania, delivering more than 3.4 million LLINs in 2016-2017, with about 3.4 million more to be

distributed each year. In order to account for every LLIN being distributed, a stringent accountability system was designed to reduce the possibility of fraud. DHIS2 indicators are integrated in a dashboard that automatically triangulates and compares the number of pregnant women that received ANC services and children who received measles vaccinations against those who received an LLIN. The dashboard generates monthly trends for review by programme planners. On a quarterly basis, the system also compares service information with commodity information, such as stock on hand from the electronic Logistics Management Information System (eLMIS). Information from the two systems is visualized in a dashboard that summarizes individual health facility performance and produces an accountability report that shows variances. The district malaria coordinators use the report to follow up with specific facilities on reported variances before they sign off and file their report. At the end of each quarter, the system produces a quarterly accountability report, which is reviewed by the council health management team. This presentation will report on the operational issues encountered during the implementation process and how the *CK* system has improved targeted supervision and monitoring of RCH-based LLIN distribution.

1864

LONG LASTING INSECTICIDAL BED NETS OWNERSHIP, ACCESS AND USE IN A HIGH MALARIA TRANSMISSION SETTING BEFORE AND AFTER A MASS DISTRIBUTION CAMPAIGN IN UGANDA

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Uganda conducted a second mass LLIN distribution campaign and Katakwi district recently received LLINs as part of this activity. This study was conducted to measure the success of the campaign in this setting, an area of high transmission, with the objectives to estimate LLIN ownership, access and use pre and post campaign implementation. Two identical cross sectional surveys, based on the Malaria Indicator Survey methodology, were conducted in three sub-counties in this district (Kapujan, Magoro and Toroma), six months apart, one before and another after the mass distribution campaign. Data on three main LLIN indicators including; household LLIN ownership, population with access to an LLIN and use were collected using a household and a women's questionnaire identical to the Malaria Indicator Survey. A total of 601 and 607 households were randomly selected in survey one and two respectively. At baseline, 60.57% (56.53-64.50) of households owned at least one net for every two persons who stayed in the household the night before the survey which significantly increased to 70.35% (66.54-73.96) after the campaign (p= 0.001). Similarly, the percentage of the household population with access to an LLIN significantly increased from 84.76% (82.99-86.52) to 91.57% (90.33-92.81), p= 0.001 and the percentage of household population that slept under an LLIN the night before the survey also significantly increased from 56.85% (55.06-58.82) to 81.72% (76.75-83.21), p= 0.001. The LLIN mass campaign successfully achieved the national target of over eighty-five percent of the population with access to an LLIN in this setting, however, universal household coverage and use were fourteen and three percent points less than the national target respectively. This is useful for malaria programs to consider during the planning of future campaigns by tailoring efforts around deficient areas like mechanisms to increase universal coverage and behavior change communication.

1865

LESSONS LEARNED FROM MONITORING INDOOR RESIDUAL SPRAYING (IRS) IN ATACORA DISTRICT, BENIN, 2011-2016

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Indoor residual spraying (IRS), an integral component of Benin's national malaria control plan, was performed in May/June in areas of northern Benin during 2011–2016. During this time period, we performed human landing catches (HLCs) to collect mosquitoes from eight homes in two villages where IRS occurred and in two villages where IRS did not occur to assess malaria vector biting and infectivity rates and to calculate entomological inoculation rates (EIR). HLCs occurred every one to three months between June 2011 and October 2016 (five months in 2011, ten in 2012, six in 2013, six in 2014, eight in 2015, and four in 2016). We assessed IRS insecticide performance by measuring the residual activity of bendiocarb and pirimiphos methyl (EC/CS) on IRS-treated walls using the WHO cone bioassay. We also monitored vector insecticide resistance in IRS villages by performing WHO insecticide susceptibility tests. During periods of high transmission (rainy season) in 2011–2016, significant decreases in malaria transmission were observed in IRS villages compared to controls: peak EIR/person/night in IRS villages was 0.04 compared to 1.5 in controls in 2011 (97% less); 0.3 v 1.6 in 2012 (84% less), 0.1 compared to 3.1 in 2013 (96% less), 0.1 compared to 0.4 in 2014 (75% less), 0.1 compared to 1.4 in 2015 (91% less), and 0.2 compared to 1.6 in 2016 (88% less). The residual activity of IRS insecticides on treated walls did not exceed four months. While large decreases in EIRs occurred in IRS villages, residual malaria transmission continued to occur with peak EIR/person/month of 11.3 in 2011, 10.8 in 2012, 8.2 in 2013, 2.8 in 2014, 5.6 in 2015, and 5.7 in 2016. *An. gambiae* sensitivity to bendiocarb was lost over time: mortality decreased from 97.8% in 2011 to 70% in 2016. Sensitivity to pirimiphos methyl remained at 100%. Our data suggests that IRS was effective in reducing malaria transmission through reducing EIRs, but that residual malaria transmission continued to occur. The low residual activity of the IRS insecticide may be resolved with new generation insecticide products and synergists.

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AGE GRADING MALARIA TRANSMITTING MOSQUITOES USING FEED FORWARD ARTIFICIAL NEURAL NETWORKS

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Near infrared spectrometry (NIRS) is currently complementing techniques to age-grade mosquitoes. NIRS can classify lab-reared and semi-field raised mosquitoes into < or ≥ 7 days old age groups with an average accuracy of 80%. This accuracy was achieved by training a regression model using partial least squares (PLS) and interpreting the model as a binary classifier. In this study, we explore whether using an artificial neural network (ANN) analysis instead of PLS improves the current accuracy of NIRS models on age-grading malaria transmitting mosquitoes. We also determined if directly training of a binary classifier instead of training a regression model and interpreting it as a binary classifier improves the accuracy. A total of 786 spectra of laboratory reared *Anopheles gambiae* were used and pre-processed according to previously published protocols. Based on ten replicates, we find that training both regression and binary classification age models using ANN yields models with higher estimation accuracies than when the same models are trained using PLS. While ANN regression model scored root mean squared error (RMSE) and mean absolute percentage error (MAPE) of 1.6 (+/- 0.2) and 30.6 (+/- 4.7), respectively, PLS regression model scored 3.7 (+/- 0.2) and 67.6 (+/- 6),

respectively. When we interpreted regression models as binary classifiers, the accuracy, specificity and sensitivity of ANN regression model were 93.7 (+/- 1.0)%, 95.6 (+/- 1.8)%, and 92.5 (+/- 1.6)%, respectively while PLS regression model scored 83.9 (+/- 2.3)%, 75.8 (+/- 5.2)% and 89 (+/- 2.1)%, respectively. We also find that a directly trained binary classifier yields higher age estimation accuracy than a regression model interpreted as a binary classifier. While a directly trained ANN binary classifier scored an accuracy, sensitivity and specificity of 99.4 (+/- 1.0)%, 99.3 (+/- 1.3)% and 99.5 (+/- 0.7)%, a directly trained PLS binary classifier scored 93.6 (+/- 1.2)%, 94.4 (+/- 1.6)% and 92.4 (+/- 1.9)%, respectively. These results suggest the use of ANN models for age grading *An. gambiae*, however validation on larger data set and on other species is required.

1867

RESPONSE OF TWO POPULATIONS OF *ANOPHELES GAMBIAE* S.L. EXPOSED TO DELTAMETHRIN, PERMETHRIN, AND PERMETHRIN WITH PBO AND DEF SYNERGISTS USING WHO AND CDC BIOASSAYS

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Benin faces widespread insecticide resistance in malaria vectors. We used WHO and CDC bioassays to quantify resistance to deltamethrin, permethrin, and permethrin with piperonyl butoxide (PBO) and S,S,S-tributyl phosphorotrithioate (DEF) synergists, which inhibit oxidase and esterase activities, respectively, with 2-5 day-old *Anopheles gambiae* from two cities in northern and southern Benin. We used PCR for species identification and genotyped *Kdr L1014F* and G119S *Ace-1R* mutations. Of 278 *An. gambiae s.l* collected, 99.3% (n=276) were *An. coluzzii*, 90.0% (n=270) had the *Kdr* L1014F gene mutation, and 3.0% (n=10) had the G119S *Ace-1R* mutation. Using CDC bioassays, 16.1% of southern and 12.3% of northern mosquitoes survived after exposure to diagnostic doses of permethrin (standardized dose corresponding to 99.9% mortality of susceptible mosquitoes as established by WHO). After exposure to doses of permethrin that were 2X higher, 14.1% of southern and 8.4% of northern mosquitoes survived; after exposure to permethrin doses 5X higher, 0% of southern and 1.2% of northern mosquitoes survived. When exposed to diagnostic doses of deltamethrin, 12.2% of southern and 13.9% of northern mosquitoes survived. At deltamethrin doses 2X higher, 3.2% of southern and 4% of northern mosquitoes survived; no mosquitoes survived after exposure to doses 5X higher. Using WHO bioassays, 23.9% of southern mosquitoes and 22.0% of northern mosquitoes survived after exposure to diagnostic doses of permethrin. When permethrin was combined with PBO, survival decreased to 12.7% and 5.1% among southern and northern mosquitoes, respectively. When diagnostic doses of permethrin were combined with DEF, southern mosquito survival remained similar at 23.4% but decreased to 11.6% among northern mosquitoes. High resistance to insecticides was identified in northern and southern sites in Benin, likely caused by several mechanisms including *Kdr L1014F* and G119S *Ace-1R* mutations and over-expression of oxidases and esterases. However, when diagnostic doses of permethrin were combined with PBO and DEF, resistance decreased by 46–77% in some mosquito populations.

1868

PREDICTIVE MODELING FOR ASSESSING BIO-EFFICACY OF LONG LASTING INSECTICIDE NETS IN GUATEMALA USING SEGMENTED REGRESSION

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Use of long lasting insecticide nets (LLINs) has had a significant impact on personal protection and in reducing malaria transmission. Therefore, nowadays, LLINs are one of the cornerstones of malaria control. The WHO cone bioassay is the recommended methodology for determining the bio-efficacy of a LLIN. However, logistically simpler alternatives are welcomed. The bromine x-ray fluorescence (XRF) has been used to measure insecticide content and has proven to be a feasible alternative to high liquid performance chromatography (HPLC). This study was conducted to assess if the results from the XRF analysis could serve as a predictor to estimate the bio-efficacy results in LLINs. Our data was obtained from a study conducted in La Gomera, Guatemala in which 174 LLINs were collected and evaluated by both techniques during three time-points (2013, 2014 and 2015). For this purpose, we developed a model using piecewise (segmented) regression. Piecewise regression is used when there are clearly two or more linear relationships in the data with a sudden, sharp change in direction. Exploratory analysis of our data showed the relationship between our response variable-proportion of mortality of mosquitoes/24 hours by the cone bioassay- and the main exposure variable-mg deltamethrin by the XRF analysis-, can be explained as two separate linear relationships where the change in the direction of the relationship occurs around the cutoff of 25 mg of deltamethrin. To develop our predictive model, we used cross validation. We trained our model using 80% of the data and validated it with the remaining 20%. This process was repeated 100 times. To assess the accuracy of the fitted model, a confusion matrix was built to calculate the proportion of correct classification. The average accuracy our model was estimated to be 89.94 %, indicating that approximately 90% of the time we can estimate the bio-efficacy of LLIN correctly based on two predictors: the results from the XRF analysis and the age of the LLINs.

1869

IS BIOKO GETTING THE HANG OF IT? EVALUATION OF A UNIVERSAL LONG-LASTING INSECTICIDAL NET (LLIN) DOOR-TO-DOOR DISTRIBUTION AND HANG-UP CAMPAIGN IN EQUATORIAL GUINEA

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Despite the known effectiveness of long-lasting insecticidal nets (LLINs) in providing protection against malaria, maintaining universal coverage and use continues to be a challenge. To maintain universal coverage, the Bioko Island Malaria Control Project (BIMCP) has applied a combination of mass free distributions and continuous distributions through multiple channels. Strategies such as door-to-door visits and hang-up activities are being integrated into mass distribution campaigns to encourage higher LLIN usage. Mass door-to-door distribution campaigns include a pre-registration of persons and sleeping spaces, sensitization, and hanging of LLIN by community volunteers to encourage high and sustained use. From February to July 2018, the BIMCP is leading a mass LLIN distribution campaign on Bioko Island with the goal of achieving universal coverage. Data on the number of sleeping spaces, LLINs previously owned, LLINs received, and LLINs hung are recorded in an Open Data Kit (ODK) based Campaign Information Management System (CIMS) that facilitates longitudinal analyses of household-level interventions. A cross-sectional malaria indicator survey (MIS) will be conducted two to five months after the mass distribution campaign to collect information on individual parasitemia, LLIN survivorship, and use. This study will explore community, household, and individual level associations between parasitemia and factors related to LLIN survivorship and use among households in which LLINs were hung and those in which LLINs were not hung. The results

will add to the evidence base for decision-making on future distribution strategies that seek to incorporate novel approaches to encourage higher LLIN usage.

1870

RAPID REDUCTION OF MALARIA TRANSMISSION AFTER INTRODUCING A THIRD GENERATION INDOOR RESIDUAL SPRAYING PRODUCT IN PREVIOUSLY UNSPRAYED DISTRICTS OF MOPTI REGION, MALI IN 2017

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Mali's national malaria control program (NMCP), working with the US President's Malaria Initiative (PMI) Africa Indoor Residual Spraying program, has had success decreasing malaria transmission using 3rd generation IRS (3GIRS) products in areas of pyrethroid resistance, primarily in Ségou and Koulikoro regions. In, 2015 national survey data showed that Mopti region had the highest under 5-year-old (u5) malaria prevalence at 53.4% - nearly twice the national average - despite having high access to long-lasting insecticidal nets (LLINs) and seasonal malaria chemoprevention (SMC). Migrations of displaced people to Mopti Region from Northern Mali, where malaria transmission is substantially lower and acquired immunity is thought to be low, has also complicated the landscape in Mopti. Accordingly, in 2016 NMCP, PMI, and other stakeholders shifted IRS activities from Ségou to Mopti. Here, we present observational analyses of this switch using routine malaria indicators. Health facility-level analysis of DHIS2 data showed a decline in confirmed u5 malaria cases following IRS: in the four months after spraying, health facilities in communities receiving IRS with pirimiphos methyl in addition to new LLINs and SMC reported 254 (37%) fewer confirmed cases per 10,000 children-months at risk compared to those receiving only new LLINs and SMC. Comparing changes in peak u5 incidence from 2016 to 2017, facilities receiving no IRS reported 20% (CI_{95} = 32% - 8%) fewer cases, while facilities receiving IRS in 2017 reported 51% (CI_{95} = 59% - 43%) fewer cases, a 31% greater decrease compared to non-IRS facilities. Entomological surveillance data also showed that reduced malaria incidence corresponded to reductions in indoor *An. gambiae* s.l. densities. Forthcoming analyses will also explore trends in malaria burden in Ségou after IRS was suspended. IRS provided significant added protection from malaria to a package that included a concurrent universal LLIN distribution campaign and high SMC coverage. These observations add to the growing evidence that 3GIRS can be a wise public health investment.

1871

FACTORS ASSOCIATED WITH REFUSAL AND RELUCTANCE TO INDOOR RESIDUAL SPRAYING ON BIOKO ISLAND, EQUATORIAL GUINEA

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Indoor Residual Spraying (IRS) has proven to be a robust control measure against malaria, and until 2015 it was the primary vector control strategy under the Bioko Island Malaria Control Project (BIMCP). However, its effectiveness strongly depends on public acceptance by the targeted populations. This study aims to describe IRS acceptance and to investigate factors related to refusal and reluctance amongst the populations of Bioko Island. A secondary data analysis of the 2017 annual Malaria Indicator (MIS) survey; which collected valid data on 4,835 random households, selected from every community on the Island was conducted. The multinomial logistic regression was used to assess factors associated with IRS refusal and reluctance. 39% percent of households were reported to have been sprayed (not all communities are targeted for spraying). When respondents were asked if they will like their houses to be sprayed during the next IRS round, 81.1% accepted, while 11.1% refused and 7.8% were reluctant (don't know). The reasons mostly evoked for refusals were "IRS causes ill effects" (50%) and "IRS is disruptive or annoying" (25%). Respondents belonging to the middle and high wealth categories were more likely to refuse or to be reluctant to IRS; those living in households with heads having post-secondary educations were more likely to refuse IRS, and those who were not sure if their households were sprayed in the past were more likely to be reluctant to IRS. However, respondents living in the districts of Baney, Luba, and Riaba were less likely to refuse or to be reluctant to IRS when compared to Malabo. Individuals living in households that have been sprayed in the past were less likely to refuse or to be reluctant to IRS, and those previously exposed to malaria sensitization messages were less likely to be reluctant to IRS. Refusal and reluctance to IRS is almost 20% in households on Bioko Island, and are associated with socioeconomic, geographic, and educational factors. There is a need to improve malaria sensitization strategies, targeting every social class to increase IRS uptake.

1872

FOLLOW-UP OF LLIN'S SOON AFTER A MASS DISTRIBUTION CAMPAIGN IN TWO URBAN DISTRICTS IN MALABO, EQUATORIAL GUINEA

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The Bioko Island Malaria Control Project (BIMCP) uses bed nets as the primary vector control strategy on Bioko Island. As recommended by WHO, every three years the BIMCP, in partnership with the National Malaria Control Program (NMCP) of Equatorial Guinea, conducts mass distribution campaigns of long-lasting insecticide treated bed nets (LLINs) on Bioko Island. As well as distributes LLINS through routine keep-up campaigns in primary schools, high-risk communities, and Antenatal Care (ANC) clinics. However, results from annual malaria indicator surveys (MIS) have been highlighting the inadequate use of bed nets. Field workers providing anecdotal evidence suggest that the population has complained about the odor, the color, and adverse effects related to the nets (itchiness) in previous campaigns. To assess bed nets utilization and apprehension prior to the annual MIS and immediately post-distribution, the BIMCP will conduct a representative household survey in 2 urban districts of Malabo on Bioko Island in April 2018. Communities within the urban districts were selected by probability proportional to sizes and households were randomly selected in each community. The survey questions will focus on LLINs utilization and apprehension, reasons for not using LLINs, educational messages heard about LLINs, and the sources of the messages. This information will contribute to a better understanding of the needs of the population regarding LLINs and will improve communication and sensitization strategies concerning LLINs, with the goal of increasing utilization on Bioko Island.

1873

COMMUNITY ENGAGEMENT AND THE USE OF HOUSEHOLD MAPPING TO TARGET SENSITIZATION AND IMPROVE IRS COVERAGE ON BIOKO ISLAND

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Since its inception in 2004, the Bioko Island Malaria Control Project (BIMCP) has relied on Island-wide IRS as one of the major malaria vector control strategies. Since 2015, the strategy has focused on targeting high-risk communities for IRS. Communities are selected by stratification based on high prevalence of malaria. However, not only does IRS coverage depends on public acceptance, but it is also highly dependent on the veracity and the accuracy of the data. It is challenging to accurately monitor IRS field operations, refusals in the population, and perform quality control on IRS activities. To reduce these challenges, the BIMCP began using spatial data to improve intervention coverage and collaborating with community leaders to support and strengthen IRS sensitization across the Island. In 2014, the Island was mapped into official government recognized communities and divided into grid sectors. Each household was then enumerated and assigned a unique identifier, which was placed on a sticker on the front door. A Campaign Information Management System (CIMS) was developed to track interventions at the household level on a longitudinal basis. IRS field data were entered directly into the CIMS tablet application and uploaded to a cloud server, enabling multiple household interventions spanning multiple years to be linked by a common unique identifier. Before mapping was introduced, IRS coverages were reportedly very high (above 90%). After mapping was introduced and data entry standardized, IRS coverage dropped to 57% during round 19 in 2014 and subsequently to 41% during round 20 the same year. After the engagement of the Ministry of Interior of Equatorial Guinea, in charge of community leaders, the support provided by these community leaders during mobilization and sensitization activities, increased IRS coverage to 81%, 71% and 82% in 2015, 2016 and 2017 respectively. The use of the CIMS, the mapping of households to systematically track IRS activities and data, led to targeted mobilization and sensitization through community leaders in identified high refusal areas for IRS, resulting in increased coverage.

1874

UTILIZING A CAMPAIGN INFORMATION MANAGEMENT SYSTEM AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR IMPROVED QUALITY CONTROL OF INDOOR RESIDUAL SPRAYING WITH ACTELLIC 300 CS ORGANOPHOSPHATE INSECTICIDE ON BIOKO ISLAND OF EQUATORIAL GUINEA

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Quality control of Indoor Residual Spraying (IRS) is necessary to ensure spray operators do not falsify spray records and that they deposit the recommended lethal dose of insecticides on spray walls. Insecticide Quantification Kits (IQK) for in-field quality control of IRS currently detects only concentrations of alpha-cyano pyrethroids and carbamates. IQK kits for in-field quality control with organophosphates are not yet developed though IRS programs are increasingly using organophosphates due to insecticide resistance to pyrethroids and DDT. This study examined the use of an advanced campaign information management system (CIMS) and high performance liquid chromatography (HPLC) for IRS quality control with an organophosphate insecticide. The Bioko Island Malaria Control Project (BIMCP) currently uses Actellic 300CS organophosphate insecticide with pirimiphos methyl as the active ingredient. A total of 17,600 households were sprayed in 2017 using 63 spray operators. The BIMCP instituted a quality control procedure to test houses randomly selected from the CIMS that maps and captures data in real time for houses reportedly sprayed each day. Insecticide samples were tape-lifted from structures reported sprayed and analyzed using HPLC. Spray operators were monitored in 2017 and 2018. During the 2017 monitoring, all the selected houses monitored had the insecticides deposits as detected by HPLC. However, 11.1% of the spray operators deposited less amount of the insecticide (<1.0g a.i./m²) on the wall, 54.0% deposited the target dose of 1.0g a.i./m² and 34.9% deposited over-dose >1.0 g a.i./m²). These results were compared with the 2018 monitoring results. The ability to randomly select, locate, and test houses sprayed reportedly within a week via HPLC has markedly improved the quality of IRS on Bioko Island, virtually eliminating falsification, and enabling the project to better evaluate its performance. The results obtained will also form the basis for developing new tool kits for in-field monitoring of organophosphates insecticides.

1875

OBSERVATIONAL ANALYSIS OF THE IMPACT OF THE REINTRODUCTION OF IRS IN DISTRICTS OF THE NORTHERN AND UPPER EAST REGIONS OF GHANA IN 2017

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Since 2008, Ghana has been implementing IRS as a key malaria control intervention in the northern savannah, including districts in Upper West, Upper East and Northern regions. The country has seen a steady decline in malaria prevalence since 2011. In 2011, the Northern and Upper East regions had a parasite prevalence in children under 5 of 48% and 44% to 39% and 25% by 2016. Organophosphate-based IRS was however suspended in Upper East in 2015 after two years of continuous implementation, but reintroduced in 2017 to three districts, Builsa North, Builsa South and Kassena Nakana West. Similarly, in 2017 IRS was re-introduced in two districts in Northern Region, Gushiegu and Karaga; both last sprayed in 2012 with a pyrethroid. The reintroduction in 2017 of IRS operations using an encapsulated organophosphate, pirimiphos-methyl in these districts offered an opportunity for an observational study of the impact of this new product. Routine epidemiological data from the national District Health Information Management System II were analyzed to calculate monthly malaria RDT-positive incidence rates at the health facility level. Changes from 2016 to 2017 in incidence rates in new IRS districts were compared to changes in incidence at health facilities without IRS in the same region to provide an estimate of intervention impact. Preliminary analysis of health facility-level data in the Upper East region in 2017 showed that there was an increase in malaria incidence in both IRS and non-IRS districts in the three months immediately after the spray campaign; however, IRS districts had approximately 155 fewer cases per 10,000 person-months of observation compared to non-IRS districts. IRS districts also showed lower rapid diagnostic test positivity rates (TPR) in the same months, with an average TPR 16% lower than in non-IRS districts. These analyses highlight the positive contribution of IRS in mitigating an overall increase in malaria cases in the north of Ghana. Routine surveillance data were useful to assess the impact of vector control interventions in operational settings and support evidence generation for IRS strategy decision-making.

1876

TESTING THE DURABILITY AND PERFORMANCE OF PERMANET 2.0 LONG-LASTING INSECTICIDE NETS AFTER THREE YEARS OF USE IN GUATEMALA

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Long-lasting insecticidal nets (LLINs) are a standard measure for the prevention and control of malaria, impacting personal protection and in reducing malaria transmission. In Guatemala, since 2000, an estimated of 2million bednets have been distributed in the highest risk malaria-endemic areas, and to date constitute the main vector control measure in the country. However, there has not been a planned evaluation to determine the timely replacement of LLINs whose effectiveness becomes diminished by routine use. To determine survivorship, physical integrity and insecticide content of the LLINS, cross-sectional surveys were conducted at 18, 24 and 32 months after PermaNet 2 deployment in a malaria *Foci* in Guatemala. The surveys were standardized based on the WHO Guidelines. A total of 988 LLINs were analyzed (290 at 18 months, 349 at 24 months and 349 at 32 months). The overall survivorship of bednets decreased over time, 86% at 18 months, 76% at 24 months and 66% at 32 months. Independently of the time of the survey, less than 80% of the bednets that were still present in the household were reported to have been used the night before. A great percentage of the bednets had been washed at least once (88% at 18 months, 92% at 24 months and 96% at 32 months), and 60% of them were soaked, mainly with detergent powder. The physical inspection of the nets showed that the median total hole area (cm^2) in the bednets was 2.4 at 18 months, 8.4 at 24 months and 34.3 at 32 months. The proportion of bednets considered in good condition, according to the total hole surface area, diminished from 77% at 18 months to 58% at 32 months. The portion of LLINS with deltamethrin concentration less than 10mg/m^2, measured by bromine x-ray fluorescence, increased over time (14% at 18 months, 23% at 24 months, and 35% at 32 months). Among the bednets in where bioassays were conducted, the number of bednets that passed the WHO bioassay criterion of >=80% mortality at 24 hr. dropped from 90% at 18 months to 68% at 24 months and then 52% at 30 months. Even though the nets are in good physical condition at 32 months, its diminished bioefficacy compromises the protection provided to the user.

1877

WATER-LEVEL MANAGEMENT REDUCES MALARIA MOSQUITO ABUNDANCE AROUND LARGE DAMS IN SUB-SAHARAN AFRICA

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Water level management has been suggested as a potential tool to reduce malaria around large reservoirs. However, no field-based test has been conducted to assess the effect of water level management on mosquito larval abundance in African settings. The objective of the present study is to evaluate the effects of water level drawdown rates on mosquito larval abundance. Twelve experimental dams (5mx5mx1m) were constructed on the foreshore of the Koka Dam in Ethiopia. These were grouped into four daily water drawdown treatments, each with three replicates: no water-level drawdown (Group 1; Control), 10 $mm.d^{-1}$ (Group 2), 15 $mm.d^{-1}$ (Group 3) and 20 $mm.d^{-1}$ (Group 4). Larval sampling was conducted weekly for a period of 12 weeks each in the wet season (June - September) and subsequent dry season (January - April). A total of 1284 *Anopheles* mosquito larvae were collected from the experimental dams during the study period. Most (64%; n=822) were collected during the main malaria transmission season while the remaining (36%; n=463) were collected during the dry season. Larvae comprised four *Anopheles* species, dominated by *Anopheles arabiensis* (48%) and *An. pharoensis* (33.2%). Mean larval density was highest in control treatment dams with stable water levels throughout the study, and decreased significantly ($P < 0.05$) with increasing water drawdown rates in both seasons. During the main transmission season, anopheline larval density was generally lower by 30%, 70% and 84% in Groups 2, Group 3 and Group 4, respectively, compared with the control dams (Group 1). In the dry season, larval density was reduced by 45%, 70% and 84% in Groups 2, Group 3 and Group 4, respectively, when compared to the control dams. In conclusion, increased water drawdown rates were associated with lower mosquito larval abundance. Water level management could thus serve as a potential control measure for malaria vectors around reservoirs by regulating the persistence of shallow shoreline breeding habitats.

1878

INCIDENCE AND ETIOLOGY OF DIARRHEA IN CHILDREN: A TWO-YEAR ACTIVE SURVEILLANCE IN AN URBAN COMMUNITY OF VIETNAM

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Diarrheal diseases remain amongst the primary causes of the mortality among children under five years in low and middle-income countries. In Vietnam, the burden of hospitalised paediatric diarrheal disease is well-described; however, the true burden and aetiology of diarrheal disease in the community are not well documented. Between 2014 and 2016, we prospectively followed a cohort of 748 children aged between one and three years living in an urban area in Southern Vietnam. Active surveillance with SMS messaging and phone calls were conducted bi-weekly to caregivers to acquire further diarrheal disease episode in their children in the preceding seven days. Children with severe diarrhea were advised to visit a study hospital. Fecal samples were collected during the diarrheal episode at home or at hospital. These samples were then examined by microscopy for parasites, by culturing for bacterial pathogens, and by the Luminex xTAG Gastrointestinal Pathogen Panel assay to identify 15 pathogens. We measured 773 diarrhea episodes, of which 304 (39%) were hospitalised and 257 (33%) were severe and contained blood or mucus. The incidence of diarrhea significantly decreased with age ($p<0.001$) and was calculated as 721/1,000 child-years of observation (95%CI: 660-788). 433/628 (69%) fecal samples tested positive by Luminex; commonly identified bacteria and parasites included *Salmonella* (140, 32%), ETEC (82, 19%), *Clostridium difficile* (80, 18%), *Campylobacter* (63, 14%), *Shigella* (30, 7%), and *Cryptosporidium* (44, 10%). Commonly detected viral pathogens included *norovirus* (163, 37%), *adenovirus* (75, 17%), and *rotavirus* (69, 16%). We conclude that the incidence of paediatric diarrhea was high in this setting but decreased with age. We identified an abundance of pathogens known to be associated with moderate-to-severe paediatric diarrhea. Our work represents the most comprehensive cohort study of diarrhea disease conducted in children aged under 5 years in transitional Vietnam. Targeted age-specific interventions targeting common pathogens are necessary to prevent the morbidity from diarrheal disease among children in this setting.

1879

GEO-SPATIAL REPORTING OF CEFTRIAXONE RESISTANT *SALMONELLA TYPI* OUTBREAK INVESTIGATION IN HYDERABAD, PAKISTAN

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There are around 21 million cases and annually around 222,000 deaths occur related to typhoid. First case of ceftriaxone resistant Salmonella typhi was reported from Hyderabad, Pakistan in November 2016. We conducted a geospatial based outbreak investigation to identify the burden and spatial distribution of the outbreak using GIS mapping technique. A matched age case control study was conducted from 1st December 2016 to 15th September 2017 in Hyderabad. Data was collected through a structured questionnaire and drinking water samples were collected from household of cases and controls and from community water supplies for microbiological and molecular analysis. For geospatial mapping, Google Map® layer was integrated in the Esri ArcGIS version 10.5 to identify famous points and localities in Hyderabad. A paper based map of Hyderabad sewerage network was also acquired from Hyderabad water board which was scanned and georeferenced in the ArcGIS software. Further co-ordinates of the households for both cases and controls and important relevant landmarks including water supply were collected through a tablet. Color coding of positive and negative cases as well as water positive cases was conducted. A total of 486 ceftriaxone resistant S. typhi blood culture confirmed cases were identified. With the help of geospatial map, clustering of cases specifically around two areas of Hyderabad namely Qasimabad and Latifabad where identified. Further by incorporating different layers of data including sewerage lines, pattern of clustering along the water sewerage lines could be clearly seen. Lastly by plotting positive microbiology samples of community water we were able to show continuous shedding of bacterium in feces and contamination of water supplies by S. typhi patients. Based on the findings it is suggested to treat water sewage plants, safe potable water supply and mass vaccination to control outbreak.

1880

MANAGEMENT OF DIARRHEA IN MALI: ASSESSMENT OF THE ADHERENCE TO WHO RECOMMENDATIONS IN THE VACCINE IMPACT ON DIARRHEA IN AFRICA (VIDA) STUDY

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Diarrheal diseases are a leading cause of under-five morbidity and mortality in developing countries. To improve management of pediatric diarrhea, WHO has provided treatment recommendations based on clinical syndromes and degree of dehydration as part of its Integrated Management of Childhood Illnesses approach. Here we assess practitioners' adherence to WHO recommendations for the treatment of diarrhea in Bamako, Mali among children who were enrolled in the Vaccine Impact on Diarrhea in Africa (VIDA) study. We identified children aged 0-59 months residing within two Bamako quartiers who were seeking care at a health center. Eligible children had a new episode (onset during the previous 7 days, after >7 diarrhea-free days) of moderate-to-severe diarrhea (diarrhea associated with sunken eyes, decreased skin turgor, dysentery, hospitalization, or IV rehydration). We assessed antibiotics, oral rehydration therapy (ORT), and zinc received or prescribed according to hydration status to determine the practitioners' adherence to WHO recommendation. From May 2015 to May 2017, 1227 eligible cases were enrolled. Among the cases 5% had "severe dehydration", 92% had "some dehydration" and 2% had "no dehydration." Most children presented with acute watery diarrhea (92%), followed by diarrhea with severe acute malnutrition (SAM, 5%) and dysentery (3%). ORT/IV antibiotics were given at the health center to 15% of children with diarrhea and SAM and 25% with severe dehydration associated with acute watery diarrhea or dysentery. Zinc was prescribed to 4% of the acute diarrhea cases; co-trimoxazole was the most widely prescribed antibiotic (72% of all cases). Only 8% cases of bloody diarrhea received a WHO-recommended antibiotic for dysentery and an additional 63% received co-trimoxazole. The management acute bloody and watery diarrhea are not in alignment with WHO recommendations for rehydration therapy, zinc, and antibiotics in Mali. Additional sensitization is needed to raise awareness about WHO recommended diarrhea case management.

1881

EVALUATING CHANGES IN DIARRHEA INCIDENCE AFTER 20 YEARS AMONG CHILDREN 0-59 MONTHS IN OSHIKHANDASS, GILGIT-BALTISTAN, PAKISTAN

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In 1989, diarrhea was the highest cause of childhood morbidity and mortality in Oshikhandass, a temperate climate region where the main water source was untreated glacier melt and toilet facility the traditional pit latrine. Interventions (water filtration plants, flush-toilets) were introduced from 1996-2002. Research Workers (RWs) trained in diarrhea management did weekly surveillance of children 0-59 months from 1989-1996 and classified diarrheal episodes by severity, gave treatment or referred, and followed up until recovery. Similar methods were utilized from 2011-2014. In the first study, 1843 children were followed for 4194 child-years (CY); 61.7% had at least one episode of diarrhea. In the second study, 1168 children were followed for 1567 CY; 43.2% had at least one episode of diarrhea ($p<.01$). Between the two study periods, diarrhea incidence remained unchanged at 0.7 episodes/CY. In the second study, children whose families migrated to Oshikhandass after 1995 had significantly higher incidence of diarrhea compared to those whose families had lived in the village during the first study (0.9 vs 0.7, RR: 1.3 95% CI: 1.2-1.5). Prevalence of diarrhea and mortality from diarrhea decreased significantly from 4.8 to 2.9 days/CY (RR: 0.61, 95% CI: 0.59-0.62) and 7 to 0.6 deaths/1000 CY (RR: 0.01, 95%CI: 0.00-0.09). Mean duration of diarrhea episodes decreased from 7.1 to 3.7 days ($p<.01$). In the first study 33.3% of episodes were prolonged (7-13 days), 8.5% were persistent (>=14 days), and 12.5% were bloody, compared to only 6.0%, 1.3%, and 5.4%, respectively, in the second study ($p<.01$). The highest incidence of diarrhea was in children 9-11 months of age in the first study (1.6 cases/CY, 95% CI: 1.4-1.7), and 18-20 months of age in the second study (1.3 cases/CY, 95% CI: 1.0-1.5). In both studies, diarrhea incidence was highest from May to September. Over the 20 years, incidence of diarrhea did not change, but mortality, mean duration, and severity decreased substantially, possibly because of improved SES including water and sanitation, and introduction of zinc. The percentage of children receiving ORS from health workers remained high.

1882

SYSTEMIC REVIEW OF OUTBREAKS DUE TO *SALMONELLA TYPHI* AND PARATYPHI IN AFRICA AND ASIA; CHALLENGES AND LIMITATIONS

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Typhoid is a serious health issue, particularly in South Asia and in some regions of Africa. In addition to the high burden of endemic cases of the disease in these countries, outbreaks of typhoid pose a major challenge for the public health system. The recent outbreak of ceftriaxone resistant Salmonella Typhi in Pakistan led us to review the literature on typhoid outbreaks in Asia and regions of Africa to identify the case definitions, diagnostic criteria and methods used for outbreak control. We searched PubMed (1964-2018) for studies describing the outbreaks of Salmonella Typhi and Paratyphi A and B in Asia and Africa. Articles on mathematical modeling, papers describing laboratory methods of the outbreak strain were also excluded. Out of a total 185 relevant articles, only 38 met the eligibility criteria.Of these, 10 outbreaks were reported from Africa and 28 from Asia. Reports from Africa were from 7 countries , 60% (6/10) were from south Africa, Zimbabwe and Uganda. The reports from Asia were from 14 countries of which 28%(8/28) were from India. More than 80% of the outbreaks were reported between 2000-2017. Varying case definitions and lab methods were used for the outbreaks. In Africa 80% of the reports used some form of lab investigation to confirm the cases, 25% used serology, 87% used a combination of blood culture and PCR. In Asia 82% used a lab method for case confirmation. Most (86%) used stool and blood cultures. The source of the outbreak was identified in 50% outbreaks in Africa of which 80% were due to contaminated food. In Asia 14% were due to contaminated food and 44%(12/27) due to contaminated water. In Africa 30% of studies took steps to control the outbreaks. In Asia 35%(11/28) took outbreak response measures, of which 45%(5/11) used WSH methods and 27% (3/11) used health education. Typhoid vaccination as control measure was used in only one outbreak in Africa and 2 in Asia. This review highlights the importance of developing standardized case definition, laboratory confirmation and guidelines for outbreak response to use in response to typhoid outbreaks as currently there are none provided by the WHO or CDC.

1883

USING ARCGIS AND SATSCAN SOFTWARE TO INVESTIGATE EPIDEMIOLOGY AND RISK FACTORS OF ENTERIC INFECTIONS IN MIRPUR, BANGLADESH USING DATA FROM THE PROVIDE STUDY

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Diarrheal disease is a leading cause of death in children under five years worldwide. In addition to a high mortality burden, diarrheal disease contributes to decreased oral vaccine efficacy and poor growth in children. Understanding risk factors, including geospatial distributions, associated with enteric pathogen transmission is essential to the design of interventions to prevent incident infections. To investigate these risk factors, data was collected on 700 infants from birth to 24 months of age in Mirpur, Bangladesh, through the PROVIDE study to examine associations between the spatial distributions of various enteric pathogen cases (*Rotavirus, Shigella, E. coli, Campylobacter, Cryptosporidium,* and *Giardia*) among PROVIDE participant households and SES factors such as monthly income, toilet type, and water source. Spatial clustering was analyzed through the use of a Bernoulli model in SaTScan software. Of the pathogens screened for in this population, *Rotavirus* was the only pathogen that exhibited significant spatial heterogeneity with 1 high-risk spatial cluster identified in the study area (relative risk = 2.18; p = 0.033). Additionally, though not statistically significant at the $\alpha = 0.05$ level, toilet sharing between households was implicated as the primary risk factor for both *Rotavirus* infection using a logistic regression model (OR: 1.69; 95% CI: 0.84 - 3.37; p = 0.140) and total pathogen burden using a Poisson model (IRR: 1.12; 95% CI: 0.99 - 1.27; p = 0.064).

1884

TRANSFER OF HEALTHY GUT FLORA FOR RESTORATION OF INTESTINAL MICROBIOTA VIA ENEMA (THRIVE) FOR THE REHABILITATIVE PHASE OF SEVERE ACUTE MALNUTRITION: METHODOLOGY FOR A NOVEL PILOT STUDY EVALUATING SAFETY, MICROBIAL ENGRAFTMENT AND NUTRITIONAL OUTCOMES

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Severe acute malnutrition (SAM) remains an important challenge to child health globally. Accumulating evidence indicates that children with SAM have impaired development of their gut microbiota. This study aims to take the first translational step in evaluating the potential role of microbiota transfer therapy (MTT) as a broad-spectrum microbial therapy for uncomplicated SAM unresponsive to standard therapy. MTT, or fecal microbiota transplantation, has emerged as a safe and effective therapy for *Clostridium difficile* infection and has shown promise in repairing abnormal gut microbial community configurations related to other diseases such as inflammatory bowel disease, metabolic syndrome or autoimmune diseases. THRIVE is a single-center, two-armed, randomized controlled trial evaluating the safety of MTT administered via enema (followed by standard of care) in South African children aged 18-60 months in the recovery phase of SAM. MTT is minimally processed stool from a screened healthy donor. Twenty children presenting to hospitals in Cape Town, South Africa who meet the inclusion criteria will be randomized 1:1 to receive MTT by enema and standard of care or standard of care only. Treatment will be dosed at 10mL/kg (maximum 150mL) of donor fecal material followed by standard ready-to-use therapeutic food. Children in the control arm who show no nutritional recovery at 8 weeks despite adequate nutritional intake will be offered MTT provided they continue to meet the inclusion criteria. Children will be evaluated through 8 weeks after randomization. Children will be assessed for the primary outcome of safety and secondary outcomes including microbial engraftment, biomarkers associated with recovery in SAM, nutritional and clinical endpoints. Stool samples will be collected at enrollment and on days 3, 7, 21 and 56 and analyzed using 16S rRNA sequencing and whole genome sequencing. This is the first study exploring broad-spectrum microbiome manipulation for SAM and could open novel therapeutic avenues for children with SAM poorly responsive to standard therapy.

1885

ETIOLOGY, CLINICAL SEVERITY AND ENTEROAGGREGATIVE *ESCHERICHIA COLI* ASSOCIATED WITH DIARRHEAL DISEASES IN INFANTS AT SEMIARID REGION IN BRAZIL: A CASE-CONTROL STUDY

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Diarrheal diseases are important cause of morbidity and mortality in children in developing countries. We aim to study the etiology and severity of diarrheal disease in children in the low-income Brazil semiarid region. This is a cross-sectional study, age-matched case-control of diarrhea in children aged 2-36 months residing in six cities from Brazil semiarid region. After informed consent from their parents or guardians, clinical and epidemiological data, anthropometric measurements, and a fecal sample collected to identify enteropathogens (Luminex Bio-Plex 200 System, Bio-Rad, USA). Positive EAEC samples were further analyzed using multiplex PCRs targeting 27 virulence related genes (VRGs). We enrolled 1,200 children, 596 diarrhea cases and 604 controls. By logistic regression including all enteropathogens, the best predictors of diarrhea were six pathogens in descending order: *Norovirus*, *Adenovirus*, *Rotavirus*, STEC, *Giardia* and EAEC. Severity diarrhea score was significantly associated with EAEC ($p < 0.001$). EAEC VRGs analysis showed *espC* gene (cytotoxic serine protease autotransporter) was associated with diarrhea (p=0.025, OR 1.82, 95% CI 1.07 3.11), and *agg4A* gene (AAF/IV fimbria unit) was associated with controls (p = 0.011, OR 0.47 95% CI 0.26 0.85). In addition, *espC* gene was further associated with increased diarrhea severity (p=0.001), while *aar* gene (negative regulator of AggR) was associated with less severity (p=0.025). Six enteropathogens, *Norovirus*, *Adenovirus*, *Rotavirus*, STEC, *Giardia* and EAEC are associated with diarrhea in children in the low-income Brazil semiarid region. EAEC was associated with diarrhea severity score, with further contribution of specific VRGs profile *espC*(+) and *aar*(-).

1886

THE CRITICAL ROLE OF ZINC IN A NEW MURINE MODEL OF ENTEROTOXIGENIC *ESCHERICHIA COLI* DIARRHEA

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Enterotoxigenic *E. coli* are major causes of traveler's diarrhea as well as endemic diarrhea and stunting in children in developing areas. However, a small mammal model has been badly needed to better understand and assess mechanisms, vaccines and interventions. We report a murine model of ETEC diarrhea, weight loss and enteropathy, and investigate the role of zinc on the outcomes. LT+ST producing enterotoxigenic *E. coli* (ETEC) given to weaned C57BL/6 mice after antibiotic disruption of normal microbiota cause growth impairment, watery diarrhea, heavy stool shedding and mild to moderate intestinal inflammation, the latter worse with zinc deficiency. Zinc treatment promoted growth in zinc deficient infected mice, and subinhibitory zinc reduced expression of ETEC virulence genes *cfa1*, *cexE*, *sta2* and *degP*, but not *eltA in vitro.* Zinc supplementation increased shedding and ileal burden of WT ETEC, but decreased shedding and tissue burden of LTKO ETEC. LTKO also caused less inflammation by fecal MPO assessment, all suggesting that, in contrast to LT which is more associated with colonization and early diarrhea, ST expression is increased with ZD and is responsible for greater inflammation. These findings provide a new murine model of ETEC infection that can help elucidate mechanisms of growth, diarrhea and inflammatory responses as well as potential vaccines and interventions.

1887

CAMPYLOBACTER JEJUNI INFECTION AND HOUSEHOLD-LEVEL FACTORS ARE ASSOCIATED WITH CHILDHOOD GROWTH IN MIRPUR, BANGLADESH: AN ANALYSIS OF THE MAL-ED STUDY

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The dual burden of enteropathogen infection and childhood malnutrition continues to be a global health concern and a leading cause of morbidity and death among children. *C. jejuni* infection, in particular, continues to be highly prevalent in low and middle income countries, including Bangladesh. We examine longitudinal data to evaluate the trajectories of change in child growth, and identify the association with *C. jejuni* infection and household-level factors. The study analyzed data from 265 children participating in the Etiology, Risk Factors, and Interaction of Enteric Infections and Malnutrition and Consequences for Child Health (MAL-ED) Study in Mirpur, Bangladesh. We applied latent growth curve modelling to evaluate the trajectories of change in height, as measured by height-for-age z-score (HAZ), amongst children 0-24 months of age. Household-level risk factors were included in the model as time-invariant covariates. Asymptomatic and symptomatic *C. jejuni* infections were included as lagged time-varying covariates. An inverse association was found between increasing age and HAZ. Greater maternal education, improved water source, and treatment of water were positively associated with HAZ. *C.jejuni* infection was more prevalent with increasing age, with over 70% of children 18-24 months of age testing positive for infection. A positive episode of infection in the preceding three-month interval was associated with decreased HAZ at 12, 15, and 18 months of age. The results indicate that maternal education and household-level factors have a positive effect on child growth trajectory while *C. jejuni* infection has a negative effect on HAZ at specific age intervals. Further analyses will explore the relationships between the household-level factors and *C. jejuni* infection.

1888

IDENTIFYING TRANSMISSION ROUTES FOR CHOLERA INFECTIONS AMONG HOUSEHOLD CONTACTS OF CHOLERA PATIENTS IN RURAL BANGLADESH

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Household contacts of cholera patients are at a 100 times higher risk of developing cholera than the general population. The objective of this study is to examine the incidence of cholera infections among household contacts of cholera patients in a rural setting, to identify risk factors for cholera infections among this population, and to investigate cholera transmission pathways using multilocus-variable-number-tandem-repeat-analysis (MLVA). This approach allowed for intervention strategies to be identified that could be used to reduce the incidence of cholera among household contacts of cholera patients. Stool from household contacts and water source and stored water samples were collected from cholera patient households at Day 1, 3, 5, and 7 after the presentation of the index patient at a health facility. Two hundred thirty clinical and water

isolates were analyzed by MLVA. Thirty-seven percent of households had at least one household contact with a cholera infection. Thirteen percent of households had *V. cholerae* in their water source, and 27% had *V. cholerae* in stored household drinking water. Household contacts with *V. cholerae* in their water source had a significantly higher odds of a symptomatic cholera [Odds Ratio (OR): 5.49, 95% Confidence Interval (CI): 1.07, 28.08]. Contacts consuming street vended food had a significantly higher odds of a cholera infection (OR: 9.45, 95% CI: 2.14, 41.72). Older age was significantly associated with a lower odds of a cholera infection (OR: 0.96, 95% CI: 0.93, 0.99). All households had clinical and water isolate that were closely related by MLVA. These findings emphasize the need for interventions targeting water treatment and food hygiene to reduce cholera infections.

1889

MODELLING THAI POPULATION DYNAMICS AND SEASONAL MOVEMENT TO ASSESS AND PREDICT THE BURDEN OF MELIOIDOSIS

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Thailand demography is in the transient phase with the aged population doubling within a decade. Melioidosis is a communicable disease, with transmission from environment, especially soil and water, with 4.96/100,000 reported cases in 2015, and eight-fold in rural areas compared to urban areas. Changes in the population structure and social impacts of life style changes reflecting in seasonal movement, and urbanization are hypothesized to significantly influence the incidence of melioidosis. Our objective is to examine changes in the age, sex, diabetes, and seasonal movement on melioidosis using mathematical modelling. A dynamic model was constructed including demographic and disease processes. The model was fitted to incidence of reported melioidosis among males and females using a Bayesian Markov chain Monte Carlo (MCMC) approach to accurately assess uncertainty in parameters of disease onset, progression, and reporting. Our model predicted higher incidence rates of melioidosis compared with the national surveillance data, reflecting under-reporting issue. Transient populations and working ages are the highest risk groups. The estimated of incidence rates among males was two-fold greater than that of females, moreover, the incidence rates in rural area was more than double compared to urban area. The predicted annual incidence rate of melioidosis between 2008-2035 was 22.1 to 23.8/100,000 with a slightly increasing trend. The estimated population mortality rate was 3.67 to 3.86, compared with 3.45 reported per 100,000 people in 2015. Our results show the incidence rates among people with diabetes was two-fold greater than those non-diabetes. Transient populations, males aged ≥ 45 years, living in a rural area, and diabetes (diagnosed or not) diabetes were independent risk factors for melioidosis. The study helps assessing the impacts of changing population structure and diabetics on melioidosis epidemiology in Thailand. The model is useful to identify high risk groups and to guide targeted strategies to disease prevention and control.

1890

SPATIOTEMPORAL DYNAMICS OF *STREPTOCOCCUS PNEUMONIAE* IN RURAL PAKISTAN

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Background and aimsKnowing how S. pneumococcus (SP) carriage varies in time and space aides in rollout of new vaccination programs through identifying potential 'hotspots' of transmission as well as estimating the level of herd-protection induced by those vaccination programs. Largely, these spatiotemporal dynamics are underexplored in developing settings. MethodsAn ongoing NP carriage survey near Karachi, Pakistan randomly selects 60 infants per month and gathers information on illness indicators, socioeconomic status, and household demographics, and records the spatial coordinates of each individual. These data were modeled as spatial point patterns and space-time clusters were identified using Ripley's K function. ResultsSignificant clusters were observed over the study area across three years. Over time, the physical distance between children vaccinated with PCV and vaccine-type SP carriage increased, giving an indirect estimate of herd immunity induced by PCV. ConclusionsClear spatiotemporal patterns of SP carriage exist in rural Pakistan, and are associated with PCV vaccinated individuals, indicating the need for uniform vaccination coverage when introducing it to new populations.

1891

DEVELOPMENT AND EVALUATION OF A LABORATORY-DEVELOPED TAQMAN ARRAY CARD (TAC) FOR ANTIMICROBIAL RESISTANCE (AMR) DETECTION

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Antimicrobial resistance (AMR) is a significant public health issue worldwide. Monitoring and surveillance of resistance is one action for addressing and preventing AMR. Phenotypic antimicrobial susceptibility testing is laborious and unable to test multiple antimicrobial agents simultaneously. A rapid high throughput AMR detection tool will be useful to monitor AMR prevalence. We designed and developed an easy-to-perform genotypic TaqMan array card with 89 sequences specific PCR reactions to detect 85 antimicrobial resistance associated genes or mutations for 10 highly important antimicrobial classes used in human and veterinary medicine. This included cephalosporins, quinolones, macrolides, penicillins, aminoglycosides, polymyxins, folate pathway inhibitors, tetracyclines, phenicols and carbapenems. The 89 qPCR assays were tested for performance against 251 well characterized (genotypic) antibacterial resistance isolates, which included 201 isolates from the FDA-CDC AR bank (CDC, Atlanta, GA, USA), 15 isolates from Antibacterial Resistance Leaderships Group (ARLG, Durham, NC, USA), and 35 isolates from American Type Culture Collection (ATCC, Manassas, VA, USA) on 384 well PCR plates. Sanger sequencing was performed for confirmation when discordances were observed. The 89 assays revealed near-perfect concordance on the 251 isolates (i.e., 2244 true positives, 20081 true negatives, 11 discrepant). Comparing the association between genotypic and phenotypic DST of 38 *E. coli* isolates for 22 antimicrobial agents, we observed an overall sensitivity of 75-100% across the 22 drugs, specificity 55-100%, and accuracy 74-97%. Sensitivity was lowest for β-lactam/β-lactamase inhibitors, and specificity low for aztreonam, ciprofloxacin, aminoglycosides and sulfamethoxazole. Kappa agreement ranged from moderate to almost perfect between the two methods (κ = 0.44 – 0.91). Accepting certain genotypic-phenotypic discrepancies that are manageable, this TaqMan array card yields an accurate susceptibility result compared to the standard phenotypic DST.

1892

DRY REAGENT-BASED LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR CONFIRMATION OF BURULI ULCER USING A PORTABLE FLUORIMETER

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Early diagnosis and treatment of Buruli ulcer (BU), a necrotizing skin infection caused by *Mycobacterium ulcerans* (MU), are crucial for case management and disease control. The use of IS*2404* PCR is recommended for laboratory confirmation of BU, but this requires well-equipped laboratories and is thus restricted to reference centres in endemic countries. As the highest burden of BU is in rural areas, point-of-care molecular diagnostic tests are urgently needed. Loop-mediated isothermal amplification (LAMP) is considered an appropriate technology for microscopy level laboratories. This study optimizes and evaluates a dry reagent-based (DRB) LAMP test for detection of MU DNA (IS*2404*) using a portable fluorimeter. Two IS*2404* LAMP primer sets were analysed using freeze-dried LAMP chemistries from OptiGene and Lucigen. A Genie III fluorimeter (OptiGene) was used for isothermal amplification, detection of LAMP products and confirmation of amplicon specificity by annealing curve analysis. Different freeze-dried primer/chemistry combinations were prepared and their performance assessed by analysis of specificity, sensitivity and time to positivity (Tp) using serial dilutions of IS*2404* plasmid standards, MU culture extracts and DNA extracts derived from swab and fine-needle aspirate samples from BU suspected cases, as well as must-not-detect samples from leprosy patients. Both primer combinations using OptiGene chemistry detected MU DNA in all MU culture extracts and clinical BU samples, and showed 100% specificity. The limit of detection was 5 and 0.5 MU genome equivalents, with 78% and 100% sensitivity in clinical samples depending on the primer set. The Tp ranged from 3 to 19. Freeze-dried primer combinations using Lucigen chemistry did not yield any positive result under our laboratory conditions. The optimized DRB LAMP test was highly specific, sensitive and robust and will now be subjected to large-scale evaluation. DRB-LAMP will contribute in filling the gap in confirmatory diagnosis that exists between reference centres and endemic *Foci* in rural areas.

1893

MOLECULAR MARKERS OF ANTIMICROBIAL RESISTANCE IN *NEISSERIA GONORRHOEAE* ISOLATES FROM GHANA

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Antimicrobial resistance (AMR) to *Neisseria gonorrhoeae* is evolving. In Ghana, gonorrhea is treated empirically, little is known about the resistance status of circulating gonococci. Identifying AMR, molecular mechanisms of resistance and *Neisseria gonorrhoeae* multiantigen sequence types (NG-MAST), are important for guiding treatment and detecting the emergence of new molecular mechanisms of resistance. There is no published record of NG-MASTs. Forty-nine isolates were collected during 2012-2015. After obtaining informed consent, a urethral swab (males) or an endocervical swab (females) was collected from patients presenting at two health facilities in Accra and three others in Sekondi and Takoradi with urethral or vaginal discharge, dysuria and intermenstrual bleeding in women, as well as abdominal pain. Each swab was inoculated on Modified Thayer Martin (MTM) agar. The agar plate was incubated at 25-27°C for 24-72 hours and checked for growth every 18-24 hours. Gonococcal isolates were identified by Gram stain, oxidase, catalase and the API-NH tests. Resistance to penicillin, tetracycline, ciprofloxacin, azithromycin, cefixime, ceftriaxone and spectinomycin was tested with the disc diffusion method and the E-test. NG-MASTs were determined. Resistance determinants to penicillin, cefixime, ceftriaxone (bla_{TEM}, *penA,* MtrR*, ponA*) and ciprofloxacin (*parC* and *GyrA*) were detected. A total 11 NG-MASTs were seen, an additional 17 were novel. The only multidrug resistant (MDR) isolate was ST1407. Isolates had either a bla_{TEM-1} or a $bla_{TEM-135}$ gene. Six *penA* amino acid sequence patterns, II, XVIII, XIV, XIX, XII and XXXIV were detected. In the MtrR repressor, the following mutations were seen, A39T, G45D and G45S as well as L421P in the *ponA* gene. Substitutions, S91F, D95A and D95G were seen in the *gyrA* regions, and D86N, S87N and E91G in the *parC* regions. Data shows genetic diversity: one isolate belongs to ST1407, which has been circulating globally with high MICs to the extended spectrum cephalosporins. This is the first report of an isolate with reduced susceptibility to cefixime in Ghana. There is a need for continued AMR surveillance.

1894

SURVEILLANCE OF ESBL-PRODUCING *KLEBSIELLA PNEUMONIAE* IN SAMPLES OBTAINED FROM TWO HOSPITALS IN THE PERUVIAN AMAZON

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Infections caused by Gram negative bacteria are highly prevalent in hospitalized patients, especially in intensive care units. However, multidrug resistant strains represent a therapeutic challenge for both community and hospital-acquired infections, leaving very few and often undesirable options for treatment. It is estimated that between 50% and 60% of the more than two million hospital-adquired infections in the United States per year are caused by resistant bacteria, leading to nearly 77,000 deaths annually. Bacterial resistance also continues to increase globally. Extended-spectrum beta-lactamases (ESBL) are of particular significance as they confer resistance to a large group of beta-lactam antibiotics, especially carbapenems, 3rd generation cephalosporins, monobactams and to a lesser but stillimportant extent the aminoglycosides. In the present study conducted from January 2016 to February 2018, 172 samples were collected and processed following standart laboratory procedures for identification and AST determination. Of those samples, 158 (92%) were positive to some pathogen: *E. coli* was the most common pathogen isolated, 70 (46%), followed by *Klebsiella pneumoniae,* 37 (24%), of which 26 (70%) were ESBL producers. Susceptibility test revealed a high resistance to Ceftriazone (100%) and Aztreonam (88%), and a high sensitivity to Imipenem (100%). These results indicate that treatment strategies in the Amazon region of Peru should reflect emerging resistances and ESBL producing strains.

1895

PREVALENCE OF ESKAPE PATHOGENS AND THEIR ANTIMICROBIAL RESISTANT IN WOUND INFECTIONS FROM HOSPITALIZED PATIENTS IN THE PERUVIAN AMAZON CITY OF IQUITOS

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Wound infections caused by multi-drug resistant (MDR) ESKAPE pathogens (*Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.)* are an increasing problem in both military and civilian populations.

Iquitos is a geographically isolated city in the Peruvian Amazon with little information regarding prevalence and antimicrobial resistance of the ESKAPEs. From July 2017 to February 2018, 180 wound samples (160 civilian; 20 military) were taken from hospitalized patients in Iquitos; 134 of which were culture-positive with 59% (79/134) yielding ESKAPE strains. In total, 229 bacterial strains were isolated, of which 40.6% (93/229) were ESKAPEs. *S. aureus* was the most common with 36 isolates (36/93, 39%), followed by *K. pneumoniae* (27%,25), *P. aeruginosa* (14%, 13), *A. baumannii* (10%, 9), *E. cloacae* (9%, 8), and finally *E. faecium* (2%, 2). Of *S. aureus* isolates, 94% (34/36) were resistand to Penicillin, 19% (7/36) were resistant to Azithromycin and Tetracycline, 17% (6/36) were methicillin resistant; 8% (3/36) to Ciprofloxacin and Clindamycin, and 8% (3/36) showed inducible clindamycin resistance. 80% (20/25) of all *K. pneumoniae* isilates were ESBL-producing; 88% (22/25) were resistant to Trimethoprim/sulfamethoxazole, 68% (17/25) to Tetracycline, 60% (15/25) to Gentamicin, 40% (10/25) to Ciprofloxacin with 48% (12/25) intermediate, and only 8% (2/25) were resistant to Amikacin. 38% (5/13) of *P. aeruginosa* isolates were MDR ande 31% (4/13) were Imipenem resistant. 67% (6/9) of *A. baumannii* isolates were MDR and 11% (1/9) was imipenem resistant. These high rates of antimicrobial resitance represent a serious public health concern for wounded and hospitalized patients, and emphasize the need for continued resistance surveillance in Peru and developing countries.

1896

RESURGENCE OF DIPHTHERIA IN HAITI: OBSERVATIONS FROM THE NATIONAL EPIDEMIOLOGIC SURVEILLANCE SYSTEM: 2014 - 2018

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Diphtheria is a vaccine-preventable disease caused by toxin-producing *Corynebacterium diphtheriae*. Globally, diphtheria cases have declined due to improvements in childhood vaccination coverage. In Haiti, childhood immunization coverage with a diphtheria vaccine has perennially been below 70%. Since August 2014, Haiti has experienced a resurgence in diphtheria cases. We reviewed epidemiological data from 2014-2018. We defined a probable case as pharyngitis, tonsillitis, or laryngitis and the presence of a pseudomembrane; a confirmed case was a probable case with laboratory confirmation of toxin-producing *C. diphtheriae* by PCR, or a probable case with epidemiologic link to a laboratory-confirmed case. From August 2014 to March 2018, 456 probable diphtheria cases were notified to the Ministry of Health (MOH), from the ten geographic departments of the country. The Median age was 8 years (IQR: 5, 11), 263 (58%) were female. Of 456 probable cases, 374 (82%) were tested by reverse transcriptase PCR and 189 (51%) were positive. Laboratory-confirmed cases have a median age of 8 year old (IQR: 5, 11); 182 (96%) were younger than 18 years and, of these, 145 (80%) were unimmunized against diphtheria. Among confirmed PCR positives, 152/189 (80%) had no history of immunization. Of patients tested by PCR, among those with no history of immunization 151/270 (56%) were PCR positive, whereas among those who received at least one dose of vaccine 38/104 (37%) were PCR positive ($p<0.00005$). Of PCR positives, 14% received diphtheria antitoxin (DAT) for treatment. The case fatality rate among confirmed case was 40/189 (21%). In response to the outbreak, the MOH has strengthened laboratory testing capacity, provided awareness, case management and outbreak response activities, improved access to diphtheria antitoxin and is conducting diphtheria supplemental immunization activities. The current surge of diphtheria cases emphasizes the need for ongoing enhanced surveillance, laboratory testing capacity, awareness among health providers, and ensuring availability of DAT.

1897

WHOLE-GENOME ANALYSIS OF *BURKHOLDERIA PSEUDOMALLEI* ISOLATE ASSOCIATED WITH A CONFIRMED CASE OF MELIOIDOSIS IN PERU

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Burkholderia pseudomallei is a gram-negative bacterium that causes the fatal disease melioidosis. An increasing number of melioidosis cases are being reported in tropical regions, including Latin America countries. In this study, we report the whole-genome analysis of Burkholderia pseudomallei isolate associated with the first confirmed case of Melioidosis in the northern of Peru. An isolate of B. pseudomallei from a clinical case of melioidosis was sequenced using both Miseq Illumina and MinION Oxford Nanopore. The raw sequences were assembled using is hybrid assembly pipeline Unicycler and gene annotation was performed using web platform RAST. Genomic sequences of global B. pseudomallei isolates were retrieved from GenBank. Phylogenomic analysis based on SNPs of the core genome was inferred with RAxML v8.2.9 using the Maximum Likelihood method and under the general evolutionary model GTR with 1000 bootstrap. Genomic analysis allow to complete the whole genome of this isolate divided in two chromosomes and also confirm the presence of a novel variant of Class D β-lactamase OXA-57 associated with multidrug-resistance. Phylogenomic reconstruction revealed that Peruvian isolate grouped into a single clade including isolates form Brasil and Ecuador resulting in the Latin America lineage based on highly informative SNPs. Overall genomic and epidemiological data confirm the report of novel multidrug-resistant Burkholderia pseudomallei isolate associated with a fatal case in Peru that would be particularly relevant in respect of the melioidosis as emerging diseases posing as public health risk.

1898

EMERGING *BARTONELLA BACILLIFORMIS*, *LEPTOSPIRA* SPP., AND *RICKETTSIA* SPP. IN THE SOUTHEASTERN PERUVIAN AMAZON BASIN

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Acute febrile illnesses (AFI) represent a major health problem in the Peruvian Amazon basin due to their diverse etiologies and low laboratory-confirmation rates. Madre de Dios is one of the most affected regions in Peru endemic for dengue, leptospirosis among other causes of AFI such as bartonellosis and rickettsiosis that remain under-reported. This study was undertaken to detect via PCR the presence of *Leptospira* spp., *Bartonella bacilliformis* and *Rickettsia* spp. in serum samples from patients with AFI from Madre de Dios, Peru. Blood samples from patients with acute febrile illness were analysis by real-time PCR for detection the presence of *Bartonella bacilliformis*, *Leptospira* spp. and *Rickettsia* spp. *Bartonella bacilliformis* was the most prevalent bacteria isolated in 21.6% (30/139) of samples, followed by *Leptospira* spp. in 11.5% (16/139) and *Rickettsia* spp. in 9 cases. No coinfections were observed between these bacteria. The most frequent symptoms associated with fever were headaches, myalgias and arthralgias across all groups, with no significant difference between the three bacteria clinical presentation. In conclusion, in our study series, *B. bacilliformis* is the most common pathogen isolated in patients

with AFI. The unspecific presentation of these bacteria highlights the need for laboratory-confirmation to strength the Peruvian epidemiological surveillance.

1899

RETROSPECTIVE COHORT STUDY OF TYPHOID AND PARATYPHOID FEVER AT REFERRAL CENTER FOR TRAVEL AND TROPICAL DISEASES IN JAPAN

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Typhoid and paratyphoid fever are rare infectious diseases in Japan. Moreover, the treatment is complicated mainly because of drug resistance. The aim of this study is to demonstrate the clinical characteristics of typhoid and paratyphoid fever at referral center for tropical infectious diseases in Japan. We reviewed medical records of the patients with typhoid or paratyphoid fever who were treated at our institute between 2000 and 2017. Patients with *Salmonella enterica* serovar Typhi or *S. enterica* serovar Paratyphi A isolated from any specimens were included. There were 32 typhoid fever cases and 23 paratyphoid fever cases. The median age was 25 years (interquartile range, 22-37 years), 60% were male and the majority was Japanese (76%). Ninety-five percent (52/55) were imported cases. Fifty-eight percent (32/55) were from South Asian countries and 33% (18/55) were from Southeast Asian countries. Eighty-nine percent were diagnosed by blood culture, and the median time to blood culture positivity was 1 day. Nine percent (5/55) were suspected multi-drug resistant (MDR) and all MDR suspected cases were typhoid fever infected in South Asian countries. Sixty-four percent were nalidixic acid (NA) resistant, which means low-level fluoroquinolone resistant, and 87% of strains from South Asian countries were NA resistant. All 3 relapsed cases were mainly treated by cephalosporin, and the relapse rate of cephalosporin treatment was 10%. There was no fatal case. Nowadays, rate of MDR cases has already declined, so the most can be treated by the conventional drug of choice, for example sulfamethoxazole/trimethoprim and ampicillin. On the other hand, many strains are still resistant for NA, so we should pay attention for fluoroquinolone treatment. Cephalosporin, for example ceftriaxone, tends to cause drug fever, drug eruption and hepatotoxicity as adverse event, and is known its high relapse rate. Furthermore, although it is known that azithromycin resistant strains are spreading, we cannot widely use sensitivity test for azithromycin. For NA resistant and cephalosporin intolerant cases, we think the conventional drugs could be reasonable choice.

1900

HIGH-THROUGHPUT INTEGRATED DISEASE SEROSURVEILLANCE USING A ONESTEP MULTIPLEX BEAD ASSAY

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Serological data indicating the presence and titer of antibodies against infectious disease and vaccine antigens can provide robust indicators of transmission patterns and immune status in a population. Laboratory testing for large-scale serosurveys is often hindered by time-consuming immunoassays that employ multiple steps for antibody detection and quantification. We present a novel approach to antibody detection by bead-based quantitative immunoassay that involves concurrent incubation with all assay reagents and the sample overnight. When compared to the standard protocol employing tandem incubation steps, this "OneStep" assay protocol was found to amplify the assay signal for IgG detection for 37 of 40 antigens tested from 29 pathogens causing disease in humans. The greatest increases in assay signal were seen at the low- and mid-range IgG titers, and was indicative of an enhancement in the analyte detection, not simply an increase in the background signal of the assay. Upon completion of the OneStep protocol, assay plates stored at 4^0C retained assay signal for up to one month, indicating the stability of the bead complex over time. To compare seroprevalence and immune protection estimates that would be generated for defined study population if using different laboratory protocols or sample types, serum and filter paper blood samples collected during an integrated serosurvey in Haiti were utilized. Estimates for seroprevalence and vaccine-induced protection in the Haiti study population were largely unchanged if different sample types or test protocols were used. This OneStep bead assay protocol has the potential to be applied to various disease group antigens for the high-throughput and timely collection of serosurvey data.

1901

INJURY AS A REASON FOR EMERGENCY DEPARTMENT VISITS IN THE AFTERMATH OF HURRICANE MARÍA IN A SOUTHERN PUERTO RICAN HOSPITAL

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Hurricane María hit Puerto Rico on September 20, 2017. This was the worst documented natural disaster in Puerto Rico's recorded history. Six months after the hurricane, we are still doing reconnaissance as to the extent of damages caused. Within the realm of public health, the aim of our study is to describe the individuals that sought care for injury in the emergency department of a tertiary teaching hospital and its associated urgent care clinic in the aftermath of the hurricane. Using a modified Natural Disaster Morbidity Surveillance Form, we collected the reason for visit of patients that accepted participation after informed consent during the period between October 16, 2017–March 28, 2018. Of the 5,094 participants recruited, 548 (11%) reported injury as the main reason for visit. Of these, 52 (9%) were self-reported to related to the hurricane or rebuilding efforts. The peak of injury cases occurred the week of October 22, 2017 and decreased thereafter. Injured participants had a median age of 52 (range 12–81) years, with 67% male. The most common type of injury in all visits was described as an abrasion, laceration, or cut (45% of all injury visits; 54% of hurricane-related visits). The next most common injury types included sprains/strains (13% of all visits; 10% of hurricane-related visits) and concussions or head injuries (8% of all visits; 13% of hurricane-related visits). The most common mechanism of injury was described as a fall, slip, or trip (268, 49%) or being hit by or against an object (87, 16%). Hurricane-related injury visits were more likely to be associated with being hit by or against an object compared with non-hurricane-related visits (33% to 14%, $p<0.05$) and less likely to be associated with a fall (21% to 52%, $p<0.01$). Most participants with injury-related visits (92%) were discharged to home after evaluation; 3% were admitted to the hospital. This information will be used to better prepare the emergency department in the likely event of a future natural disaster as well as to develop targeted strategies for patient injury prevention.

1902

PREVALENCE OF FLOOD-ASSOCIATED INFECTIOUS DISEASES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Flooding is a natural disaster with major direct and indirect consequences on humans' health. Several studies focused on infectious diseases that increase due to floods. However, none reported the prevalence of post-flood infections and their associated risk factors. Here, we aim to investigate the prevalence of these diseases A comprehensive literature search was performed using nine databases. Data were extracted and results were presented as percentages. We assessed the risk of bias using Cochrane Collaboration's tool. The protocol was registered in PROSPERO (CRD42016043939). Cholera following floods has the highest prevalence among other infections with 47.04%. Leptospirosis showed the widest geographical distribution, overall prevalence (27.72%). Malaria was also frequently reported (13.27%). A wide range of GIT infections were detected as well including bacterial (E-coli, Salmonella, Shigella), viral (Rotavirus) and miscellaneous ones. Skin infections recorder a relatively higher prevalence (23.19%) when compared with respiratory (18.30%), ENT (3%) and ophthalmologic (3.95%) ones. The most frequently reported risk factor is exposure to contaminated water. In Conclusion, several post-flood infections recorded high prevalence including cholera, leptospirosis, malaria and diarrheal infections. Understanding the epidemiological and clinical aspects of these infections can help healthcare responders better anticipate the needs and overcome the challenges that they face during and after floods.

1903

ELECTROCARDIOGRAPHIC FINDINGS IN ADULTS WITH DENGUE FEVER

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There are few data on the electrocardiographic changes in acute dengue fever. We performed serial 12 lead ECGs (during admission and once at follow up) on adult Vietnamese inpatients with confirmed dengue. A total of 143 (77 males) patients, median (range) age 23.5 (range 16-72) years, were admitted with confirmed dengue on illness days 1-8 (median 5). Only 4 patients had 2007 WHO defined severe dengue. The majority of patients had three ECGs done and the Fridericia corrected QT interval had the least association with the heart rate (adjusted R^2=0.0061, p=0.07). The mean heart rate reached a nadir on D10, mean=64 (95% CI: 60.6-67.4)/min, the same day as the peak QTcF: 401 (392 - 410) msec. These nadir and peak times occurred later than the days of nadir platelet count (D5) and the peak haematocrit (D4). No patient had an uncorrected QT exceeding 500 ms and 1 patient had a QTcF of 501 msec. Three females and 8 males had QTcF values exceeding the upper limit of normal (ULN) of 450 and 430 ms, respectively. The proportions of patients with bradycardia (heart rate less than 60/min) rose from 1/12 (8.3%) on D4, peaked at 9/34 (26.5%) on D10 and fell to 3/19 (15.8%) on D30. The mean PR interval (150 ms) changed little over time and 4 patients had 7 episodes of an increased PR interval. By mixed effects modelling for repeated measures, only temperature was a significant explanatory variable. Most ECG parameters were within the normal range. These QTcF values represent the natural QTcF variability in nonsevere dengue.

1904

A SIX YEAR REVIEW COMPARISON PROFILES OF INFECTIOUS DISEASES IN UNDER-NOURISHED AND WELL-NOURISHED CHILDREN IN RURAL SUB-SAHARAN AFRICA PRIMARY CARE CENTER

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This study aimed to compare pattern of infectious diseases recorded in malnourished children compared to well-nourished children in a free rural primary care clinic in Kiang West District The Gambia.The interrelated nature of the occurrence of infectious diseases amongst malnourished and well-nourished children is still among the topics that need a thorough research. Severe acute malnutrition (SAM) and moderate acute malnutrition (MAM) are associated with increased severity of common infectious diseases. Death amongst children with severe acute malnutrition is almost always as a result of infection. Malnutrition is common in Kiang West District particularly during the "hunger" season from the month of August to October every year. We retrospectively analysed data from the Keneba Electronic Medical Records System looking for records of all children 5 years and below seen with ICD-10 codes for various prevalent of infectious diseases between January 2010 and December 2017. Data of children with normal anthropometry characteristics was compared against those with moderate acute malnutrition (WHO z score -2 to -3) and severe acute malnutrition (WHO z score greater than -3). Both moderate acute malnutrition and severe acute malnutrition were compared against each other and in reference to the well-nourished children. Common colds were the most prevalent infectious diseases in all groups followed by pneumonia. Overall, all infectious diseases diagnosed apart from common colds, intestinal helminthiasis and acute otitis media were more common among malnourished children compared to well-nourished children. There was an increasing gradient in relative frequency of pneumonia, HIV, septicaemia, UTIs, malaria, giardiasis and tuberculosis from well-nourished through moderate acute malnutrition to severe acute malnutrition. The relative frequency of prevalent infectious diseases among children of age 5 years and below in this study differed by their state of nutrition. More severe forms of infectious diseases occurred more frequently with worsening malnutrition in children 5 years and below in Kiang West District.

1905

THE LAST STEP BEFORE STOPPING TRACHOMA MDAS IN CAMEROON

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Cameroon is on the brink of eliminating trachoma as a public health problem by 2020. Initial mapping in 54 health districts (HDs) showed that 5 HDs were not endemic (trachomatous inflammation - follicular (TF)

0%), 28 HDs had TF≤5%, 5 HDs had a TF prevalence of 5-9.9% and the remaining 16 HDs had TF ≥10%. Mass drug administration (MDA) started in the 16 HDs with the highest TF prevalence followed by the 5 HDs with TF 5-9.9. In 2016, 7 HDs (out of 21) met the criteria to stop MDA as trachoma impact survey (TIS) showed TF <5%. In 2017 the other 14 HDs carried out TIS with funding from USAID/RTI. This abstract presents the findings of the last TIS to be performed in Cameroon. The TIS were carried out via a cross-sectional study based on a stratified random sampling. In each of these HDs, 30 communities each representing a cluster were selected; then, 30 households were selected in each community. Each team included a grader and a recorder, and all teams were provided with training before field data collection. Data were collected using android smartphones and transferred to a Tropical Data (TD) platform. These data were analyzed by both National Program staff and the TD Data Manager. Overall 390 communities were surveyed, 30 communities per HD. Teams visited 11,664 households and they examined 54,947 people. In all the surveyed HDs, the number children aged 1-9 years who were examined reached the sample threshold required for a valid TF estimate. In the surveyed HDs, the prevalence of TF in children aged 1-9 years varied from 0% to 2.5% (CI: 2.3-2.7). All these prevalence are below the endemic threshold of 5%. The decrease of the TF prevalence in these 14 HDs after effective MDA rounds demonstrates the efficacy of the «A» component (from the SAFE strategy) as well as the effectiveness of its implementation. These results from this last group of eligible HDs indicate National Program can stop MDA in these HDs, meaning that trachoma MDA is no longer required in Cameroon. However, to maintain the TF prevalence below the endemic threshold, the country should continue to implement the "F" and the "E" components from the SAFE strategy.

1906

CHARACTERIZING UNDERNUTRITION AMONG INFANTS AGED 1 TO 6 MONTHS IN HIGH MORTALITY AFRICAN SITES WITHIN THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK

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Globally, an estimated 3.8 million infants <6 months (U6M) are severely wasted. However, analyses often exclude infants U6M, as no consensus exists on reliable undernutrition indicators in this age group. Anthropometric measurements in infants U6M may reflect gestational age (GA), intrauterine factors, or postnatal nutritional status. Growth standards for preterm infants exist but rely on accurate assessment of GA, which is lacking in many settings. The Child Health and Mortality Prevention Surveillance (CHAMPS) Network aims to improve understanding of child mortality in high-burden settings. We aimed to create a standard approach to identify undernutrition in deceased CHAMPS cases 28 days to U6M and compare different assessment methods. We applied CHAMPS postmortem measurements to three indicators of undernutrition: severe underweight (weight-for-age z-score <-3) based on WHO Child Growth Standards (WHO-GS); severe underweight based on INTERGROWTH-21st Newborn Size Standards (IG-NS), which account for GA; and severe acute malnutrition (SAM) based on mid-upper arm circumference (MUAC) <11cm. Of 84 CHAMPS cases 28 days to U6M, only those with GA available from clinical records (n=36) or with GA unavailable but birthweight ≥2500g (n=6; considered term for analysis) were included. Of the 42 cases, 64% were preterm (GA <37 weeks). The proportion of cases with severe underweight was 74% (n=31) according to WHO-GS and 50% (n=21) according to IG-NS. Among the 27 preterm infants, WHO-GS classified 89% as severely underweight whereas IG-NS classified 52%. Based on MUAC, 74% of all cases and 82% of preterm cases had SAM. By accounting for GA in cases 28 days to U6M, IG-NS likely generated a more reliable estimate of the true undernutrition burden and resulted in a 32% decrease in cases with severe undernutrition compared to WHO-GS or MUAC. Overall, all three methods suggest severe undernutrition is common in this population. With increasing survival of premature infants, standardized anthropometric indicators in infants U6M are needed towards reliably characterizing undernutrition when GA is unknown.

1907

ALTERNATIVE STRATEGY FOR THE DEWORMING OF SCHOOL-AGED CHILDREN IN THE NORTHWEST AND THE SOUTHWEST REGIONS OF CAMEROON IN AN INSECURE SOCIO-POLITICAL CONTEXT

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The Cameroon NTD program carries out mass drug administration (MDA) for soil-transmitted helminths (STH) and schistosomiasis (SCH) with mebendazole (MEB) and praziquantel (PZQ) respectively, to school aged children (SAC) aged 6-14 regardless if they are enrolled. In 2017, a teachers' strike and socio-political unrest in the South-West and North-West regions led to the cessation of school classes. Consequently, the planned school-based deworming activities could not take place in schools. The South-West and North-West regions have 18 and 20 health districts (HD) respectively. Both are endemic with STH and 12 HDs are endemic with SCH. To ensure continuity of treatment, the health authorities along with Sightsavers, adopted a community-based distribution as an alternative deworming strategy. MDA was carried out using a door-to-door method and supervision was provided as well as data monitoring during the MDA. The regions were able to mobilize additional funds, increasing the initial global grant by 35%. 2570 community drug distributors (CDDs) were provided with training in the North-West and 2399 in the South-West region. Importantly, the therapeutic coverage reached 85% for MBD and 86.9% for praziquantel PZQ)in the North-west, and 87% for MBD and 84% for PZQ in the South-West. These results show that the community-based approach is an effective alternative to the school-based approach. The advantages of this strategy include allowing the CDDs to reach out-of-school children normally difficult to reach through the school-based strategy. It also reduced data reporting delays and it allowed strengthening capacity though health system pyramid including communities. Moreover, the therapeutic coverages were improved using this strategy than have been achieved in the last two years: (South-West: 50.93% for MBD, 49.10% for PZQ - North-West: 96% for MBD, 73% for PZQ) and in 2016 (South-West: 51.58% for MBD, 53.34% for PZQ - North West: 89.6% for MBD, 66.17% for PZQ). Despite these improvements it must be noted that the community based approach is more expensive and it should be accompanied by quality assurance mechanisms.

1908

SCREENING FAILURES: HOW TO IMPROVE COVERAGE OF VACCINATION

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Evaluation of the PfSPZ vaccine has been conducted in Equatorial Guinea (EG) since 2015. A Phase I and a Phase II trial has been completed. Currently preparations are ongoing to conduct a Phase II and Phase III trial in EG. As part of the preparatory activities an assessment will be done on the proportion and profile of potential trial subjects excluded from enrolment based on inclusion and exclusion criteria set for the two trials. The potential participants of the two trials will be invited for screening and detailed information about health history and social background will be collected. Physical examination will be done and blood samples collected for laboratory analysis. Based on the protocol guidelines and eligibility criteria eligible will be invited for enrollment. Participants excluded prior to any vaccination are considered as "screening failures" and the reasons for their exclusion are various. Data from the screening failures will be analyzed in comparison to those enrolled and the total number of screened participants to gain insight on the contributing factors for non-participation for individuals who consider themselves healthy in the community. The proportions of screening failures will be calculated and categorized with respect to age group and reason(s) for exclusion. These will be extrapolated to get an estimate of how many people on the Island of Bioko would potentially *not be* eligible for future biomedical research like a vaccine study. The implication of the screening failures on the logistics of the conduct of the trials and potential options for optimizing recruitment for the trials or any additional supportive measures will be discussed.

1909

A POST MASS DRUG ADMINISTRATION COVERAGE EVALUATION AND KNOWLEDGE, ATTITUDES AND PRACTICES SURVEY IN TWO HEALTH DISTRICTS IN MALI

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Mali has implemented preventive chemotherapy for neglected tropical diseases (NTDs) for the last 10 years. A post mass drug administration (MDA) coverage survey was conducted in Bandiagara and Barouéli health districts (HDs) in July 2017 as difficulties in achieving sufficient coverage had been reported. The MDA for schistosomiasis (SCH) and soil-transmitted helminths (STH) was conducted with both a school-based and community strategy (school enrolment rate is around 50%) targeting school-aged children (SAC). Sampling was done using the WHO protocol with probability proportional to estimated size with segmentation "PPES-Segmentation" methodology and the Coverage Survey Builder. The target population for the survey was SAC as they were the targets of the MDA. Data were captured electronically. A total of 3,500 SAC were recruited for the survey: 1,637 in Bandiagara and 1,863 in Barouéli HDs. The survey showed that, using SAC as the denominator, 96.2% (1,605/1,637) in Bandiagara and 86.2% (1,575/1,863) of SAC in Barouéli received drugs, while 96.1% (1,573/1,637) in Bandiagara and 85.7% (1597/1863) in Barouéli reported taking the drugs (swallowing). 320 SAC did not receive drugs during the MDA (62 and 258 SAC in Bandiagara and Barouéli, respectively). The main reasons cited were: unaware of the MDA, 30.9% (99/320); the community drug distributors (CDDs) did not come to their home, school or public place, 24.4% (78/320) and absence from the home during the MDA 21.3% (68/320). Side- effects were reported by 14% of surveyed children (<1% were moderate - interfered with daily activities) and there were no serious adverse events. SAC were informed about the MDA by CDDs, family members, community leaders and community radios. These results show that the surveyed coverage rates were lower than those reported: 103.9% in Bandiagara and 100.1% in Barouéli during the MDA, but were still above the minimum threshold of 75% recommended by WHO. These results also indicate that a combined school and community based MDA strategy is effective in Mali for SCH and STH MDA.

1910

TRACHOMA SITUATION IN 2017 ONE YEAR BEFORE ELIMINATION DATE IN MALI

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Mali has implemented the World Health Organization's SAFE strategy for trachoma elimination since 1997 and has committed to eliminating trachoma as a public health problem in 2018. Trachoma mapping conducted in 1996/1997 showed that all regions in Mali were endemic. The prevalence of trachomatous inflammation-follicular (TF) in children under 10 years of age ranged from 23.1% to 46.7% and that of trachomatous trichiasis (TT) in women over 14 years of age ranged from 0.65% to 3.9%. Based on the results of the mapping, the backlog of patients requiring TT surgery was estimated at 85,000 adults. Mass drug administration (MDA) was conducted in endemic health districts (HDs) from 2002 to 2016 and mass TT surgery started in 1999. In addition, radio messages focused on trachoma prevention have been widely broadcasted and slabs for latrines provided to some households in high TF prevalence areas. The objective of this study is to describe the current trachoma situation in Mali one year before the planned elimination date. The study evaluated the results of the SAFE strategy implemented from 1997 to 2017 as shown by results from impact and surveillance surveys conducted according to WHO guidelines. The TT surgical backlog was revised each year based on results of TT prevalence surveys and the number of patients operated. At the end of 2017, all 66 of Mali's HDs endemic for TF at baseline have achieved the elimination threshold (<5% in children 1 - 9 years of age). The threshold of TT elimination (<0.1% all ages) was reached in 48 of 57 HDs originally endemic for TT. The backlog of people requiring TT surgery has decreased by 93% (from 85,000 to 5,893) and is now located in only 9 of the 57 HDs. Based on these results, Mali is poised to eliminate trachoma as a public health problem by the end of 2018. The country must now complete the pre-validation surveys (including in some insecure areas), conduct surgery to eliminate the TT backlog, and prepare the trachoma elimination dossier for WHO.

1911

MONOCYTE SUBSETS AND CO-STIMULATORY MOLECULES IN LEPROSY REACTIONS

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Leprosy reactions are acute inflammatory events associated with several clinical manifestations leading to disabilities and permanent deformities due to peripheral nerve damage. Type 1 reactions are the main cause of neuritis, whereas type 2 reactions are characterized by systemic involvement and symptoms like fever, arthritis, myalgia, neuritis and erythema nodosum. Understanding host immune pathways associated with tissue damage during reactions are of upmost importance to the development of immune intervention strategies in order to develop better treatment or prevention. The participation of monocytes in leprosy reactions was evaluated by determining the frequency of monocyte subsets and the degree of cellular activation through the expression of MHCII and the co-stimulatory molecules CD40, CD80, CD86. Leprosy subjects with or without reactions were included in this cross-sectional study. Peripheral blood mononuclear cell were isolated and stained *ex-vivo* to determine monocyte subsets and the degree of cellular activation by flow cytometry. Intermediate monocytes were increased in leprosy patients with reactions when compared to patients without reactions. Although no difference was detected in the frequency of monocyte subsets between type 1 and 2 reactions, the expression of CD80 was increased in monocytes from patients with type 1 reactions and CD40 was higher in PB subjects presenting type 1 reactions. Moreover, CD86 and MHC II expression were higher in intermediate monocytes when compared to the other subsets in leprosy reaction types 1 and 2. Our data indicates that intermediate monocyte activation with CD86 and MHCII expression is involved with both type 1 and 2 reactions, whereas CD80 and CD40 expression is related to type 1 reactions.

1912

A CROSS-SECTIONAL STUDY OF HEMATOLOGIC AND INFECTIOUS MORBIDITY IN KENYAN CHILDREN WITH SICKLE CELL ANEMIA

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Sickle cell anemia (SCA) afflicts over 200,000 newborns annually in sub-Saharan Africa, where it is highly prevalent in malaria-endemic areas. In these settings, it causes early childhood deaths owing to infectious and hematologic complications. To mitigate these risks, supportive care for children with SCA typically entails measures to directly prevent infections and anemia and indirectly prevent painful crises, delayed development, hospitalizations, and death. In Kenya, SCA is common in children in malarious regions bordering Lake Victoria, but the burden of SCA complications is poorly understood in these populations. We measured infectious, hematologic, and functional complications in children with SCA who were enrolling in a clinical trial of malaria chemoprevention regimens in Homa Bay, Kenya. Participants were enrolled if they were 1y-10y, had hemoglobin SS disease confirmed by electrophoresis, and lacked ongoing chronic medical conditions that would interact with trial procedures. From January 2018, we enrolled over 150 participants meeting these criteria and conducted comprehensive medical and social histories, reviews of systems, physical exams, electrocardiograms, splenic ultrasounds, blood counts and electrolyte measures, and testing for malaria parasites. Our study confirms the Kenyan children with SCA have high burdens of multi-system morbidity. This group of children with poor health outcomes in tropical settings requires focused exploratory, comparative, and implementation studies in order to prolong their survival and enhance their healthspan.

1913

EMERGENCY VISITS TO A HOSPITAL SYSTEM IN SOUTHERN PUERTO RICO IN THE AFTERMATH OF HURRICANE MARIA

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During September 2017, Hurricane Maria, a category 4 storm, made landfall in Puerto Rico. This event created an environment with countless health hazards, increasing the risk of injuries, wound infections, communicable diseases, and complications of chronic illnesses and mental illnesses. In the aftermath of this natural disaster, the Sentinel Enhanced Dengue Surveillance System (SEDSS) established a syndromic surveillance study on October 2017 at the emergency department of a tertiary care teaching hospital and associated urgent care clinic located in the southern area of the island. A modified CDC's Natural Disaster Morbidity Surveillance Form was used to collect the occurrence of injuries and illnesses and detect outbreaks during a disaster. We offered enrollment to all patients who presented to the emergency department or urgent care clinic during the staff coverage period from October 16, 2017 to March 28, 2018. After obtaining informed consent, surveillance personnel interviewed participants briefly to categorize their reason for visit and later obtain outcomes through the electronic medical record. A total of 5,116 participants were enrolled; 1,405 (27.5%) were between the ages of 1-18 and more than half of them (51.0%) were male. One hundred (2.0%) respondents reported the visit as a result of disaster work or response activities. The most common reason for visit was acute illness (78.8%) and 570 (11.1%) of study participants were hospitalized. Among the participants, 13 had a Leptospirosis rapid test performed, of which 4 (30.8%) were positive cases. In addition, 1,487 Influenza rapid test were performed and 392 (26.4%) resulted positive. Active post-event morbidity surveillance provides a quickly scalable model to provide decision makers with actionable information following a disaster that may reduce mortality and morbidity. Furthermore, the information gathered through this surveillance can be used for developing disaster ready public health systems and educational campaigns to better address the immediate needs of those affected by future natural disasters, such as hurricanes.

1914

SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS CONTROL IN SCHOOL-AGED CHILDREN IN NIGER

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Worldwide, about 1.5 billion people are infected with soil-transmitted helminths (STH) and 206 million people require treatment for schistosomiasis (SCH). In Niger, 69% of the population was found to be infected with STH or SCH in 1995, and in 2004, the National Program for the Control of SCH and STH was established. In 2006, Niger drew up a national plan for the integrated control of neglected tropical diseases (NTDs), one of the objectives of which was to reduce the morbidity caused by SCH and STH. Deworming for SCH and STH began in 2004 and was integrated into national scale mass drug administration (MDA) for NTDs in 2007. Districts have received between four and ten rounds of treatment with praziquantel and albendazole. In 2016, a cross-sectional sentinel site survey was conducted to determine SCH and STH prevalence in school-aged children after years of mass treatment. Sixty children aged from 6 to 8 years from each of 17 sentinel sites (schools) were selected and urine and stool samples were examined through urinary filtration and the Kato Katz techniques. Among the 1,020 children examined, the overall average prevalence of SCH was 33.5% (95% confidence interval [CI]: 19.2%-

47.9%), ranging from 1.7% in Filingue to 100% in Tera among districts. This was a 52.3% reduction from the 2011 surveys which showed an average prevalence of 70.2% (95% CI: 63.6%-76.8%), ranging from 44% to 96% among districts. Three types of intestinal worms were reported: Ascaris lumbricoides, Trichuris trichiura and Ancylostoma duodenale. The overall average STH prevalence was 6.8% (95% CI: 4.0%-9.5%), ranging from 0% to 20% among districts. After several rounds of treatment, there was a marked decrease in the prevalence of urogenital schistosomiasis, except for Yelwani, Boudoum, Doungouram, and Bangoukoukou sites. STH prevalence is generally low with 8 of 17 sites with a prevalence <5%. Multiple rounds of MDA and sensitization on hygiene and sanitation all appear to contribute to reduced SCH and STH in these sites.

1915

INAPPROPRIATE ANTIBIOTIC PROVISION FOR DIARRHEA AMONG HEALTH PROVIDERS IN RURAL MALI

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Clinically inappropriate provision of antibiotics is of growing global concern given the recent rise in antibiotic resistance. However, the dynamics of antibiotic provision for pediatric illnesses in West Africa are not well understood. From December 2016 to February 2017, mothers of 19,556 children under age five were interviewed in a baseline household survey for a cluster-randomized trial across seven health catchment areas in rural Mali. Respondents provided information about whether children experienced diarrhea with or without bloody stools in the two weeks preceding the survey, care sought, and treatment received. We used mixed-effects regression models to assess predictors of receipt of antibiotics for children reporting recent diarrheal illness without bloody stools by patient characteristics (distance to facility, child age and sex, household wealth, and maternal education) and provider characteristics (sector, facility type, catchment area). Among 2,136 children with diarrheal illness (no bloody stools), 45.9% sought any care outside the home, 26.7% of whom received inappropriate antibiotic treatment versus oral rehydration therapy (ORT) and zinc. Provision of antibiotics for diarrheal illness is concentrated among trained providers: 45.2% of children attending a hospital, primary health facility, or community health worker received antibiotics versus 15.3% who attended untrained providers ($p<0.001$). Inappropriate antibiotic provision rates were higher than provision rates of ORT and zinc across sectors. There was no difference in cost of treatment among children who received incorrect antibiotics versus those who received ORT and zinc. We find no effect of distance to facility or socio-demographic characteristics on likelihood of antibiotic receipt for diarrheal illness. Given that trained providers are significantly more likely to prescribe antibiotics for diarrheal illness without bloody stools than untrained providers, and to provide antibiotics more frequently than ORS and zinc, our findings suggest better antibiotic stewardship and remedial training for trained providers are needed.

1916

A POST MASS DRUG ADMINISTRATION COVERAGE SURVEY FOR TRACHOMA TREATMENT IN GUINEA

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Mass drug administration (MDA) for trachoma started in Guinea in 2013. Treatment coverage has always been reported as reaching 80% or more except in 2014 when coverage was below 80% due to the outbreak of Ebola virus disease. With the use of the new population census in 2016, underestimations of population were reported in the majority of health districts (HDs) treated. The national program carried out a post-MDA coverage survey to confirm the high reported coverage rates. A cross-sectional survey using a cluster sample design was conducted in July 2017 in randomly selected areas in 4 health districts (HD) in Guinea to validate the administrative coverage obtained during the trachoma MDA undertaken in May 2017. In each district, the sample size was calculated using the Coverage Survey Builder (CSB) tool. Survey households were selected using the segmentation method recommended by WHO. All individuals in selected households were presented with a drug sample and asked about taking the drug during the campaign. Data were collected electronically using an Android device via Open Data Kit (ODK) application. Survey coverage was then compared with administrative coverage. A total of 1,249 households were surveyed, of which, 1,243 (99.5% agreed to participate in the survey). A total of 7,901 (3,776 males and 4,125 females) individuals were present, of which 5,121 adults and 2,780 children under 10 years of age. The survey coverage varied from 78.6% (95%CI: 76.5 - 80.4%) to 96.7% (95%CI: 95.9 - 97.4%) where one of the four surveyed HD reported insufficient coverage below 80%. The survey coverage results revealed a significant difference between reported coverage and survey coverage: From 96.3% to 78.6% in Boffa, from 94.8% to 88.3% in Forécariah, from 90.4% to 96.7% in Kissidougou and from 88.7% to 82.1% in Kouroussa. Even though reported coverages were not validated by the survey coverage, all survey coverages were greater than 80% in all HDs except in Boffa. The reasons for this low coverage below 80% in Boffa should be investigated to ensure sufficient coverage will be achieved in future MDAs.

1917

TORCH SEROPREVALENCE IN PREGNANT WOMEN FROM PERI-URBAN IQUITOS IN THE AMAZON JUNGLE OF PERU

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Some infections acquired during pregnancy can be transmitted to the infant causing abnormal development. These infections include toxoplasmosis, rubella, cytomegalovirus (CMV), herpes simplex (HSV) and others (TORCH). As part of clinical care in Peru, it is mandatory to test for syphilis and HIV during pregnancy; however, there is no routine testing for the TORCH infections listed above. As a part of a prospective, international, multisite cohort study of the incidence and outcomes on Zika virus infection during pregnancy (ZIP study), 350 pregnant women have been enrolled in their first or early second trimester from peri-urban communities in Iquitos, Peru. Blood samples have been collected at enrollment to test for syphilis, HIV and TORCH. We determined the seroprevalence of toxoplasmosis, CMV, rubella, and HSV in 244 women using a commercial ELISA for IgM. The mean age of the women enrolled was 26 ± 6.6 years, the gestational age at sample collection was 13.1 ±

3.2 weeks, and 30% were primigravida. One (0.4%) sample was positive for syphilis and 3 (1.2%) for HIV. Sixteen (6.6%) samples were positive for *Toxoplasma gondii*, 54 (22.1%) for CMV, 12 (4.9%) for rubella, and 75 (30.7%) for HSV 1 or 2. In addition, 9 (3.7%) women had equivocal results for toxoplasmosis, 14 (5.7%) for CMV, 5 (2%) for rubella, and 20 (8.2%) for HSV 1-2. All equivocal samples are being retested. Almost half of the women (107, 43.9%) had negative IgM for all TORCH infections, and only one (0.4%) was positive of all. The most frequent profile was a IgM positive only for HSV (37, 12.5%) and only for CMV (20, 8.2%). Although these results need to be confirmed with additional testing (e.i. IgG avidity test for toxoplasma), the high IgM seroprevalence of TORCH infections found in this study provides evidence to support local routine testing during pregnancy by the Ministry of Health. In addition, in order to maintain the elimination of congenital rubella in the region, it is important to strive for high immunization rates with MMR (measles, mumps and rubella).

1918

IDENTIFICATION OF BARRIERS TO HYDROCELE SURGERY: CASE STUDY OF THE BIREME AND KARA-HAY HEALTH DISTRICTS IN CAMEROON

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In 2017, the USAID-funded Morbidity Management and Disability Prevention (MMDP) Project supported the Cameroon Ministry of Health to conduct a pilot project for the management of lymphatic filariasis-related morbidity cases in five health districts of Cameroon. As a part of this effort, a study to identify patient-reported barriers to hydrocele surgery was conducted in two health districts (Bireme and Kara-Hay). From 405 suspected cases of hydroceles identified in Bireme and Kara-Hay districts, a convenience sample of 86 patients was selected from the four health areas in each health district that reported the highest number of suspected cases, for a total of eight health areas selected. Data was collected using a standard questionnaire asking about knowledge of health facilities providing hydrocele surgery, means of transportation to these facilities, and initial thoughts on the costs and other factors that could influence the decision to have surgery. Less than one third of the respondents (27 of 86, or 31%) said they were able to afford the cost of surgery. Of the remaining 59 respondents, 44 reported that they would be able to cover indirect surgery costs (e.g., transportation, food), and 15 reported being unable to support any direct or indirect costs. Fear of surgery and of adverse effects after surgery were also identified as potential obstacles. Specifically, 4% of respondents feared the possibility of erectile dysfunction following surgery, 1% feared not being able to wake up following general anesthesia, and 1% were afraid sterility following surgery. These results highlight cost as a major obstacle to hydrocele surgery and underline the importance of considering universal health coverage or subsidies for essential surgeries such as hydrocele surgery. Fears raised by patients, though cited less frequently than cost, will also be an important obstacle to address through the development of behavior change communication strategies.

1919

STRENGTHENING THE QUALITY OF TRACHOMATOUS TRICHIASIS SURGICAL SERVICES: USING AN INTEGRATED SUPPORTIVE SUPERVISION APPROACH

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Supportive supervision is critical to ensuring quality of trachomatous trichiasis (TT) surgery interventions, impacting both productivity and quality of surgery. The USAID-funded Morbidity Management and Disability Prevention (MMDP) Project has supported national trachoma programs to strengthen supportive supervision and train national TT surgeon supervisors in project-supported areas. A key component of the approach was the development of a supportive supervision checklist for both general and technical aspects of TT management service provision. The general checklist can be completed by non-technical staff trained in preferred practices who assess social mobilization, patient counseling, infection control and health care waste management. The technical component must be assessed by surgeons trained specifically as supervisors who assess pre-, peri- and post-operative procedures. The checklists have been implemented in three countries in areas supported by the project, each with very different disease burdens. As few as three or as many as 128 individual surgeons or surgical teams operate in project-supported areas and, therefore, require supportive supervision. To date, between 30% and 100% of these surgeons or teams have received technical supportive supervision visits, with the proportion naturally highest in areas with fewer surgeons or teams operating. Supportive supervision enables close monitoring of TT surgeon performance. The approach has identified high performing surgeons who have been trained as supervisors, and provides the opportunity to triangulate findings with 3-6 months post-operative outcome data for operating surgeons. In 2018, the curriculum for a refresher training for operating surgeons in Cameroon was tailored to focus on findings reported through supportive supervision and 3-6 month outcome data. Given the utility and feasibility of integrating supportive supervision into the project-supported areas, this experience supports introduction of these activities as a routine component of TT management services for program strengthening.

1920

MALARIA AND VIRAL INFECTIONS OF THE UPPER RESPIRATORY TRACT IN CHILDREN PRESENTING WITH RESPIRATORY DISTRESS AT TWO DISTRICT HOSPITALS IN GHANA: EXPLORING HOW CLINICAL PRESENTATIONS RELATE CONFIRMED DIAGNOSES

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Overlap in the clinical features of malaria and viral respiratory infections in children present diagnostic and therapeutic challenges in resource-limited settings. We analyzed data on the clinical and laboratory parameters of children presenting with acute respiratory distress at two district hospitals in Ghana. Between Jan 20, 2014, and Dec 5, 2015, we enrolled children one month to five years of age presenting with acute respiratory distress. *Plasmodium falciparum* malaria was tested using rapid diagnostic test (mRDT). Blood smears for microscopy were obtained. Nasopharyngeal

swabs obtained at time of presentation were tested by the BioFire FilmArray PCR assay for 17 viral pathogens. We determine the accuracy of mRDT as compared to blood smears and performed one-way ANOVA to explore differences in clinical parameters. Two thousand children (44.3% female) with a mean age of 17.5months (IQR 9.5-29.0) were enrolled. Mean weight, respiratory rate, pulse, temperature, oxygen saturation and hemoglobin levels were 9.6 (+3.2) kg, 57 (11.2) breaths/min, 152.1 (23.1) beats/min, 37.6C (1.1), 96.9 (+5.0) % and 8.0 gm/dl respectively. Compared with microscopy, mRDT had a sensitivity and specificity of 94% and 60%, respectively. Viral yield was 59%. The main isolated organisms were rhinovirus/enterovirus (36%), respiratory syncytial virus (11%) and parainfluenza (7%). Viral infection among children with or without malaria was 53% and 67% respectively (P<0.01). Significant (P<0.01 in all) differences were observed between children with viral infections only , malaria only , and those with co-infections, respectively as follows; means of weight (8.4kg, 10.5kg, 9.8kg), respiratory rate (59/min, 56/min, 57/min), temperature (37.3C, 37.8C, 37.7C), oxygen saturation (96%, 98%, 97%), hemoglobin levels (8.9gm/dl, 7.2gm/dl, 7.4gm/dl) and total WBC (13.8, 11.5, 13.2). Systematic exploration of the parameters of clinical presentation matched against confirmed diagnosis may improve the accuracy of diagnosis and targeting of medications in resource-limited settings.

1921

HIGH PREVALENCE OF ANXIETY AND DEPRESSION AMONG PATIENTS WITH DENGUE AND NON-DENGUE ACUTE FEBRILE ILLNESS IN SRI LANKA

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Acute fever, notably dengue is a common cause of hospital admission in Sri Lanka and is regarded with fear by the general public. We assessed the prevalence and features associated with anxiety and depression among patients hospitalized with acute fever using the Hospital Anxiety and Depression Scale (HADS) validated for Sri Lanka. We enrolled consecutive patients ≥12 years admitted with acute fever to a tertiary care hospital in Sri Lanka, Jul- Oct 2017. We recorded epidemiological, clinical data and the HADS score. Patients' satisfaction with hospital care and knowledge about illness was recorded using a 5-item Likert scale with 0-3 score on each item. A HADS score of ≥8 was indicative of anxiety or depression. Chi square/ Fisher exact tests and t-test/ Kruskall-Wallis tests were used to identify associations between sociodemographic and clinical variables and anxiety or depression. Of 193 patients, 86 (44.6%) were male; median age was 33 years (IQR 25- 53). A total of 41.4% had anxiety, 42.5% had depression and 26.9% had both. Mean anxiety and depression scores were 7 (IQR 4- 9) and 7 (IQR 4-10), respectively. Patients with anxiety were more likely to have lower income (46.2% vs 30.9, p=0.03), know their diagnosis (95%vs 84.5%, p=0.02), anorexia (81% vs 67%, p=0.03), diarrhea (36% vs 21%, p=0.02), and arthralgia/myalgia (81% vs 68%, p=0.04). Patients with depression were more likely to have headache (83% vs 60.4%, p<0.001), anorexia (85.3% vs 63.9%, p<0.001), vomiting (62.2% vs 41.4%, p<0.01, and diarrhea (39% vs 18.9% p<0.01). Patient knowledge about illness and hospital satisfaction score were not associated with anxiety or depression. Of 121 (62.7% of total) patients with dengue, prevalence of anxiety and depression was similar to no dengue group (43.8% vs 37.5%, p=0.39 and 40.5% vs 45.83%, p=0.46, respectively). Patients hospitalized for acute fever had high levels of anxiety and depression, with similar prevalence among dengue and other fever groups. Anxiety and depression were associated with symptoms and lower economic status. Further measures to improve psychological wellbeing of patients hospitalized with acute fever need to be explored.

1922

DELIVERING CAUSE OF DEATH INFORMATION AT HOUSEHOLD LEVEL IN THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) IN MOZAMBIQUE: IMPLICATIONS FOR MORTALITY SURVEILLANCE DESIGN AND ACTION

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CHAMPS was established in a rural area in Mozambique to ascertain causes of child death (CoD) in order to inform policy and public health action. Minimally Invasive Tissue Sampling (MITS) for histopathology and microbiological CoD assessment are ongoing at the Manhiça District Hospital. Families of deceased under-5 children from the catchment area are approached for consenting to MITS, and CoD results returned to the family. We describe the receptiveness, practicality and usefulness of the CoD results feedback to families for defining public health action. From 12/2016 to 02/2018, 98 of 120 in-hospital MITS-eligible deaths were approached for consent and MITS performed in 80 (82%). CoD were assigned to 46 cases and results returned to 32 families; social behavioral observations of results feedback sessions were conducted on 26 cases. The number of family members present in the sessions varied from 1 to 4, including mothers (in all visits), fathers (in 23% of visits), grandparents (in 54%), and uncles/aunts (46%). Occasionally neighbors and children also participated. The following language was used to explain various causes of death: cerebral malaria (with emphasis on fever effects on the brain); deficient intra-uterine growth; HIV/AIDS; infections in the mother affecting the fetus; loss of breath upon birth; problems in the lungs; limited intake of nutrients; lengthy delivery. The following reactions by family members were captured: the majority felt thankful, linked to sensation of closure, clearance of witchcraft suspicion, the learning experience. However, many blamed themselves (improper child feeding, vertical transmission and delayed treatment-seeking) or the health system (delayed initiative to perform C-section, use of instruments during labor). Results delivery frequently triggered immediate action on the side of families: initiation of antenatal care for new pregnancies, HIV treatment to both parents, accepting treatment of other family members (e.g. syphilis, hypertension), protecting pregnant women from heavy domestic chores. These results have implications for defining strategies to improve health services.

1923

FEBRILE PATIENT MANAGEMENT WORLDWIDE: ANALYSIS OF EXISTING GUIDELINES AND ALGORITHMS TO INFORM NOVEL DIAGNOSTIC AND ALGORITHMIC SOLUTIONS

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Fever is one of the most frequently encountered problems worldwide. Clinicians in peripheral clinics and district hospitals (Level 1 & 2 facilities, respectively) in low- and middle-income countries (LMICs) with limited diagnostic capabilities are challenged to manage febrile patients presenting without clear differentiating features. International and national guidelines have been published to improve management of these patients utilizing existing resources. Medecins Sans Frontieres (MSF) and

FIND have partnered to improve febrile patient management by exploring development of a diagnostic system (MAPDx) capable of testing for several pathogens and analytes simultaneously. To inform the use case for this system and to support the development of an MSF febrile illness algorithm, we conducted a landscape analysis of clinical algorithms and guidelines used to manage febrile patients in LMICs with specific attention to the recommended diagnostics. We performed a literature review of PubMed and Cochrane Library and searches of the WHO archives and national Ministry of Health websites. Only documents published in English were included and those intended for Level 1 & 2 facilities were prioritized. We analyzed 4 WHO and 18 national guidelines, 8 algorithms from literature or reference texts, and 7 electronic algorithm applications. WHO guidelines for Level 1 facilities have been widely adopted with minimal changes, while WHO hospital guidelines have not been uniformly adopted and large variation remains across countries. Successful implementation of guidelines contributed to a significant reduction in patient mortality in some settings, while overarching challenges for implementation remain (e.g. poor training, un-sustained investments). Given the envisioned diagnostic system in the ecosystem of existing guideline recommendations, the implementation of such an aspirational diagnostic might best fit within the context of Level 2 facilities. However, substantial additional work is required to enable simple integration and development of improved febrile illness algorithms in multiple settings including at MSF sites.

1924

HEMECHIP: A PORTABLE, AFFORDABLE POINT-OF-CARE DIAGNOSTIC TECHNOLOGY FOR DETECTING HEMOGLOBIN DISORDERS

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Genetically inherited hemoglobin (Hb) disorders are of global public health concern. There are more than 700 hemoglobin disorders: HbS, HbC, and HbE are the most widespread Hb variants. HbS and HbC are associated with sickle cell disease. Sickle Cell Disease (SCD) affects between 300,000 to 400,000 newborns every year, and more than 75% of these infants are born in sub-Saharan Africa and India. Of these, WHO estimates that between 50-80% die before reaching age 5. Both the World Health Assembly and the United Nations recognize SCD as a public health priority and have called on countries to tackle the disease. HbE, another structural hemoglobin variant, occurs widely throughout the eastern half of the Indian subcontinent, Bangladesh, Myanmar, and east and southeast Asia. The current clinical standards for diagnostic testing, such as High Performance Liquid Chromatography (HPLC), are associated with high cost per test as well as high cost of infrastructure. Moreover, these clinical standards require centralized laboratories, resulting in delayed turnaround times (several weeks in some regions) and logistical complexities. The geographical regions prevalent with these Hb disorders include some of the lowest-resourced countries in the world, and therefore, early diagnosis of hemoglobin disorders remains a challenge. To address this need, we developed HemeChip, a mass-producible, low-cost version of electrophoresis on a microchip, able to detect and quantify these Hb variants. HemeChip is a robust, highly reliable point-of-care technology that can separate, detect and quantify Hb variants in blood. This rapid (<10 minutes) and easy-to-use test can be performed by minimally trained personnel using only a finger-prick volume of blood. HemeChip can categorize a blood sample as Normal (HbAA), Sickle Cell Trait (HbAS), Sickle Cell Anemia (HbSS), Hemoglobin SC disease (HbSC), and HbE variants. In preliminary tests, with a sample size of 122 (42 HbAA, 36 HbAS, 34 HbSS, and 10 HbSC), HemeChip yielded a high accuracy (> 96%) compared to standard laboratory tests.

1925

QUANTIFYING THE BURDEN OF DIARRHEA AMONG CHILDREN AND ADULTS: INCIDENCE, HOSPITALIZATIONS, AND MORTALITY IN THE GLOBAL BURDEN OF DISEASE STUDY 2017

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Despite being entirely preventable, diarrhea is the second leading cause of infectious disease globally and the eighth leading cause overall. The Global Burden of Disease study 2017 (GBD 2017) is a systematic, scientific effort to quantify health for all ages, both sexes, every geography, and from 1980-2017. In this presentation we will share methodology and updated results for diarrhea in GBD 2017, focusing on deaths, incidence, and hospitalizations. Deaths, incidence, and hospitalizations were modeled separately using vital registration, verbal autopsy, survey, health records, and scientific literature data and these regression models were strengthened by covariates and by hierarchical space-time trends. Diarrheal diseases were the eighth leading cause of death among all ages in 2017 and the second leading cause of mortality due to infectious diseases (1,540,000 deaths, 95% Uncertainty Interval [UI] 1,140,000-2,250,000). Most of these deaths occurred in children under 5 years (480,000, 95% UI 420,000-530,000) and adults over 70 years (637,000 deaths, 95% UI 370,000-980,000). Diarrhea deaths have decreased by 50% since 1990 among all ages and 75% among children under-5. Despite this dramatic decline globally, deaths have increased by 600% among adults over 70 years old in high-income countries. We estimated 8 billion episodes of diarrhea in 2017 among all ages (95% UI 7.7-9.0 billion) and 13,710,000 hospitalizations due to diarrhea (95% UI 3,470,000 - 52,530,000). The Global Burden of Disease study provides a timely, comprehensive, and detailed picture of the health loss associated with diarrhea and provides public health experts, policy makers, and health officials with evidence for decision making and a roadmap for reducing the burden of diarrhea.

1926

PRELIMINARY PERFORMANCE OF THE BIOFIRE® FILMARRAY® TRAVEL-RELATED FEBRILE ILLNESS PANEL AT THE MAHOSOT HOSPITAL IN VIENTIANE, LAOS

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Fever is a common symptom in returned travelers and an important marker of potentially serious illness. While many of the symptoms of febrile patients are non-specific and over-lapping, it is important to distinguish between viral syndromes, bacterial or parasitic infections, or non-infectious causes of fever to timely determine correct treatment. BioFire Diagnostics, LLC has developed a polymerase chain reaction (PCR), high-resolution melting analysis instrument called the FilmArray. The BioFire Travel-Related Febrile Illness (TRFI) Panel is a reagent pouch in development for use with the FilmArray instrument that contains freeze-dried reagents to perform nucleic acid purification and multiplex PCR for the identification of nine viral, seven bacterial, and eight parasitic pathogens that cause systemic infections in febrile patients from a single specimen. Three hundred and twenty whole blood samples, prospectively collected from patients admitted to Mahosot Hospital with acute, undifferentiated fever were tested using the BioFire TRFI Panel. When possible, BioFire TRFI Panel testing results were compared to those from the hospital Microbiology Laboratory molecular diagnostic testing.

BioFire TRFI Panel testing resulted in 110 (34%) positive detections and included Dengue virus (97), *Leptospira* spp. (2), *Orientia tsutsugamushi* (6), *Plasmodium vivax* (1), *Rickettsia* spp. (2), and *Salmonella* spp. (2). For samples in which BioFire TRFI Panel and hospital laboratory results could be compared, positive agreement for the detection of Dengue virus was 98.7% (78/79) with a negative agreement of 69.0% (20/29). Negative agreement comparisons were available for *Leptospira* spp. (21/21), *O. tsutsugamushi* (4/6), *Rickettsia* spp. (20/20), *Salmonella* spp. (7/7), Chikungunya virus (107/107), and Zika virus (86/86). These results indicate that the BioFire TRFI Panel could provide a fast, multi-pathogen testing platform for aiding in the etiological diagnosis of acute travel-related febrile illness. This abstract contains information regarding assays that have not been reviewed by regulatory agencies for *in vitro* diagnostic use.

1927

SUSCEPTIBILITY OF *CANDIDA* ISOLATES FROM FEMALES PRESENTING WITH CANDIDAL VAGINITIS AT THREE GHANAIAN HOSPITALS TO FORMULATIONS OF *CRYPTOLEPIS SANGUINOLENTA* ROOT

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Following claims that *Cryptolepis sanguinolenta* (Linn.) Schltr has antimicrobial activity against some infectious microbes, an *in vitro* anticandidal activities of two formulations of *C. sanguinolenta*, *C-Root-A* and *C-Root-B* were evaluated against nine clinical isolates of *Candida* species and a standard strain of *Candida albicans,* using the agar well diffusion method. The results obtained from the present investigation revealed that both *C-Root-A* and *C-Root-B* formulations exhibited antifungal activity against all test *Candida* species with the highest activity exhibited by the *C-Root-A* against *Candida spp (*K'bu-1) at the concentration of 200 mg/ml (zone of inhibition 28.9 ± 0.3 mm). Antifungal activities were recorded for all the test concentrations of *C-Root-A* with the diameters of zones of inhibitions decreasing with decreasing concentrations of the product (200 mg/ml to 12.5 mg/ml). Period of incubation (24 – 72 h) had no significant effect on the diameter of zones of inhibition ($p \leq 0.05$), suggesting the products may have fungicidal effect against the test organisms. The study confirmed that of *C. sanguinolenta* has anticandidal activity and could be used to formulate products for the treatment of candidal infections.

1928

IMPACT OF MALARIA, PNEUMONIA AND DIARRHEAL ILLNESS ON CHILD GROWTH IN PAPUA NEW GUINEA

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The first 1000 days of a child's life is a critical period for child development, where inadequate nutrition and infectious disease can not only increase risk of serious illness and death but have long term adverse health, developmental and socioeconomic outcomes. In many low to middle income countries including Papua New Guinea (PNG), infectious diseases and undernutrition are highly prevalent and are major contributors to high rates of early childhood illness and death. Undernutrition and infectious diseases are believed to have a bi-directional causal relationship but the impact of infectious diseases on growth is complex and poorly understood. Using passive case surveillance data from a large malaria prevention trial in PNG, we investigated the impact of the three commonly occurring infectious diseases in PNG (malaria, pneumonia and diarrheal diseases) on child growth. Children were enrolled at 3 months of age and actively monitored every 3 months for a total of 2 years (720 days) with weights measured at every three-monthly visit and heights were only taken at 15 and 27 months of age. Analysis included 1605 children with a total of 8950 illness episodes with 1885 malaria, 2611 pneumonia and 1655 diarrheal disease episodes recorded over the study period. A total of 11352 weights and 2060 height measurements were taken with corresponding WHO standardized z-scores. A total of 28 lags of 30 days each were defined. Preliminary analysis fitting a basic hierarchical distributed lag model with linear basis for the lags suggested an impact of malaria on weight the longer the lag was. However, this model will be extended to allow for non-linear associations between lag times for the different number of episodes of malaria and weight. Analysis for the effect of pneumonia and diarrheal illness will also performed and the final results will be presented at the meeting.

1929

RETURNING TRAVELER WITH AN ULCERATING BUG BITE: LEISHMANIASIS MISDIAGNOSED AS SQUAMOUS CELL CARCINOMA

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We present a case of cutaneous leishmaniasis that was misdiagnosed as a squamous cell carcinoma. We also review the literature on the adverse outcomes associated with this misdiagnosis and discuss the clinical challenge of skin disease in the returning traveler. A 48-year-old female who had recently traveled to Panama presented to a clinic with an ulcerated nodule on the left dorsal forearm. Her initial work-up and treatment included negative bacterial cultures and a course of oral valacyclovir without clinical response. She eventually underwent a skin biopsy and was diagnosed with a poorly differentiated squamous cell carcinoma. She was treated with mohs micrographic surgery, but the surgeon felt the tumor was still present at the margin despite extensive surgery. She was then referred to the University of Minnesota for additional mohs micrographic surgery. At the time of her evaluation the patient had developed several new subcutaneous nodules in the left antecubital fossa, and the case was re-evaluated by dermatopathology. Review of the biopsies demonstrated pseudoepitheliomatous hyperplasia, which on histopathology can mimic squamous cell carcinoma. However, within the inflammation under the reactive skin there were multiple scattered intracellular amastigotes. The patient's ultimate diagnosis, which was confirmed by the Center for Disease Control, was *leishmaniasis panamensis*. After 4 weeks of oral miltefosine her symptoms completely resolved. There are at least five case reports of leishmaniasis misdiagnosed as squamous cell carcinoma. Many of the patients underwent large unnecessary surgical procedures prior to receiving the correct diagnosis. Skin disease is common in the returning traveler with 19.5% of travelers reporting skin disease in the GeoSentinel Surveillance data. Despite the prevalence of dermatologic diseases in returning travelers these medical errors still occur. This is likely due to limited familiarity with cutaneous tropical diseases, non-specific clinical presentations, the paucity of available disease specific diagnostic tests, and the lack of clinicians trained in tropical skin disease.

1930

THE ETIOLOGIES AND OUTCOMES OF SEPSIS IN PATIENTS WITH ACUTE FEBRILE ILLNESS IN INDONESIA: RECOMMENDATIONS FOR FUTURE DIRECTIONS

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Case fatality rate of acute febrile illness (AFI) is often high when sepsis occurs. In low resource settings it is often difficult to establish a diagnosis of sepsis from the clinical presentations and physical examination, since sepsis share similar clinical features with other disorders. Ideally, a guideline for sepsis treatment should be adjusted based on epidemiology data built from national surveillance studies. To identify the etiology of AFI and the clinical outcomes, an observational study, Acute Febrile Illness Requiring Hospitalization (AFIRE), was conducted at 8 top-referral hospitals across Indonesia from 2013 to 2016. Hospitalized subjects were enrolled and visited 24 hours, 14-28 days, and 3 months after enrollment. Blood, other biological specimens (if available) and clinical data were collected during these visits. Later the specimens were screened for more than 50 different pathogens using molecular and serological methods. 59 from 1486 subjects were enrolled with clinical diagnosis of sepsis (38 subjects) or had sepsis (21 subjects) during hospitalization. The mean period of fever onset in subjects with sepsis was 5.3 days (SD 3.2 days) and the mean period of hospitalization was 7.8 days (SD 7.9 days). Twenty-one subjects (35.5%) discharged from the hospital and thirty-eight (64.4%) died during the hospitalization, among which 27 (71.0%) had underlying diseases such as HIV, TB, malnutrition, anemia, congenital malformation, cardiovascular and metabolic disease. Thirty-five (59.3%) had confirmed etiologies of sepsis, 27 were bacterial pathogens (8 *R. typhi,* 3 *Salmonella spp.,* 3 *S. aureus*, 2 *Leptospira spp.,* 2 *Escherichia coli,* 2 *M. tuberculosis,* 2 *S. pneumoniae,* 1 *E. aerogenes,* 1 *E. faecalis,* 1 *M. leprae,* 1 *P. aeruginosa, and* 1 *S. faecalis*), and 7 were viruses (3 influenza, 2 dengue, 1 HHV-6, and 1 acute HIV infection), and 1 parasite (*A. lumbricoides*). The findings of this study will aid the national health policy program to develop guidelines for diagnosis and treatment of sepsis caused by AFI and highlight the importance of enhancing diagnostic capacity on infectious diseases in Indonesia.

1931

EFFECT OF COMORBIDITIES ON LINEAR GROWTH: A COHORT STUDY OF CHILDREN AT RISK FOR ENVIRONMENTAL ENTERIC DYSFUNCTION

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Pakistan has high prevalence of stunting (44%) in children <5y of age. Stunting (height for age Z score (HAZ)<-2)/ linear growth faltering is associated with irreversible physical and neurocognitive damage these children. Lower birth weight is known to be a major determinant of poor linear growth and increased frequency of infectious comorbidities in subsequent years. Insulin like growth factor (IGF) is protein that plays critical role in the growth hormone/IGF-1 axis. We aimed to study the relationship of birth weight with serum Insulin like growth factor (IGF), comorbidities and linear growth (as measured by HAZ). 380 newborns were prospectively enrolled in a community-based cohort and followed longitudinally with monthly anthropometric measurements and weekly surveillance for diarrheal days, fever and cough or runny nose. IGF was assessed from serum samples at 6 and 9m. Data was analyzed by SPSS 21. We correlated birth weight with HAZ at 18m and frequency of diarrhea, cough & runny nose and fever. Lower birth weight significantly correlated with higher frequency of fever at 6m and 9m(r=-0.135;p=0.009,r=-0.124;p=0.027), diarrhea at 9m (r=-0.154;p=0.006) and HAZ at 18m(r=0.328;p<0.001). Increasing frequency of fever showed a negative correlation with IGF at 6m only (r=-0.111;p=0.051). However, significant positive correlation was observed for IGF at 6 and 9m with HAZ at 18m (r=0.237;p<0.001, r=0.314;p<0.001). An interesting finding of note was the correlation among comorbidities at both 6m [diarrhea with fever (r=0.262;p<0.001), diarrhea with cough & runny nose (0.251;p<0.001) and fever with cough & runny nose (r=0.478;p<0.001)] and at 9m [diarrhea with fever (r=0.323;p<0.001), diarrhea with cough & runny nose (0.347;p<0.001) and fever with cough & runny nose (r=0.542;p<0.001)]. Yet, non-significant association was observed between comorbidities and HAZ at 18m. Linear growth faltering/ stunting is a multifactorial process driven by birth weight which may be influenced by repeated infections with or without the involvement of IGF.

1932

PERSISTENTLY LOW VIRAL LOAD FOR HBV IN PATIENTS WITH HEPATOCARCINOMA IN PERU 2013 - 2015

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Before the implementation of the immunization program against Hepatitis B, the Amazon region and some inter-Andean valleys in Peru used to be considered as HBV hyperendemic areas. Nonetheless, some people infected before the application of the HBV vaccine remain as chronic carriers. The internationally standardized recommendation is to start treatment if the viral load of HBV is greater than 2,000 IU / ml. In order to determine viral load levels in patients with a diagnosis of hepatocarcinoma, 35 patients with histopathological diagnosis were evaluated, from the National Institute of Neoplastic Diseases (Lima-Peru). Blood samples were obtained to determine the serological markers for HBV using the ELISA technique and the HBV viral load (qRT-PCR). Demographic data such as sex, age, origin and history of illness were collected in a clinical record. The average age was 30.4 years, (CI 18 -72 years). 65.7% were male and 20% (7/35) were from hyperendemic areas of hepatitis B (Cusco, Ayacucho and Apurímac). 96.55% of the 35 participants were HBsAg (+), 100% anti HBc total (+), 100% HBc-IgM (-) and 100% HBeAg (-). The viral load was undetectable in 7/35 people, <20 IU / mL in 13/35; between 50- 1163 IU / mL in 12/35 and only in 1/35 (3%) had a viral load for HBV> 2,000 IU / mL. In conclusion, based on the international parameters (> 2,000UI / mL), only 1 of 35 (3%) patients with hepatocarcinoma would require treatment according to established norms, which suggests that the viral load parameter should be re-evaluated as a criterion of treatment for patients with chronic hepatitis B in the studied population.

1933

UTILITY OF Q PCR ASSAY AS A CLINICALLY RELEVANT DIAGNOSTIC TEST

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Quantitative Polymerase Chain Reaction (qPCR) has been tested in archived samples successfully as an early diagnostic test for leptospirosis. We carried out a prospective hospital-based study in Teaching hospital Anuradhapura, the third largest hospital in Sri Lanka to provide routine qPCR diagnosis for leptospirosis as a clinically relevant diagnostic test. Febrile patients were consecutively recruited on the first encounter and a previously validated qPCR assay targeting 16s ribosomal gene of pathogenic *Leptospira spp* was done using CFX96 real-time PCR detection system (Bio-Rad). Those samples with a Ct value<40 and a melt curve in duplicate wells were considered as confirmed cases. First 207 samples included all fever patients without a diagnosis and both serum and whole blood (WB) were tested for comparison. The rest were possible leptospirosis patients. Altogether, 362 undifferentiated febrile patients; 30(8.3%) from OPD and 332(91.7%) from wards were recruited. Of them, 1 (3.3%) and 79 (23.8%) were confirmed with qPCR from OPD and wards respectively. The Ct values ranges from 24 to 40 in positive cases, with a mean of 35. Among 75.7% of the positive patients, sample collection was done within the first five days of illness. For all urgent samples, results were issued within 24 hours of request. In the first 207 samples, only 7(3.4%) were positive in WB. Only four of those were positive for serum. The Ct value for the serum and whole blood ranged from 29-37 and 25-39 respectively. Whole blood samples consistently produced early signals with an average Ct difference of 4 compared to serum. The three samples, which were only positive with WB, had Ct values between 38 and 39. An independent spiking experiment with serum and WB confirmed higher sensitivity of WB in which the standard curves showed an average Ct different of 2 in lower concentrations. We demonstrated that qPCR could be used as a clinically relevant diagnostic tool in early diagnosis of leptospirosis, even in remote settings with minimal facilities.

1934

SCALING-UP ACCESS TO OXYGEN IN 3 STATES IN NIGERIA: A PROGRAM EVALUATION

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Hypoxemia, or lack of oxygen in the blood, is a fatal condition commonly found in pediatric patients with pneumonia. The WHO recommends pulse oximetry for detecting hypoxemia and oxygen for treatment. In Nigeria, access to these medical interventions is limited. The Clinton Health Access Initiative (CHAI) supported the state governments of Kano, Kaduna, and Niger to install oxygen systems in pediatric wards of 30 facilities and trained healthcare providers to use the system and biomedical engineers on maintenance. We evaluated whether the program increased the use of pulse oximetry among pediatric patients presenting to the study facilities with respiratory illness and the use of oxygen therapy for treatment of hypoxemia. The study design was a pre- vs. post-intervention comparison. We conducted monthly reviews of medical records for all pediatric patients under age 5 and with a respiratory illness diagnosis. Data was collected from Jan 2017 through Aug 2018 with the pre-intervention period defined as Jan-Aug 2017 and the post-intervention period as Jan-Aug 2018. We excluded Sep-Dec 2017 since the interventions occurred during this period. The primary outcomes of interest of the study were (1) whether patients diagnosed with a respiratory illness had a documented arterial blood oxygen saturation measurement, SpO2, in their medical records, and (2) whether patients with clinical signs of hypoxemia were prescribed oxygen therapy. We reviewed 3,354 medical records at baseline. Baseline results show that prior to the intervention, 6.2% (95% CI 2.4%, 14.6%) of all under-5 respiratory illness cases had a SpO2 measurement. Among the records reviewed, 580 (17.29%) had documented signs of clinical hypoxemia, and at baseline, 16.5% (95% CI 7.3%, 33%) of hypoxemic patients were prescribed oxygen therapy. Data collection is ongoing for the post-intervention period and will be available by October 2018.

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FLUBENDAZOLE: ROBUST PRECLINICAL DATA SUPPORT DECISION TO DISCONTINUE DEVELOPMENT IN FILARIAL WORM INFECTIONS

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Onchocerciasis, caused by the filarial parasitic worm, *Onchocerca volvulus*, is a major cause of infection-related skin disease and blindness in Africa. It is a Neglected Tropical Disease targeted for elimination. Since regimens used in Mass Drug Administration kill the larvae, but not the adult worms, a macrofilaricidal drug would accelerate progress towards elimination. Flubendazole (FBZ) is a benzimidazole anthelmintic used to treat intestinal worm infections. Subcutaneous (sc) administration is very effective in killing adult filarial worms and is generally used as a positive control in animal efficacy studies. To explore its efficacy after oral administration, Johnson & Johnson developed an Amorphous Solid Dispersion, and studied its preclinical toxicology, pharmacokinetics (PK) and efficacy. The efficacy and PK of single-dose sc administrations were also tested. Efficacy studies were performed in well-established models at Bonn University (A Hoerauf, M Hübner), UCLA (J Sakanari) and LSTM (J Turner). Oral administration of FBZ required high doses and/or long treatments to reach sufficient macrofilaricidal effect. Single sc injections led to sustained systemic exposure and were highly effective. Toxicology studies showed that, in line with its mechanism of action as a tubuline inhibitor, FBZ targets cell division and foetal development, with potential for carcinogenicity, embryotoxicity and testicular effects. Long exposures to systemically available FBZ are therefore not recommended. The company concluded that no efficacious dose could be selected that would be safe to enter the clinic, and decided to discontinue development of FBZ in filarial diseases. The authors will present the development path of FBZ and will explain that, even if the outcome was discontinuation, the project is considered successful, since the decision was based on robust data and new insights, that can help advance the field. In parallel with preclinical development, a search for clinically relevant *Onchocerca*-specific biomarkers was undertaken. The results of those efforts will be presented separately.

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ATYPICAL PATTERNS OF *LOA LOA* MICROFILARIA DENSITIES IN GABON

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Loa loa are microfilariae producing roundworms highly endemic in west and central Africa. These microfilariae can be diagnosed in peripheral blood by direct examination or after concentration techniques. Classically, a diurnal periodicity with microfilariae density peak around noon was described and thought to be an adaptation to the diurnal behavior of the main vector. Based on this observation blood withdrawals for *L. loa* microfilariae diagnostics are recommended to be performed between

10am and 3pm. However, these fluctuations of microfilariae counts have only scarcely been studied. Recent case series have challenged this feature of loiasis. The objective of this study was therefore to reevaluate the dogma of diurnal periodicity of *L. loa* microfilaremia. The study was performed at the Centre de Recherches Médicales de Lambaréné, situated in central Gabon. Patients diagnosed to harbor *L. loa* microfilariae within another study were explained the rationale of the project and invited to participate. If participants accepted, four consecutive finger pricks were performed within a 24hours time period. Finger pricks were performed at 6am, noon, 6pm and midnight. From each finger prick, two thick blood smears using each 10ul blood were prepared. Finger pricks were dried and stained using Giemsa stain. Thick blood smears were then read by two trained microscopists, which were blinded to each other's results. Absolute microfilariae counts of both thick blood smears were added and extrapolated to a Mf/mL count. Readings of 22 participants showed a diverse picture of midday microfilariae densities with counts between 50 and 100,900 Mf/mL. No clear circadian periodicity of microfilaremia was observed during the different observation points (p=0.54; ANOVA). Interestingly, only one patient became negative for *L. loa* microfilariae during night time, while in the others microfilariae remained detectable. These results show an atypical pattern of microfilariae densities within the studied population and question the classical dogma of clear diurnal periodicity of *L. loa* microfilaremia.

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A BURDEN OF DISEASE ASSESSMENT OF *LOA LOA* INFECTION IN GABON

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Loiasis is a parasitic infection highly endemic in west and central Africa. Previously often considered as a benign infection studies have recently suggested that high microfilaria loads in *L. loa* patients may be associated with raised mortality. The true impact of loiasis on affected communities is unknown. Burden of disease studies including calculation of disability adjusted life years are a well proven tool to assess objectively the impact of a disease on an affected population. However, in-depth studies about clinics and on disease impact in endemic populations have never been performed for *L. loa*. Therefore, further studies are needed to increase current knowledge and to evaluate the impact of this parasitosis. This cross-sectional study was performed in rural Gabon to establish the burden of disease of loiasis. Inclusion criteria were local residency for at least two years and an age above two years. Participants were interrogated a standardized questionnaire covering loiasis specific symptoms, history of eye worm and health care seeking behavior. History of eye worm was assessed using the standardized RAPLOA questionnaire and local vocabulary was employed if needed. In case of children, parents were interviewed or assisted during the questioning. At the same time *L. loa* microfilaria diagnostics were performed including thick blood smear preparation and concentration techniques. Peripheral blood withdrawals were done between 10am and 3pm. 980 participants were recruited, of which 56% were female and aged between 2 to 98 years. *L. loa* microfilariae were detectable in 27% of all participants. Microfilariae densities ranged from 1 to 76250 Mf/mL. Overall a positive history of eye worm was reported in 56%, with 25% of participants reporting eye worm passage within the last year. Of those, 93% stated that eye worm passage was accompanied by severe local pain and 76% reported concomitant visual disturbances. Furthermore, 60% had stayed at home during worm passage, not being able to follow their daily work. Based on these data a burden of disease analysis was performed establishing the disability adjusted life years for affected communities.

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MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN AN URBAN AREA OF CROSS RIVER STATE NIGERIA

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The challenges of conducting mass drug administration in urban settings is well documented. These challenges include, but are not limited to, lack of trust, inadequate numbers of health workers, migrant populations, and disorganized poor urban settlements. Calabar South local government area (LGA) in Cross River State, Nigeria, faces these challenges. The LGA

has a population of 235, 388. Mapping of neglected tropical diseases (NTDs) showed that the entire population is at risk for lymphatic filariasis (LF). The LGA is also endemic for soil transmitted helminths, however prevalence is low and treatment is not required. The LGA has conducted at least seven annual rounds of mass drug administration (MDA) for LF with Albendazole and Ivermectin since 2011, and has only successfully reached the target 65% of people at risk, three times. Reports from the community drug distributers (CDDs) and supervisors from the LGA revealed that many people in Calabar South go to work in neighbouring LGAs and therefore the CDDs would find nobody at home when they conducted MDA. Through discussions with the community and LGA leaders, they mapped internal migration patterns - residents of Calabar South go to work in Calabar North during the day and many residents frequented the market and places of worship during the weekends. With this information, the LF program set out to mobilize not only community drug distributers but also faith-based organization' members, Rotary club members, and university students to distribute treatments in homes, places of worship and markets. LF increased from 43% in 2016 to 97% in 2017. Because if these efforts, Calabar South has now achieved four effective rounds of LF MDA and is on the path to stop treatment in 2018 and become eligible to conduct pre-Transmission assessments survey in 2019.

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A RANDOMIZED CLINICAL TRIAL TO STUDY THE IMPACT OF TREATMENT WITH SEMIANNUAL ALBENDAZOLE FOR LYMPHATIC FILARIASIS IN COTE D'IVOIRE

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Implementation of mass drug administration (MDA) with ivermectin (IVM) plus albendazole (ALB) for lymphatic filariasis (LF) has been deferred in many parts of Africa where *Loa loa* is co-endemic because ivermectin can induce serious adverse events in people with high level *Loa loa* microfilaremia. WHO now recommends ALB MDA alone with vector control to reduce LF in areas with co-endemic loiasis. This study tested the efficacy of semiannual treatment with ALB 400mg or ALB 800mg. We conducted an open-label randomized clinical trial in which *Wuchereria bancrofti*-microfilaremic adults in Cote d'Ivoire were assigned to treatment with IVM/ALB annually x 3 (n=48), ALB 400mg semiannually x 6 (n=45), and ALB 800mg semiannually x 6 (n=47). Clearance of blood microfilaria and adult worm nests was measured at 6, 12, 24 and 36 months. 36 months results will be collected in July 2018. IVM/ALB cleared Mf in 15 of 45 (33%), 10 of 43 (23%) and 22 of 40 (55%) at 6, 12 and 24 months respectively. At the same time points ALB 400mg cleared Mf in 2 of 40 (5%), 7 of 36 (19%) and 12 of 34 (35%, risk relative to IVM/ALB at 24 months =1.4, P=0.09). ALB 800mg cleared Mf in 1 of 40 (3%), 7 of 42 (17%) and 11 of 37 (30%, risk relative to IVM/ALB at 24 months=1.6, P=0.03) at 6, 12, and 24 months respectively. At 24 months IVM/ALB, ALB 400mg and ALB 800mg produced 93%, 77% and 71% reduction in Mf levels compared to baseline. Among participants that had worms nests at baseline, 50%, 19% and 25% of individuals had complete inactivation of detectable worm nests at 24 months for IVM/ALB, ALB 400mg and ALB 800mg arms respectively. The overall reduction Mf levels at 12 and 24 months in positively correlated with number of worm nests inactivated (r^2 = 0.52, P=0.007). Semiannual treatment with ALB alone produced a significant reduction in Mf levels, but was inferior to annual IVM/ALB at 24 months. ALB 800mg provided no benefit compared to ALB 400mg.

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IDENTIFYING RESIDUAL TRANSMISSION OF LYMPHATIC FILARIASIS AFTER MASS DRUG ADMINISTRATION - COMPARING SCHOOL-BASED VERSUS COMMUNITY-BASED SURVEILLANCE, AMERICAN SAMOA, 2016

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Under the Global Programme to Eliminate Lymphatic Filariasis (LF), American Samoa conducted seven rounds of mass drug administration (MDA) from 2000-2006. The WHO recommends post-MDA surveillance using Transmission Assessment Surveys (TAS). Recent studies have suggested TAS may not be sufficiently sensitive for post-MDA surveillance in low antigen (Ag) prevalence settings. We compared the effectiveness of two surveys for post-MDA surveillance: a systematic school-based TAS of Grade 1 and 2 children (mostly aged 6-7 years) in all elementary schools (N=29) and a community-based cluster survey in 28 villages of people aged ≥8 years on the two main islands of American Samoa. Blood samples were collected from each participant, and tested for circulating filarial Ag using the Alere™ Filariasis Test Strip. We estimated crude and adjusted Ag prevalence for both surveys, and evaluated the utility of the school-based survey for identifying villages with high Ag prevalence, using threshold ranging from 1% to 20%. The school-based TAS (n=1143) identified 9 Ag-positive children in 5 schools with adjusted Ag prevalence of 0.7% (95% CI 0.3-1.8), of whom one was microfilaria (Mf) positive. The community-based survey (n=2507, 711 households) identified 102 Ag-positive people with adjusted Ag prevalence of 6.2% (95% CI 4.5-8.6), and Mf prevalence of 25.6% in Ag-positive persons. School-based TAS had limited sensitivity (range 0-23.8%) but high specificity (range 83.3-100%) and positive predictive value (range 0-100%) for identifying villages with high Ag prevalence. American Samoa failed the school-based TAS, and the community-based survey identified higher than expected numbers of Ag-positive people. School-based TAS was logistically simpler, but results were not indicative of the overall Ag prevalence in older persons. The community-based survey was operationally more challenging, but as countries reach elimination targets established by the Global Programme, consideration should be given to testing older persons, including opportunistic and cost-effective methods of screening community members.

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POST-ELIMINATION SURVEILLANCE OF LYMPHATIC FILARIASIS IN SRI LANKA: CAN SEX- AND AGE-STRATIFIED CASE DATA BE USED TO DEMONSTRATE TRANSMISSION INTERRUPTION?

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The Global Program for Eliminating Lymphatic Filariasis (GPELF) was launched in 2000 by the World Health Organization. Initially 81 countries were identified as endemic for LF, but substantial progress has been

made, with 11 countries has successfully completed WHO recommend strategies and awaiting validation and 10 countries validated as having eliminated LF as a public health problem, including Sri Lanka. Following elimination validation in 2016, the Sri Lanka Anti Filariasis Campaign has still been detecting remaining cases in localised areas. Moving from mass drug administration (MDA) to intensive surveillance is a paradigm shift, with very little global precedent to guide the best approach. Developing improved methods for case detection and data interpretation will be a key factor in the drive towards total elimination and during this process it is important to understand whether cases arising through enhanced surveillance represent ongoing transmission or the presence of routinely missed individuals from previous MDA programs. Results from analysis of five years of post-intervention data from the Sri Lanka Anti Filariasis Campaign demonstrate a drastic difference between the age-distribution of cases in males and females, with the majority of cases found in older adult males. Mathematical modelling methods are used to disentangle the probability of ongoing transmission and the burden of residual cases, enabling vital insights into the state of LF transmission in Sri Lanka. We find that the majority of cases represent residual infections that may have been missed by previous programs or resulted from non-adherence to treatment, particularly in adult males, although there is some evidence of ongoing transmission in specific regions, such as Galle. We conclude that total elimination of LF in Sri Lanka should be achievable, but may require intensive effort in regions with higher case incidence.

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LYMPHATIC FILARIASIS ELIMINATION IN NEPAL: IDENTIFYING THE BARRIERS AND SOLUTIONS FOR THE LAST MILE

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Sixty-one of Nepal's 75 districts, covering 25 million people, are endemic for Lymphatic Filariasis (LF). Mass drug administration (MDA) with DEC and Albendazole started in 2003; by 2018, all districts had implemented six rounds. To date, 37 districts achieved stop-MDA criteria according to WHO guidelines, of which 19 passed the second transmission assessment survey (TAS II) and five passed TAS III. All 24 districts still requiring treatment should be under post-treatment surveillance by 2021. The program has experienced many challenges: 14 districts have failed Pre-TAS and/or (re)-Pre-TAS, one district failed TAS I, and one district failed TAS II. The program is working to overcome the challenges. Coverage surveys conducted in 2011, 2015, and 2017 and an LF Experts' Meeting in March 2018 identified barriers to high treatment. These include treatment in urban areas, compliance issues in minority populations, variability in sub-district coverage, lack of directly-observed treatment, and fear of side-effects (68% of those non-compliant reported this reason). To overcome these, Nepal has implemented media orientation at the district and central level so health journalists understand the campaign, developed comprehensive SAE management plans at village level, and is developing district-specific activities to improve coverage. Banke district, which has failed (re)-Pre-TAS, is addressing low coverage, particularly in the minority Tharu and Muslim communities and in urban areas. This is done using sub-district level data for planning, mobilizing health workers in addition to volunteers as they are more trusted to respond to adverse events, and community-focused activities like orienting target groups, culturally-appropriate MDA messaging for religious leaders, and community videos of people with LF. Health workers mobilized in 14 low coverage communities directly observed treatment. These resulted in a coverage increase of 13.7% (range; 1.2% - 25.6%) from the previous year. Lessons from this approach can be useful in special community settings with low coverage, and for other countries looking to address coverage issues.

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PROGRESS IN STOPPING MASS DRUG ADMINISTRATION FOR ONCHOCERCIASIS - A REPORT FROM THE CARTER CENTER

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The Carter Center (TCC) has assisted ministries of health in their fight against onchocerciasis through community-based distribution of Mectizan® (ivermectin) in Africa and the Americas since 1996 To date, the total exceeds 320 million treatments supported across ten countries. This effort has been primarily characterized by expansion. TCC's programs only reached 3.9 million people in 1996; they hope to reach 41 million in 2018, more than a ten-fold increase in scope. Programs hope to provide 73.4 million ivermectin treatments in 2018, in a combination of annual, semi-annual, and quarterly distributions. Much of this increase is due to a change from once- to twice-per-year mass drug administration (MDA) as African programs shifted from control of onchocerciasis to elimination of transmission. The geographic extent has expanded as well, beginning with 109 implementation units (IUs—districts or *Foci*) in 1996 and growing to 260 in 2018. Remarkably, 17% of these units—including four countries—no longer require treatment because they have demonstrated, according to WHO guidelines, that they have either interrupted or eliminated transmission of onchocerciasis. More than 6 million people no longer need treatment. They hail from Nigeria (2.6 million), Uganda (1.9 million), Ethiopia (1.1 million), Guatemala (196,000), Sudan (190,000), Mexico (170,000), Venezuela (107,000), Ecuador (26,000), and Colombia (1,500). While this is the largest number of treatments stopped for onchocerciasis by a non-governmental development organization to date, it represents only 15.3% of the people targeted by TCC-assisted programs. Studies to determine whether treatment may be stopped are pending in three countries (Ethiopia, Nigeria, and Uganda). Activities must focus on determining which areas under MDA can undergo large-scale "Stop MDA" surveys, both in terms of assessing progress and in determining the unit of evaluation.

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REVAMPING THE STRATEGY FOR MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN URBAN METROPOLITAN AREAS OF PORT AU PRINCE, HAITI

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To achieve global goals to eliminate lymphatic filariasis (LF), the Haiti NTD Control Program (HNTDCP) follows WHO's recommendations to implement rounds of annual mass drug administration (MDA) of diethylcarbamazine and albendazole for at least five consecutive years among at risk populations to halt LF transmission by 2020. In 2018, 84% of the 140 communes are under surveillance. Among the 23 communes that have not achieved the criteria for stopping MDA, five are in the densely populated Port au Prince metropolitan area where MDA coverage has declined each year since 2012; from an average of 86% to 44% by 2017. Two surveys were conducted after the 2017 MDA: use of the supervisor's coverage tool in Tabarre commune and a social mobilization survey in a neighboring commune (Croix des Bouquets). With these results, HTNDCP and its partners organized a workshop to analyze the situation and adopt improved strategies tailored for the urban setting to improve

coverage. During microplanning meetings, coverage zones were mapped and previous distribution posts were geolocalized, which identified gaps in supervision areas, while others overlapped. As a result, visibility and access to the drugs will be improved by increasing the pool of community volunteers with updated communication materials. One additional day has been added to the four distribution days with timing adjusted to catch hours of high foot traffic. Going forward, the community sensitization will be intensified with revamped IEC messages including TV and radio spots, which have been tested by focus groups and then modified. Supervision will be reinforced with clear plans (random and purposive selection of distribution posts) and tailored tools. Data collection tools have been updated to facilitate rapid daily reporting for key indicators and mobile technology will be used for real time reporting. Further, a hotline will be available to respond to community questions and help with side effect management and referrals. HNTDCP and its partners expect improved coverage during the April and May 2018 MDA with results expected in June 2018.

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IMPACT OF ENHANCED MDA WITH DEC PLUS ALBENDAZOLE FOR ELIMINATION OF LYMPHATIC FILARIASIS IN COASTAL GALLE DISTRICT, SRI LANKA

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The Sri Lankan Anti-Filariasis Campaign (AFC) distributed 5 rounds of MDA (DEC/ALB) according to WHO guidelines to some 10 million people in 8 endemic districts between 2002 and 2006. Sri Lanka met WHO validation criteria for elimination of lymphatic filariasis (LF) as a public health problem in 2016. Comprehensive post-MDA surveys identified areas in coastal areas in Galle district with low-level persistence of LF. AFC distributed additional rounds of MDA in 2014 and in 2015 to 14 of 20 MOH areas in Galle (target population 850,000) with relatively moderate coverage (~78%), but this failed to eliminate the infection. In 2016 AFC provided one round of enhanced MDA (eMDA) in 11 MOH areas with participation of more than 3000 people from the Ministry of Health, private and public institutions, and volunteers. Intensive social mobilization and awareness activities (SMS, media, flyers, meetings) were conducted prior to MDA. Approximately 600,000 people aged ≥ 2 yr in 11 MOH areas were targeted, and MDA was provided over 2 days. AFC reported excellent MDA and DOT compliance rates of 90% and 47%, respectively. An independent assessment based on 1100 persons surveyed in 30 clusters found MDA and DOT compliance rates of 89% and 37%. Understanding the purpose of MDA was significantly correlated with drug consumption ($P < 0.001$). 7% of the surveyed population reported mild or moderate adverse events after treatment. Limited molecular xenomonitoring (MX) studies performed before and after eMDA showed a trend toward reduced parasite DNA rates in mosquitoes, but this was not statistically significant: maximum likelihood filarial DNA rates were 1.3%, CI 1.0-1.5 before MDA and 0.92%, CI 0.7-1.2 after MDA. These rates are higher than our provisional MX target for LF elimination (0.25%, upper CI 1%). These results show that eMDA can lead to very high measured compliance, but the MX results suggest that a single round of eMDA may not be sufficient to reach true elimination of LF across the evaluation unit. AFC has increased test and treat activities and other interventions to clear infections in remaining problem areas.

1946

LYMPHATIC FILARIASIS ELIMINATION IN URBAN AFRICA: MICRO-STRATIFICATION OVERLAP MAPPING, RISK DELINEATION AND TRANSMISSION ASSESSMENT SURVEYS

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The city of Dar-es-Salaam in Tanzania has a significant risk of lymphatic filariasis (LF), which is transmitted by *Culex* spp. mosquitoes that thrive in poor human domestic environments. At the start of the National LF Elimination Programme, the overall infection rate was ~10%, with nearly double the risk found in informal settlements and peri-urban areas. Since 2013, mass drug administration (MDA) with ivermectin and albendazole has been scaled up successfully, and many areas of the city have reached high coverage rates and significant reductions in prevalence. The National LF programme is preparing to scale down MDA and planning to implement Transmission Assessment Surveys (TAS) in selected low risk areas. Identifying low risk areas to start TAS will be challenging in such a large dynamic city. The aim of this study was to use programmatic data together with satellite imagery and remote sensing data to categorise the different areas of the city into risk zones. All LF sero-prevalence, morbidity data, MDA distribution points and treatment coverage rates were georeferenced and mapped at ward and/or health centre level. Firstly, micro-stratification mapping methods and programmatic data were used to develop composite maps and risk categories as i) high LF risk/low MDA coverage ii) high LF risk/high MDA coverage iii) low LF risk/low MDA coverage and iv) low LF risk/high MDA coverage. Secondly, high resolution satellite imagery, and topographical and population maps were used to further delineate risk across the city and within these categorised areas to help ensure that the future scaling down of MDA is successful. The results of this study will reduce the population and geographical area at risk, and also help to optimise resources by targeting interventions to where they are needed most.

1947

RETHINKING ELIMINATION STRATEGIES FOR LYMPHATIC FILARIASIS TRANSMISSION HOT SPOTS IN HAITI

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Based on baseline mapping (2000/2001) in Haiti, lymphatic filariasis (LF) was determined to be endemic throughout the country. Ninety percent of 140 total communes had low prevalence (below 4.5%), while 15 communes, categorized as "red zone," had prevalence ranging from 10 to 45%. While there has been significant progress to date, with 85% of communes in post-MDA surveillance phase, LF transmission still persists in a total of 14 communes in the red zone, including Arcahaie, Cabaret, and Croix des Bouquets in the West department. In order to conduct a transmission assessment survey (TAS), WHO guidance recommends at least 5 MDA rounds of > 65% coverage of total population and sentinel and spot-check site results with < 2% antigenemia. Sentinel and spot-check site surveys in 2015 found continued high antigenemia prevalence of 41%, 8.1% and 8.7% in Arcahaie, Cabaret and Croix des Bouquets, respectively; therefore, the MDA in 2016 and 2017 was changed to increase social mobilization in those sites with >2% prevalence. However, in 2017 repeated sentinel and spot-check sites still found more than 20%

antigenemia in Arcahaie and 8% in Cabaret and Croix des Bouquets. MDA coverage has been decreasing over the years and by 2017, coverage was marginal in Croix des Bouquets (median: 68) and Arcahaie (median: 57). These results underline a need for improved strategies. In North department, a triple drug study is being carried out in select red zone communes and could be considered in these communes per 2017 WHO guidelines. Additionally, pre-TAS results suggest a need for a more targeted MDA approach followed by a mini-TAS in the hot spot zones. This can include conducting a microplanning exercise to identify challenges and missed opportunities to improve access to MDA, generate demand and support communes through better tools (supervisor checklists and guidelines) as a way to increase the coverage rate in the hot spot areas.

1948

ADVERSE EVENTS FOLLOWING SINGLE DOSE TREATMENT OF LYMPHATIC FILARIASIS: OBSERVATIONS FROM A REVIEW OF THE LITERATURE

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WHO's Global Programme to Eliminate Lymphatic Filariasis (LF) uses mass drug administration (MDA) of anthelmintic medications to interrupt LF transmission in endemic areas. It has recently been shown that a single dose combination of ivermectin (IVM), diethylcarbamazine (DEC), and albendazole (ALB) is markedly more effective than the standard two-drug regimens (DEC or IVM, plus ALB) for achieving long-term clearance of microfilaremia. This IVM/DEC/ALB triple therapy (IDA) has been approved by WHO for programmatic use after large safety studies found equivalent rates and severity of adverse events with IDA compared to DEC/ALB. To provide context for the results of the IDA safety studies, we searched Ovid Medline for studies published from 1985 - 2017 that reported adverse events (AEs) following treatment of LF with IVM, DEC, ALB, or any combination of these medications. Studies that reported AE rates by treatment group were included. We reviewed 162 published manuscripts, 55 of which met inclusion criteria. Among these, 34 were clinic or hospital-based clinical trials, and 21 were community-based studies. Reported AE rates varied widely. The median AE rate following DEC or IVM treatment was greater than 60% among microfilaremic participants and less than 10% in persons without microfilaremia. The most common AEs reported were fever, headache, myalgia or arthralgia, fatigue, and malaise. Mild to moderate systemic AEs related to death of microfilariae are common following LF treatment. Post-treatment AEs are transient and rarely severe or serious. Comparison of AE rates from different community studies is difficult due to inconsistent AE reporting, varied infection rates, and varied intensity of follow-up. A more uniform approach for assessing and reporting AEs, such as that outlined in the CONSORT checklist for reporting harms in clinical trials, applied to LF community treatment studies would be helpful.

1949

EVALUATING THE IMPACT OF 17 YEARS OF ANNUAL IVERMECTIN MASS DRUG ADMINISTRATION IN THE MAHENGE ONCHOCERCIASIS TRANSMISSION FOCUS IN TANZANIA

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The Tanzania NTD Control Program's (TZNTDCP) goal is to eliminate onchocerciasis (OV) by 2025 in line with World Health Organization (WHO) targets, and as guided by the updated WHO guidelines for OV elimination. OV is endemic in seven *Foci* across 28 districts in six regions in Tanzania. By 2017, all districts had received 10 to 17 rounds of Ivermectin mass drug administration (MDA) with effective coverage. Four districts in Morogoro region make up the Mahenge focus area. In 2017 in Mahenge focus, the TZNTDCP evaluated the impact of 17 rounds of annual ivermectin MDA in the four districts by surveying the prevalence of OV among children <10 years old. Purposively, a total of 7 first-line villages (that are close to the known simulium breeding sites) were selected. At each village, the sampling universe was constructed from household registers for systematic sampling. A total of 1799 children were tested using the OV16 RDT that detects IgG4 antibodies against the Ov16 antigen in human blood samples. Dry blood spots were also collected for further OV16 ELISA testing. OV16RDT tests were completed in the field following manufacturer guidelines and results were provided to participants immediately. Prevalence of positive OV16 RDT tests ranged from 0%-5.8% across all test sites. Overall 33 out 1799 children tested were positive. Mgugwe and Uponera villages reported the highest (5.8% and 4.1% positive children respectively). These results indicate an ongoing transmission of infection among the communities as children born well after the treatment program started are still being infected. While there remains a need to investigate the validity of high reported MDA treatment coverage and the status of infection in the vectors (black flies), the OV16 ELISA testing will take place in June 2018 and these results will further inform the program on proper strategies to accelerate OV elimination for all OV endemic districts in Tanzania.

1950

COST-EFFECTIVENESS OF INTEGRATED VECTOR CONTROL FOR LYMPHATIC FILARIASIS ELIMINATION IN TAMIL NADU, INDIA

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Vector control is a potentially effective intervention to augment annual mass drug administration (MDA) towards the elimination of lymphatic filariasis. We conducted cost-effectiveness analysis of MDA alone and augmented by VC using data from a cluster randomized trial and historical controls. We built on an effectiveness trial. It randomized 36 villages in the State of Tamil Nadu, India, at risk of LF transmission into three groups of 12 villages each: MDA alone (the standard of care); MDA plus expanded polystyrene beads (MDA+EPB) for covering the water surface in wells and cesspits to suppress the filariasis vector mosquito Culex quinquefasciatus; MDA plus integrated vector control combining EPB with insecticidal pyrethroid impregnated curtains (MDA+EPB+PIC) over windows, doors, and eaves. Economic costs in 2010 US$ combined village and higher levels. Outcomes were antigen prevalence (AGP), microfilaria prevalence below the level for elimination (MFP<1%) from 2010 to 2013, and modeled Disability Adjusted Life Years (DALYs). The estimated annual cost per village resident was US$0.53 for MDA alone, US$1.02 for MDA+EPB, and US$1.83 for MDA+EPB+PIC. MDA and MDA+EPB+PIC increased the percentages of villages with MFP<1% by 67% and 75% of villages, respectively, although not statistically significant. The resulting incremental cost-effectiveness ratios per DALY averted were $7.2 and $141, respectively. The findings confirm that both MDA and MDA+EPB+PIC are both effective and very cost-effective strategies for eliminating LF. As MDA alone lowered MFP substantially and AGP takes years to change, the study had limited power to estimate the incremental benefits of vector control. While MDA+EPB+PIC costs three times as much as MDA alone, its substantially greater effectiveness makes it more cost-effective. This VC combination accelerates LF elimination by about one year and may contribute to LF elimination in defined communities.

1951

THE RELATIVE SAFETY OF A PROMISING TRIPLE DRUG MASS DRUG ADMINISTRATION REGIMEN VERSUS A TWO-DRUG REGIMEN FOR LYMPHATIC FILARIASIS ELIMINATION IN INDIA

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India launched a programme to eliminate lymphatic filariasis (LF) in 256 endemic districts in 2004 with annual mass drug administration (MDA) of a single dose of diethylcarbamazine with albendazole (DA). Since more than 50% of these districts have not met elimination targets despite more than 10 rounds of MDA, new strategies are needed if LF is to be eliminated by 2020. Recent clinical trials have shown a single dose of ivermectin with DA (IDA) is superior to DA for clearing microfilaremia (Mf). As part of a multicentre study, our group conducted a community-based study in an endemic district in Karnataka state to compare the safety and efficacy of IDA with DA. Pre-intervention filarial antigenemia (Ag) and Mf prevalence were 25% and 6%, respectively in the study area despite 12 prior rounds of MDA with DA. Treatment assignment was randomized by village, and consenting residents >5 years of age were treated with IDA (4782) or DA (4273). Adverse events (AE) were actively assessed by house-to-house follow-up of participants for 2 days followed by passive follow-up for an additional 5 days. More than 90% of participants had active follow-up on days 1 and 2. AEs were significantly more frequent after IDA (8%) than after DA (6.2%). AEs were more frequent in females (8.6%) than in males (5.5%), and this was independent of age. As expected from prior treatment studies, AE rates were much higher in participants with Mf and higher after IDA (40%) than after DA (20%). Most AEs were mild after IDA (90.3%) and DA (97.7%). The most common AEs were fever, headache, dizziness, nausea, vomiting and fatigue. All AEs were self-limiting or resolved after simple symptomatic treatment. There were no serious AEs, and no participant required hospitalization. One participant had severe diarrhoea that started 6 days after IDA, but this was not considered to be treatment related. Thus, while AEs were more common after IDA (especially in Mf positives), our results suggest that IDA is safe and feasible for use as a MDA regimen in India to hasten LF elimination.

1952

FIELD IMPLEMENTATION OF A NOVEL TOOL FOR THE COLLECTION OF MOSQUITO EXCRETA AND FECES FOR THE MOLECULAR XENOMONITORING OF FILARIAL WORMS AND MALARIA IN GHANA

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Current molecular xenomonitoring methods for the detection of parasites such as filarial worms require processing large numbers of mosquitoes, resulting in increased time, cost and labour required. Screening the mosquito excreta/faeces (E/F) rather than whole carcasses allows for a higher throughput by removing the need to discriminate vector species, since also non-vectors release ingested pathogens and their DNA in E/F, and sensitivity of detection, enabling larger numbers of mosquitoes to be processed per pool. We recently developed a superhydrophobic cone allowing for the efficient collection of E/F from live mosquitoes. We performed the first field implementation of this tool in two endemic rural communities in Ghana to assess its applicability in determining the presence of filarial worms and malaria parasites in the community. The presence of parasite DNA was compared between mosquito E/F and whole insects, and to parasitological detection in human blood via rapid diagnostic tests, microscopy and real time PCR. We successfully detected the presence of filarial and malaria parasites in mosquito E/F as well as in mosquito carcasses, and we also reported the presence of the midge-borne filarial parasite *Mansonella* in both E/F and a large number of people. In this talk, we will also present the relationships between parasite positivity in the E/F and mosquito carcasses and human samples. Detection of pathogen DNA in mosquito excreta on the field is possible even for parasites which are not vectored by mosquitoes, and this approach represents a new and promising molecular xenomonitoring tool.

1953

HUMORAL IMMUNE RESPONSE INDUCED BY HELMINTH AND MALARIA PARASITE COINFECTION IN CENTRAL GHANA

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Factors from the environment and host characteristics affect the immune response elicited by host to helminth and malaria parasite co-infection in endemic area. We studied the humoral immune response by helminths and malaria infection in the middle-belt of Ghana, West Africa. Cross-sectional community survey of 1826 participants were randomly recruited at household level. Biological samples were collected over 12-month period. ELISA techniques were employed to estimate the concentrations of immunoglobulins (IgG, IgG1 and IgM) in the plasma using crude parasite antigens and also with BD Cytometric Bead Array (CBA) for IL-2, IL-4, IL-6, IFN-γ, TNF-A and IL-5. From supernatant of PBMCs cultured with *Pf parasitized RBCs*, BD CBA Human Thl/Th2/Th17 Cytokine kit was used to estimate cytokine (IL-2, IL-4, IL-6, IFN-γ, TNF-A, IL-17A, and IL-10) concentration using the BD LSRFORTESSA X20. Concentration of IgG to *NF54* antigens (p=0.007) and IgG to hookworm antigens (p=0.018) were significantly different among no infection, helminth, malaria, and helminth-malaria co-infected in the study. Concentrations of plasma cytokines TNF-A (p=0.007), IL-4 (p=0.01), IL-5 (p=0.009) and IL-6 (p<0.0001) were significantly different when compared among the infection status of participants. From the culture supernatant, compared to those with no infection, IL-6 was significant among hookworm-malaria co-infected (p=0.011). IL-4 concentration was different among the various infection groups (p=0.021) and the significant difference was between hookworm and hookworm-malaria co-infected then malaria and hookworm-malaria co-infected individuals. Hookworm infection increased significantly IgG1 concentration with malaria parasite crude antigen. IgG1 to hookworm cross-react with *Pf* parasite antigens. In hookworm infected individuals, IL-17A correlates with IL-6, IL-10, and IFN-gamma. Also, pro-inflammatory cytokines positively correlate in hookworm infection. Among individuals infected with malaria, upon exposure to malaria antigens, they unpredictably induce IL-4 cytokine.

1954

MOLECULAR EPIDEMIOLOGY OF *ANISAKIS SPP.* IN BLUE WHITING *MICROMESISTIUS POUTASSOU* IN EASTERN WATERS OF SPAIN, WESTERN MEDITERRANEAN SEA

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The infection of blue whiting *Micromesistius poutassou* from the western Mediterranean Sea, off the eastern coast of Spain, with larvae of *Anisakis* spp. was studied. Between April 2016 and April 2017, 140 fish were analyzed. Total epidemiological data showed that the prevalence of *Anisakis* spp. was 29.3% and the mean intensity 1.8. Of the 74 larvae

collected, 60.8% were type I and the remaining 39.2%, type II. Of the former, 91.1% were molecularly identified as *Anisakis pegreffii* (P=19.3%; MI=1.4), 2.2% as *Anisakis simplex s.s.* (P=0.7%; MI=1.0), while the rest (6.7%) showed a recombinant genotype between the two (P=2.1%; MI=1.0). All the type II larvae analyzed were molecularly identified as *Anisakis physeteris* (P=10.0%; MI=2.1). Only three fish (2.1%) were found to have larvae in the muscle, while two were found with 1 larva of *A. pegreffii* and one with two larvae (1 *A. simplex s.s.* and 1 *A. pegreffii*). Statistical analysis showed that the prevalence of *Anisakis* spp. in blue whiting is higher in spring than in autumn (p<0.001), probably due to the greater size (and age) of the fish then related with factors as diet shift, accumulation with age, higher food intake. On the other hand, the analysis of the data suggests that blue whiting is first infected with *Anisakis* type I (mean age 2.3 years) and later with *Anisakis* type II (mean age 2.7 years), probably due to the diet of the blue whiting with age, incorporating into its diet the paratenic/intermediate host species of these parasites. In any case, the public health authorities must continue to emphasize the need for suitable thermal treatment (freezing or cooking) of the fish prior to consumption. Funded by Spanish State Research Agency and European Regional Development Fund (ERDF) [grant number CGL2013-47725-P]

1955

ANTHELMINTIC TREATMENT RESPONSE AND EMERGENCE OF ANTHELMINTIC RESISTANCE IN HUMAN POPULATION OF SOUTHERN MOZAMBIQUE INFECTED WITH SOIL-TRANSMITTED HELMINTHS

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Over one billion people are infected with soil-transmitted helminths (STH) worldwide, nearly a quarter of the world population. STH infections (*Ascariasis lumbricoides*, *Trichuris trichiura*, hookworm and *Strongyloides stercoralis*) can contribute to anemia, malnutrition, and delayed development among others. The cornerstone for controlling STH is mass drug administration (MDA) programs with benzimidazole drugs. However, several factors limit this strategy: low coverage, high re-infection rates and low efficacy against some STH infections (e.g. *T. trichiura* and *S. stercoralis*). Furthermore, since STH MDA strategy is a monotherapy, benzimidazole resistance emergence is a concern. The aim of this study was to design and implement a pilot surveillance platform for the genotypic and phenotypic characterization of anthelminthic resistance to albendazole in Manhiça district, Southern Mozambique. We conducted a cross-sectional study in Manhiça district where we recruited 400 people older than 15 years of age censed in Centro de Investigação em Saúde da Manhiça Demographic Surveillance System. We used grid sampling methodology to obtain spatial representativeness. Two stool samples from two consecutive days were collected from every participant. Regarding STH infection positive participants, a third and a forth stool samples were collected a day before and twenty-one days after treatment respectively. Stool samples were analyzed by Telemann concentration technique and Kato-Katz thick smear technique. A proportion of 21% of participants were infected with at least one STH. Phenotypic albendazole resistance will be detected by cure rate and egg reduction rate. Genotypic albendazole resistance will be evaluated by pyrosequencing, in order to detect mutations in the beta-tubulin gen related with anthelmintic resistance to benzimidazole drugs. The SaTScan method will be employed for identification of pockets of risk and clustering of STH infection and albendazole resistance. Krieging will be used to estimate general spatial trends in infection. Final results are still under analysis.

1956

SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS IN TWO HEALTH DISTRICTS OF THE KWILU PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO: EVALUATION OF THE PREVALENCE AMONG SCHOOLCHILDREN AND IN THE COMMUNITY

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Schistosomiasis (SCH) and soil-transmitted helminths (STH) control relies on Mass Drug Administration (MDA) campaigns and the World Health Organization (WHO) promotes a school-based strategy. Epidemiological data for the Democratic Republic of the Congo (DRC) are rather scarce to adequately plan MDA campaigns. We conducted two prevalence surveys to provide estimates of the disease burden. Both surveys were conducted in the Health Districts of Mosango and Yasa Bonga, Kwilu province, DRC. First, school children were surveyed in 2016 using the WHO recommended methodology. One stool and one urine sample were collected for parasitological examination by Kato Katz and urine filtration technique. Subsequently, a community based survey was held in 2017 using cluster sampling to include young children (1-5years), schoolchildren (6-14years) and adults (>18years). One stool sample was examined. 526 school children enrolled in the school-based survey and results show a *S. mansoni* prevalence of 8.9% (95%CI: 3.5-13.2) in both districts and no *S. haematobium* infection was found. The most prevalent STH infection was hookworm with 52.9% (95%CI: 29.3-62.4) in both districts, followed by *A. lumbricoides* (9.3%; 95%CI: 5.8-15.4) and *T. trichiura* (2.1%; 95%CI: 0.9-4.9). 1652 participants enrolled in the community survey. Preliminary results show that *S. mansoni* infection was not found. For STH, hookworm infection was most prevalent in the population (28.8%; 95%CI:26.7-31.1) followed by *A. lumbricoides* (2.2%; 95%CI:1.5-2.9) and *T. trichiura* (1.5%; 95%CI:0.9-2.1). The distribution of any STH infection was 25,5% in young children, 31,3% in schoolchildren and 35,2% in adults. Based on these results, both districts are categorized as low risk communities for SCH, however, no *S. mansoni* infection was found in the community survey. This could be explained by the disease being clustered in the vicinity of rivers. For STH, both districts need MDA in schoolchildren twice a year. However, preliminary results of the community survey suggest that adults and young children are equally affected by STH. Community-wide MDA seems therefore more suitable in this area.

1957

RISK FACTORS FOR HUMAN HOOKWORM INFECTION IN A TRIBAL POPULATION: RESULTS FROM THE BASELINE SURVEY OF THE DEWORM3 INDIA STUDY SUBSITE

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Global estimates for infection with the soil transmitted helminths (STH), *Ascaris lumbricoides*, *Trichuris trichura*, and hookworm are 819, 464.6 and 438.9 million respectively with 67% of these infections occurring in Asian populations. The DeWorm3 study on testing the feasibility of interrupting STH transmission using bi-annual community-wide MDA compared to

school-age targeted MDA is a multi-country study including a series of cluster randomised field trials. In India, the field trial has 2 subsites, Timiri and Jawadhu Hills in Tamil Nadu. We present the results from baseline surveys of the Jawadhu Hills subsite. This site is a hard to access, hilly tribal area with poor health care and sanitation and with most adults engaged in migrant labour. The subsite has 8 study clusters censused, and has a mean population of 4100 (range 2800-6000) per cluster. Approximately 150 subject in each cluster were randomly selected to participate in a longitudinal monitoring cohort (LMC) in an age stratified manner with 30 preschool age children (PSAC), 30 school age children (SAC) and 90 adults per cluster. A total of 1220 fecal samples collected from participants in the LMC were tested for STH ova by the Kato-Katz test. The prevalence of hookworm in PSAC, SAC and adults was 9.7%, 24.5% and 52.6% respectively. *Ascaris* (4 individuals) and *Trichuris* (1 individual) infections were rare in this community. In a multivariable logistic regression that used generalised estimating equations for risk factor analysis, increasing age and open air defecation were independently associated with hookworm infection (Odds Ratios (OR): 1.03 (95% CI: 1.02-1.04) per year of age and 2.95 (1.46-5.97) respectively). Other risk factors analysed such as not being dewormed, not wearing footwear, availability of soap at hand washing facility and gender were not significantly associated with hookworm infection. Studies on risk factors associated with transmission of STH especially hookworm in these marginalized communities with cultural barriers to the use of toilets are extremely important to formulate effective preventive strategies.

1958

PARASITES DETECTED BY METAGENOMIC BARCODING BOTH CONFIRM MICROSCOPY AND IDENTIFY ADDITIONAL ORGANISMS

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Although microbiome research has led to an explosion of knowledge about prokaryotic communities and their influence on host health, a parallel revolution for eukaryotic communities has yet to be realized. The field of parasitology in particular would benefit from a metagenomic method for characterizing eukaryotes. We used a metagenomic barcoding approach to detect eukaryotic parasites in fecal samples using published pan-eukaryotic primers. After creating a validation set of parasites, we extracted genomic DNA, amplified a common barcode region (18S ribosomal RNA gene), and deep sequenced the amplicon. This approach identified 100% (n=6 helminths, n=6 protozoans) of organisms in the validation sample, 75% (9/12) to the species level and 25% (3/12) to the genus level. We then repeated the process with samples from wild nonhuman primates where matched fecal samples had been previously characterized by microscopy in published studies, adding steps to enrich for parasite DNA. Results were analyzed with a combination of mothur, qiime, and custom python scripts using the SILVA132 database. The percentage of host reads ranged from 2.77% to 5.1% with a mean= 3.79% and median= 3.45%. In one representative sample, 5.24% of filtered reads mapped to a ciliate species found via microscopy and 4.42% mapped to a protozoan not identified by microscopy but expected to be present based on PCR-based diagnostics. This work demonstrates the potential of a metagenomic barcoding approach for identifying eukaryotic gastrointestinal parasites.

1959

POOLING STRATEGY VALIDATION AND PREVALENCE-BASED MODELING FOR COST-EFFECTIVE AND EFFICIENT MONITORING OF SOIL-TRANSMITTED HELMINTH INFECTIONS

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The strategy of pooling stool specimens has been extensively used in the field in order to facilitate the screening of large numbers of samples for the detection of soil-transmitted helminths whilst minimizing the oftentimes prohibitory cost associate with large-scale PCR-based molecular studies. It is important for such a tool to be tailored and standardized to any given study or circumstance in order to be most beneficial. The development and subsequent validation of a standard operation protocol for pooling (with focus on pools of 5) was employed as part of the Deworm3 project, where model-based community-wide mass drug administration is being tested to break transmission of STH infections. Within a recommended confidence, field samples with known real-time PCR results at an individual level were used (strategic pooling) in order to ratify the robustness of the strategy while maintaining the sensitivity and specificity of the real-time PCR assay. A key goal of pooling is to minimize the samples screened and reduce the cost of the overall DNA extraction and real-time PCR testing for the detection of soil-transmitted helminths. However, there are cases where pooling is likely not to be the most practical or time-efficient tactic to follow. In regions/clusters, where the prevalence for a targeted infection is high, the majority of the pools created in the laboratory setting will be positive for that infection. In cases where the focus is on identifying infected individuals within a pool, revisiting individual samples ('spin-outs') in high prevalence areas increases the cost of the DNA extractions and PCR testing significantly. The validation of a robust pooling strategy along with a model that demonstrates cost-effectiveness across different prevalence settings (e.g. as estimated by microscopy) would be beneficial not only for reliable screening and molecular testing at a community level, but will significantly reduce the overall cost as well.

1960

DEWORM3 INDIA STUDY SITE: DESCRIPTION OF THE STUDY POPULATION AND PREVALENCE OF SOIL-TRANSMITTED HELMINTHS

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Soil-transmitted helminths (STH) affect more than 1.45 billion people in low resource settings worldwide. The current WHO strategy of targeted deworming of preschool and school aged children is to control morbidity associated with STH. The DeWorm3 study is a series of cluster randomised trials being conducted in three countries (Benin, India and Malawi) to test the feasibility of interrupting STH transmission using bi-annual community-wide MDA compared to school-age targeted MDA. In India, the study is being carried out in 2 sub-sites, Timiri in Vellore district and Jawadhu Hills in Thiruvannamalai district of Tamil Nadu. A baseline census conducted from October to December, 2017, enumerated a total

of 37,246 households in Timiri (28,357) and Jawadhu Hills (8,889). The censused population (143,789) was fairly evenly distributed between males and females (p=0.996) and the age pyramid is similar to the country's age pyramid with the largest subgroup being young adults (15-39 years, 42.7%). Among the enumerated households, mud flooring was more common in JH (33%) compared to Timiri (6%). Household water supply also differed with 56.3% having piped drinking water in Timiri compared to only 2.6% in Jawadhu. High levels of open air defecation with no toilet in the household were documented in both study sites (Timiri, 56.8%) and (Jawadhu, 93.8%). A subset of 150 individuals from each cluster were recruited to a longitudinal monitoring cohort in age stratified sample (preschool age children, 30 PSAC 30, school age children, 30 SAC and 90 adults) accounting for a total of 6108. The prevalence of hookworm was higher in Jawadhu than Thimiri: 9.7%, 24.5%, 52.6% and 0.51%, 1.9%, 17.2% among PSAC, SAC and adults respectively. *Ascaris* and *Trichuris* infections were uncommon in both sites. In India, socioeconomic indicators, water and sanitation facilities are diverse, this coupled with significant variation in STH prevalence was evident in our study. Understanding these factors and cultural practices in endemic areas is essential to develop and implement appropriate community based strategies to interrupt STH transmission.

1961

HIGH PREVALENCE AND INTENSITY OF HOOKWORM IN AN INDIGENOUS COMMUNITY FROM PUERTO IGUAZU, MISIONES, ARGENTINA

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Literature on soil-transmitted helminths (STHs) in Argentina covered 16 localities from only nine of the 25 Argentinian provinces. Previous studies from Misiones have detected varying prevalences of all four STHs (hookworm, *Strongyloides stercoralis*, *Ascaris lumbricoides* and *Trichuris trichiura)*, but none of these measured intensity of infection. The aim of this study was to determine the prevalence and intensity of STHs in an indigenous community from the city of Puerto Iguazú. The Fortín Mbororé community is composed of around 200 families of the Mbyá-Guaraní ethnicity and the primary source of income is from guided tours organized to visit the village, handcrafts and social plans. A total of 63 houses were visited, georeferenced and characterized through the use of a questionnaire. From the 240 containers distributed for the collection of stool samples, a total of 218 samples (90.8% participation) were received for coprological analysis. Age of participants ranged from 1 to 87 years old (mean = 20) and 53.2% of them were female. Stool samples were processed through sedimentation and Baermann techniques. Samples positive for STHs with any of the two techniques, except for those with mono-infections by *S. stercoralis*, were thoroughly processed with a Kato-Katz slide. The prevalence of STHs was 65.1%, being hookworm the most prevalent parasite (64.2%). Hookworm infections were either light (46.8%), most in a mean age of 23 years old, or heavy intensity (41.0%), most in a mean age of 20 years old. Other STHs found included *S. stercoralis* (n=22, 10.1%), *A. lumbricoides* (n=2, 0.9%) and *T. trichiura* (n=1, 0.5%). This study has detected a hyperendemic area for hookworm in Puerto Iguazú. Further studies will aim to study the association with environmental and socioeconomic variables as well as the effectiveness of mass drug administration in conjunction with preventive measures such as health education.

1962

EXPLORING SOCIOECONOMIC EQUITY IN THE REDUCTION OF HOOKWORM INFECTION PREVALENCE AND INTENSITY DURING THE TUMIKIA TRIAL

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The TUMIKIA project was a three-armed randomised controlled trial investigating the effectiveness and cost-effectiveness of alternative treatment strategies and delivery systems to interrupt transmission of soil-transmitted helminth infection in Kwale County, Kenya. The trial demonstrated that both prevalence and intensity of hookworm infection were reduced by expanding anthelmintic treatment with albendazole to all community members either annually or twice a year, in comparison to the current standard of providing children aged 2-14 with treatment once a year through schools. Both school- and community-based deworming are typically thought of as pro-poor interventions. Here we investigate socio-economic equity in the impact of these alternative control strategies. Defining SES in low and middle income settings is not straightforward, and current approaches based on asset indices may fail to adequately stratify individuals in very poor communities. We therefore explore the relative impact of the trial across alternative measures of SES, with a focus on those harbouring the heaviest infections. SES is a recognised risk factor for hookworm infection, and remains so throughout this trial with individuals from households of lower SES being at substantially greater risk of infection at baseline and after two years of treatment in all three arms. However, we did observe an equitable reduction in the prevalence of HK infection across the poorest, middle and least poor groups in all arms of the trial by the end of the study. SES also continued to be a risk factor for high intensity infections, however the poorest groups appear to have experienced a greater reduction in the risk of high intensity infection in comparison to the middle and least poor groups. Understanding the impact of intervention strategies in these high risk groups can help programmes to improve vertical equity by tailoring interventions to reach the most vulnerable.

1963

THE IMPACT OF DATA UNCERTAINTY ON MODEL PREDICTIONS FOR SOIL-TRANSMITTED HELMINTHS

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Anthelminthic therapy is effective against *Ascaris* and hookworm. And the World Health Organisation (WHO) recommends treating 75% of all SAC by mass drug administration (MDA) to reduce STH induced morbidity. However, this target is not always achievable due to a variety of reasons including school absence or limited access to remote areas. Mathematical models can assist in optimising the treatment strategy to achieve the WHO morbidity goals. We have developed a parasite transmission model that includes density-dependent fecundity, age-intensity profiles and aggregation of worm burden. The data needed to fit the model parameters are scarce and its quality varies widely across different settings. An example of this is the MDA coverage, which is often difficult to estimate as the underlying demography is unknown. In this study, we investigate the impact of the uncertainty of MDA coverage data. The model is fitted to baseline prevalence data (assuming no historic treatment) and endline prevalence data (data collected after a MDA program). We fit all model parameters using Gibbs-sampling MCMC techniques and assume different achieved coverage levels for the MDA program. Additionally, we make forward projections with the fitted models to investigate the impact of data uncertainty on model predictions. Our

results illustrate that uncertainty of MDA coverage data plays a crucial role in determining the efficacy of MDA programs and highlight the need of high quality coverage data.

1964

QPCR DIAGNOSTICS FOR *ASCARIS LUMBRICOIDES*: CHARACTERIZING THE CONNECTION BETWEEN QPCR, KATO KATZ AND WORM BURDEN

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For a number of helminth parasites, new diagnostic techniques have been developed and are now starting to be used in the field. Traditional techniques involving egg counts are being superseded by antigen-based (e.g. CCA for schistosomes) approaches and PCR methods. To achieve continuity as diagnostics change, it is important to understand the relationship between the different diagnostic results. For example, the WHO treatment guidelines for control are in terms of prevalence as measured by egg-counting techniques and community-level morbidity assessments are often based on intensity thresholds from the same techniques. How will these guidelines be affected by new diagnostics? The data used in this analysis is from a study carried out in western Kenya, comprising a baseline infection survey and a follow-up survey several months after mass drug administration. We analyse *Ascaris lumbricoides* infection data from both time points, consisting of matching Kato-Katz, qPCR and expulsion data. We construct a model of host infection and diagnosis that is, as far as possible, based on realistic biological mechanisms. Bayesian methods are employed to fit our model to the data, employing prior estimates of key parameter values. Our underlying mechanistic model allows us to characterise the distribution of both egg counts and qPCR output as a function of the underlying worm burden of the host. It is then possible to construct the statistical relationship between qPCR readings and Kato-Katz egg counts, allowing an approximate conversion between qPCR output and standard thresholds of light, medium and heavy infection at the individual level. Applying these results at the population level, we show the implications for the sensitivity of the Kato-Katz diagnostic technique over a wide range of prevalences. Results for very low prevalences are particularly important in light of the increasing interest in elimination of soil transmitted helminths.

1965

PREVALENCE OF STRONGYLOIDIASIS IN SOLID ORGAN TRANSPLANT PATIENTS AND POST-TREATMENT TEST-OF-CURE EFFICACY

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Immunosuppression of a host with chronic *Strongyloides stercoralis* infection can lead to hyperinfection syndrome or dissemination. Screening for *S. stercoralis* in transplant patients is done with commercial crude-antigen enzyme-linked immunoassays (ELISAs) known to cross-react with other helminths. NIE is a specific recombinant *S. stercoralis*-specific antigen. We used *S. stercoralis*-specific NIE Ss IgG ELISA to assess the prevalence of strongyloidiasis in transplant patients. We prospectively recruited 150 solid organ transplant patients from endemic regions. Serum was sent for commercial *Strongyloides* IgG ELISA and a recombinant NIE Ss IgG ELISA was performed on all serum samples at our institution. Metadata including demographics, transplant status, absolute eosinophil count, and immunosuppressive regimen were recorded. Preliminary data yielded a median age of 64 years old with 76% of patients were from Latin America, 12% from the Middle East, 7% from Asia, and 4% from Africa, and 1% from Eastern Europe. Commercial *Strongyloides* ELISA yielded 10.7% positives and NIE Ss IgG ELISA was positive in 5.9% of all patients (80% pre-transplant, 20% post-transplant). There was a significant increase in *S. stercoralis* IgG using the NIE Ss ELISA compared to commercial assays (geometric mean 18.3 vs 9.84, p=0.0001). Eosinophilia was higher for patients with positive NIE Ss ELISA or commercial ELISA versus negative assays (geometric mean 560, 383, and 122 cells/µl respectively, p=0.0096). NIE Ss IgG ELISA had a specificity of 97% and a 92% negative predictive value (p=0.001) for *S. stercoralis* infection in this population. We found an endemic prevalence of strongyloidiasis at our institution in the solid organ transplant population. The NIE Ss IgG ELISA is specific for *S. stercoralis*. Eosinophil degranulation proteins will be assayed and associated with activated eosinophils in transplant patients with strongyloidiasis. Post-treatment efficacy will be completed prior to conference. We recommend screening all at-risk solid organ transplant patients for strongyloidiasis, especially those with eosinophilia.

1966

PREVALENCE OF STRONGYLOIDIASIS IN GUATEMALA INCLUDING AN AT-RISK IMMUNOSUPPRESSED POPULATION

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Strongyloidiasis is a neglected tropical disease likely underestimated to affect 100 million people worldwide. While the majority of disease burden relates to a chronic insidious effect on health, loss of immune control can lead to potentially fatal hyperinfection syndrome, a condition particularly associated with corticosteroid treatment. The aim of this study was to investigate seroprevalence of *Strongyloides stercoralis* in two populations in Guatemala, including a group of individuals potentially at risk of hyperinfection syndrome. In phase I, serum was collected from consecutive patients presenting with fever symptoms to a health centre in a rural area. In phase II, existing serum samples from patients seen in a rheumatology clinic in Guatemala City were randomly selected from computerized clinic records. Serum samples were analyzed for antibodies to *Strongyloides stercoralis using* a recombinant antigen NIE-ELISA assay, which has been validated to be more sensitive and specific for strongyloidiasis than other somatic antigen assays. The NIE-ELISA has no cross-reactivity to other helminths, which are endemic in Guatemala. In phase I, 49 out of 446 individuals were positive (seroprevalence in rural cohort = 11%). There was no significant association between antibody titre and age or gender. In phase II, 7 out of 106 individuals were positive (seroprevalence in urban-immunosuppressed cohort = 6.5%). We report high *Strongyloides stercoralis* prevalence in Guatemala, including in rheumatology patients who are taking immunosuppressive steroids and at increased risk of hyperinfection syndrome. Further work looking at risk-factors, infection intensity, and treatment response is needed to inform optimal testing and treatment strategies for *Strongyloides stercoralis* in this group of patients.

1967

DETECTING THE PREVALENCE OF CHAGAS DISEASE AMONG PEOPLE LIVING WITH HIV/AIDS IN LOS ANGELES COUNTY, CALIFORNIA

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Chagas disease (CD) is a well-known tropical parasitic disease with a historical footprint in Latin America. Unfortunately, a qualified detection system to identify CD is largely missing in the global north, with over 300,000 estimated cases in the United States alone (Meymandi et al. 2017). In a recent analysis of foreign-born Latin American patients being screened in Los Angeles County (LAC), CD was detected in 1.24%

of the population (Meymandi et al. 2017). CD is known to follow an insidious course, leading to a number of chronic outcomes, including cardiomyopathy. Special populations, including people living with HIV and AIDS (PLWHA) may be at a higher risk of CD and its progression to adverse outcomes. This hypothesized risk calls for a large sample size to explore the risk of CD among PLWHA in the United States. The objective of this study is to (1) measure the prevalence of CD among Latin American foreign-born PLWHA residing in LAC, CA and (2) investigate the associated with CD. Retrospective data analysis of Latin American foreign-born PLWHA aged 18 and above years seeking services at AIDS Healthcare Foundation (AHF) Healthcare Centers (HCCs) between 05/01/2017 and 03/31/2018. Descriptive analyses were performed using frequency distributions and nonparametric chi-square tests to examine the prevalence of CD. Two clients out of a subpopulation of 179 were identified as CD-positive ($p < 0.01$): one male and one female, both originating from Honduras. The prevalence documented at AHF Healthcare Centers (1.11%) is slightly lower than the 1.24% prevalence reported previously in LA County (1.24%) reported by Meymandi et al. (2017). While this is a relatively small sample size, the statistically significant results suggest developing CD testing and treatment guidelines among Latin American foreign-born PLHWA—a prominent subpopulation in LAC.

1968

ASSOCIATION OF FUNCTIONAL GENOTYPES AND HAPLOTYPES IN ADIPONECTIN GENE WITH BMI, VIRAL LOAD AND CD4⁺ T CELL COUNT AMONG HIV-1 INFECTED ANTIRETROVIRAL TREATMENT NAÏVE AND EXPERIENCED KENYAN INJECTION SUBSTANCE USERS

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Human immunodeficiency virus (HIV) and injection drug use have both direct effect on and epigenetic effects on genes. These effects could be beneficial or detrimental in defining disease outcomes. *ADIPOQ* gene is key in modulating metabolic and immunoregulatory functions. Understanding the effects of HIV and injection drug use on the gene in the context of antiretroviral therapy is important for predicting disease outcomes. This cross-sectional genetic study determined polymorphisms in the promoter region of the *ADIPOQ* gene. Two loci of *ADIPOQ* gene were analyzed rs2241766 and rs266729.The polymorphisms were associated with clinical markers of disease outcome; BMI, viral load and CD4+ T cell count. The selected SNPs were amplified via PCR then genotyped via random fragment length polymorphism. The GG genotype at locus rs266729 was associated with low BMI (OR = 2.03; 95% CI; 1.11-3.70; P = 0.021). GC haplotype was associated with low CD4+ T cell count (OR = 1.91; 95% CI; 1.54-3.51; P = 0.036), while GG haplotype was associated with a high viral load (OR = 2.14; 95% CI; 1.27-4.26; P = 0.011). TC haplotype was associated with a high BMI (OR = 1.79; 95% CI; 1.86-3.16; P = 0.042) and TG haplotype was also associated with a high BMI (OR = 1.97; 95% CI; 1.12-3.48; P = 0.019). The study revealed functional genotypes and haplotypes of the *ADIPOQ* gene at loci rs2241766 and rs266729 that could determine disease outcomes in HIV-1 antiretroviral therapy naïve and experienced injection drug users.

1969

FACTORS ASSOCIATED WITH PMTCT UTILIZATION AMONG HIV-INFECTED WOMEN

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Prevention of mother-to-child transmission (PMTCT) has led to significant decreases in the number of infants acquiring HIV infection. For barriers to be addressed and PMTCT programs to reach their target, a better understanding of women not being reached and at high risk of transmitting HIV to their infants is needed in the era of option B+ and in diverse settings. A prospective study was conducted of HIV-infected pregnant women delivering their infants at seven health facilities in Southern Province, Zambia from February 1, 2016 to January 31, 2018. The health facilities included Macha, Livingstone Central Hospitals and associated health centers. Mothers were administered a questionnaire, a chart review was completed and a dried blood sample was collected from the infant and sent to the central laboratory for HIV DNA testing. Of the 802 mother-infant pairs enrolled in the study, 794 had complete data and were included in the analysis. 92% of mothers received PMTCT. Infants born to mothers who did not receive PMTCT were more likely to have detectable HIV DNA at birth (3.0% vs. 1.0%; p = 0.17). Mothers who did not receive PMTCT were younger (28 vs. 30 years; p= 0.06), less likely to have received antenatal care (3.0% vs. 0.7%; p = 0.05), and more likely to report having been diagnosed with HIV infection during pregnancy (62.5% vs. 32.8%) or during labour (7.1% vs. 0.1%; p<0.0001). Among all mothers, 84.9% reported having been tested and knowing their status before the pregnancy. Among mothers diagnosed with HIV during pregnancy (n=275), 60.4% reported having been tested prior to the pregnancy. Among mothers diagnosed with HIV during labour (n=5), 80.0% and 20.0% reported having been tested prior to and during the pregnancy, respectively. PMTCT programs were effective in identifying and treating HIV-infected pregnant women. Few women eligible for PMTCT were missed as most women who did not receive PMTCT were diagnosed during pregnancy or labour. Frequent HIV testing of women should be emphasized to facilitate early diagnosis and treatment and prevent HIV transmission to their infants.

1970

THE USE OF DIGITAL DATA CAPTURE SYSTEMS IN EVALUATING THE IMPACT OF COMMUNITY HEALTH WORKERS INTERVENTION ON ANTIRETROVIRAL THERAPY RETENTION AND ADHERENCE IN TANZANIA

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The introduction of numerous HIV/AIDS prevention projects and campaigns by the Ministry of Health and donor parties have made significant gains in the scale-up of antiretroviral (ART) usage, thus the number of people on ART has been increasing since 2010. However, the adherence of infected people in attending comprehensive treatment centre (CTC) clinics on a monthly basis has not been consistent due to factors such as stigma, seasoned agriculture activities and lack of money for transport given that some people live far from their CTC clinics. Our study is evaluating ART program retention and viral load measures from clients receiving the existing standard of care CTC clinics, compared to clients receiving community health worker support for delivering ART medicines to HIV infected people in their communities. Multiple digital applications, including community mobile data collection via the online data kit (ODK), an online data portal, and a routine data archiving system have been implemented for this study to make sure all required data are well-captured. To better understand the adherence of participants in both study arms the study setup 20 sites (10 control and 10 intervention) in three regions of Tanzania. A total of 1986 participants were interviewed in the baseline phase, from whom tablet-based ODK forms were used to capture survey information, CTC record, and viral load data. Currently the study is collecting 12-month data, where behavioral surveys, clinic records, and viral load measures are again captured for comparison with the baseline data. The online portal was designed to provide summary information on the collected data which project managers and the data collectors can access the system at any time. As the study continues, the use of these multiple digital applications has proven to be highly effective in providing accurate data, secured data and reduced time lag between data collection, analysis and sharing with the partners.

1971

COÏNFECTIONS *OF SCHISTOSOMA HAEMATOBIUM* AMONGST HIV-SEROPOSITIVE AND -SERONEGATIVE WOMEN: KISANTU HEALTH ZONE, DEMOCRATIC REPUBLIC OF CONGO

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Schistosoma haematobium (SH) infection is a significant cause of morbidity amongst females in endemic regions, causing development of urogenital lesions which leave women vulnerable to the acquisition of HIV. However, HIV-SH co-infection has been poorly characterized in endemic zones, despite the fact that the parasite poses a serious health concern. In Kisantu, the reported prevalence of *S.mansoni* is 61.8%, yet *S.haematobium* is not monitored, making the scope of the problem (and its potential consequences) in this population difficult to ascertain. We conducted a cross-sectional analysis between October 2016 and February 2017 in the Kisantu Health Zone, DRC, to compare rates of *Schistosoma haematobium* infections between HIV-seropositive and -seronegative women. Interviews based on standardized questionnaires were administered to 383 consenting women who tested negative and 63 women who tested positive for HIV; afterward, urine and blood samples were collected and analyzed. *Schistosoma* infection was confirmed via urine microscopy and HIV infection was confirmed using rapid tests listed in DRC's 2017 National Algorithm for HIV Diagnosis (Determine™, Uni-Gold™, and Double Check™). The overall prevalence of SH among all study participants was 18.2% (95% CI: 15.0-22.2). The rate of SH co-infection amongst women positive for HIV was 37.3% (95% CI: 32.8%, 41.8%), while only 15.2% of women negative for HIV were found to harbor SH infection (95% CI: 11.9%, 18.5%). This study demonstrates significantly higher prevalence of SH infection in HIV-positive women than in HIV-negative women and emphasizes the importance of continued SH testing and communal treatment schistosomiasis-endemic regions, especially among vulnerable populations.

1972

IMPROVING CONTINUUM OF CARE IN DOMINICAN REPUBLIC FOR HIV-INFECTED PATIENTS CO-INFECTED WITH HEPATITIS B AND C

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The estimated prevalence of hepatitis B and C among people living with HIV/AIDS is 5–20% and 6% respectively in Latin America, representing 2 to 4 million people[1]. Liver diseases has become in the ART era the major cause of morbidity and mortality among PLWHIV[2]. The effective management of HIV people co-infected with hepatitis B and C is a public heath priority requiring testing service, linkage to care and access to treatment[3]. The objective of this study was to evaluate indicators involved with optimal cascade of care for HBV and HCV co-infected PLWHIV in Dominican Republic. Patients enrolled in care in two community-based clinics in Santo Domingo and Santiago, DR were evaluated for clinical indicators of HBV, HCV and HIV infections. Socio-demographic determinants and serological/chemical data were collected from clinical files, and statistical analyses were used to assess their distribution in different key populations like MSM, Trans, and drug users. A total of 2787 HIV-infected patients were evaluated. Co-infection with HBV was found in 28 patients, and 100% were followed up, 85.7% (n=24) of them were retained in care and ARV containing tenofovir were prescribed to 71.4% (n=20). HCV co-infection was identified in 8 patients, and 87.5% (n=7) were followed, while 75%% (n=6) were retained to care without receiving treatment for HCV. This study identified significant differences between the continuum of care of HBV and HCV co-infections. Access to HCV treatment in co-infected populations is crucial for care and in turn to decrease new infections. However, seems to be a subregistry on hepatitis status compared with the current epidemiological data from the region. A more systematic screening for viral hepatitis among PLWHIV will be essential for early management and treatment. Increased detection, linkage to care and long-term follow up with access to medication and HBV vaccination are public health priority to improve patient outcome and curb these epidemics.

1973

IMPROVING THE MONITORING OF TUBERCULOSIS INFECTIONS IN PEOPLE LIVING WITH HIV IN THE DOMINICAN REPUBLIC: A CASCAADE OF CARE MODEL

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Among the opportunistic infections in people living with HIV (PLWHIV), infections with M. tuberculosis appear to be the most common detected. To the date it is estimated a total of 268,500 patients per year in Latinamerica, and the Dominican Republic it estimated 59.8 cases per 100,000 in the general population, and 12% of co-infection TB/HIV has been reported by 2016[1,2]. This study is aim to propose a cascade of care for the TB/HIV for monitoring of interventions.We collected epidemiological data from three HIV primary care units from the two larger provinces in DR: Santo Domingo, and Santiago. Criteria for a positive TB infection were obtained from clinical records, based on clinical manifestations, and (+) baciloscopy/X-rays. Also, the criterion for a TB infection was not limited to pulmonary, but also included extrapulmonary infections (ganglionar, renal, etc). We analysed data by key populations (MSM/TG, Female Sexual Workers (FSWs), Drug uses (DU), and Migrants. A total of 5537 HIV (+) patients were enrolled in these three centres. Of those, 1% was confirmed as TB (+) (n=71). Among those TB/HIV (+) 41% received treatment, and 11% was considered cured (Figure 1). Among key populations, treatment access was higher (40% GP vs 46% KPs), however, cure rate was lower among KPs (8%) compared with GP (13%). Within those KPs: FSWs and MSM/TG were lower compared with migrants and DU (0% and 9%, respectively). This study revealed a disparity on access to care for tuberculosis treatment between general population and key populations; early access to care, and specific barriers of retention and early management might influence this. When compared between each KP, the lower rate of cure was observed in FSWs and MSM/TG, these findings are consistent with other studies from the DR where we also observed lower rates of retention in care and not achieving the final goal of finalizing TB treatment. Seems to be a subregistry of TB/HIV cases based on current epidemiological data from the region. This propose model of cascade of care is based on data reported and It is necessary a more intensive screening and retention to achieve the goal of a free TB-generation by 2020.

1974

A RARE CAUSE OF OLECRANON BURSITIS IN AN IMMUNOCOMPROMISED PATIENT: *PROTOTHECA WICKERHAMII*

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Human protothecosis is a rare algal infection caused by *Prototheca* spp; it is a ubiquitous achlorophyllic algae, and is the only known algae to cause human disease. Currently, the pathogenesis remains unclear and no treatment options have been adequately studied. Here, we present a case of olecranon bursitis caused by *Prototheca wickerhamii* in an immunocompromised patient. Six months prior to his presentation to our institution, a 45-year-old presented with left elbow pain after reportedly scraping his left elbow on a tree while doing yard work. He reported significant pain and swelling of the elbow after injury, which resolved on its own without intervention. He was diagnosed with HIV at that time and subsequently started on antiretrovirals. A short while later, he experienced recurrent elbow swelling and pain; an incision and drainage was performed and cultures demonstrated *P. wickerhamii*. He was unsuccessfully treated with oral voriconazole, and an attempt at treatment with IV amphotericin and oral doxycycline was made. However, he left against medical advice and presented to our institution. At presentation, both IV amphotericin and doxycycline were instituted with planned outpatient bursectomy. He clinically improved on that regimen but left against medical advice again prior to completing his anti-infective agent course. A six-week course of IV amphotericin and oral doxycycline was slated for recommendation for the *Protothec*a infection. Given the rarity of this pathogen, no official treatment guidelines exist and there are few studies analyzing the susceptibility of *Prototheca* to medication. Treatment is challenging due to slow-growing nature of the algae, the paucity of research studies, and the limited susceptibility of this pathogen. Furthermore, patients diagnosed with disseminated *P. wickerhamii* can have a mortality rate upwards of 67%. This case adds to the limited body of literature on this global organism by demonstrating the clinical presentation of protothecosis and highlighting the pathology and current treatment options.

1975

CRACK LUNG MIMICKING MILIARY TUBERCULOSIS AND FUNGAL DISEASES IN A HIV-INFECTED PATIENT

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A 45-year-old female, HIV-infected patient, with a history of abusive use of inhaled cocaine, crack and marijuana was admitted to our service presenting with weight loss, fever, dyspnea, productive cough, sometimes with hemoptysis, during the last year. Her symptoms had worsened in the last few days before admission evolving to a severe dyspnea. At admission, the patient presented with oxygen saturation level of 85% at room air, diffuse wheezing and fine rales, and a respiratory rate of 26 breaths per minute. Chest x-ray showed a diffuse miliary infiltrate and a cavitated lung lesion at left lung. Due to a low CD4+ cell count, fluconazole for oral candidiasis and treatment for miliary tuberculosis and *Pneumocystis jiroveci* pneumonia were initiated. Sputum and gastric fluid smears for *Mycobacterium tuberculosis* were negative. Xpert MTB/RIF evaluation of the same samples was also negative. A higher dose of fluconazole was prescribed after a detection of a titer of 1:16 to *Paracoccidioides brasiliensis* and biopsy of a skin lesion showing the presence of fungi suggestive of *Cryptococcus neoformans*. Bronchoscopic examination showed a diffuse airway inflammation and histopathological evaluation of pulmonary biopsies revealed foreign body filamentary structures resembling suture material, lymphoplasmocitary infiltrates, exuberating peribronchial anthracotic pigmentation suggesting a ulcerated lymphocytic bronchitis. All laboratory investigations were negative for the presence of pathogens in the bronchoalveolar lavage. No granulomas were observed and staining for fungi, mycobacteria or other bacteria was also negative. Considering the diagnosis of crack lung, tuberculosis treatment was discontinued but treatment for either Paracoccidioidomycosis or Cryptococcosis with fluconazole remained. The patient experienced a great clinical improvement and further cultures (sputum, gastric fluid, bronchoalveolar lavage and skin biopsies) remained negatives for mycobacteria and fungi reinforcing the diagnosis of crack lung presenting with pulmonary features of either miliary tuberculosis or Paracoccidioidomycosis.

1976

SUCCESSFUL TREATMENT OF DISSEMINATED STRONGYLOIDIASIS WITH SUBCUTANEOUS IVERMECTIN

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A 35-year-old male, HIV-infected patient was admitted to our hospital complaining of a watery diarrhea of about 10 episodes a day, initiated four months from admission. Ambulatory treatment with Co-trimoxazole had been initiated at the beginning of the disease, but as there was no improvement of the symptoms it was suspended. Four days before admission, the patient started with nausea and incoercible vomiting accompanied by tachypnea and fever. On clinical examination, he was pale and with fine rales at lung bases. As CD4+ count was 22 cells/mm^3, empirical treatment for *Pneumocystis jiroveci* pneumonia was prescribed. After two days, he needed orotracheal intubation with further improvement of respiratory symptoms within the next 9 days. He then developed gastrointestinal bleeding and an endoscopic examination showed ulcerated lesions at gastro-esophageal junction without signs of active bleeding. The duodenal mucosa was friable and with multiple bleeding spots. Parasitological evaluation showed *Ancylostoma* sp eggs and *Strongyloides stercoralis* larvae. Other common causes of diarrhea were excluded. Gastric mucosa biopsies revealed countless helminthes on lumen of mucosal glands suggestive of *Strongyloides stercoralis*. Oral treatment with albendazol was initiated but soon switched to ivermectin by the rectal route because the patient had copious gastric daily drainage of more than 1,500 mL of gastric content. Oral ivermectin was prescribed after gastric drainage diminished, but after 10 days of treatment the parasitological analysis of the gastric fluid remained positive for *Strongyloides stercoralis* larvae. Subcutaneous veterinary-grade ivermectin was prescribed for 7 days. Parasitological analyses of feces and gastric fluid were negative the day after the end of treatment. Gastrointestinal bleeding ceased and the patient condition improved without need for repeating the treatment. This case report underscores the importance of disseminated *Strongyloides stercoralis* infection in immunosuppressed patients and that, sometimes, drastic approaches, such as using veterinary drugs, are needed for a successful treatment.

1977

ANTIBIOTIC RESISTANCE IN CHRONIC SUPPURATIVE OTITIS MEDIA IN A HIGH PREVALENCE HIV SETTING IN SWAZILAND: TIME FOR CHANGE

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Many HIV-infected patients in Swaziland have perforated tympanic membranes and deafness from recurrent or unresolved ear infections despite having completed multiple short courses of the same ineffective antibiotic based on country guidelines. Chronic infections cause severe

disability and morbidity in resource poor countries. In Swaziland, there is no consistent medical record keeping or continuity of care. Patients do not have ready access to specialty services and there are limitations in available antibiotics. School performance is compromised and there are no regular hearing screening or services for the deaf in country. We conducted a quality improvement project to understand the antimicrobial susceptibility patterns in chronic, recurrent ear infections among HIV+ patients who seek care at the Baylor College of Medicine's pediatric AIDS clinics. Patients were selected based on complaints of ear drainage, re-occurring otitis media and hearing loss. Ear drainage samples from 24 HIV patients who had received at least one course of treatment per Swaziland guidelines without prior cultures ages 2-24 yrs (mean 12 yrs) with a range of CD4 counts 257-1766 cells/uL (mean 870) were collected and sent for culture and sensitivity. Preliminary results from April-May 2017 show primarily gram negative bacterial pathogens like *Proteus* and *Pseudomonas. Proteus* had variable sensitivity but *Pseudomonas* remained sensitive to most anti-pseudomonal agents. Two *Staphylococcus aureus* isolates were resistant to penicillin and sulfamethoxazole-trimethoprim. There was resistance to third and fourth generation cephalosporins and even meropenem. As expected, resistance rates reflected the preferred antibiotics listed in the country guidelines for regimens to treat otitis media. Treatment particularly in refractory cases should be guided by antibiotic susceptibility testing. This data can contribute to the development of an antibiogram informing medication supply purchasing, availability as well as treatment guidelines in this immunocompromised population not addressed in current guidelines.

1978

CARBON NANOTUBE AS A NOVEL HIV-1 VACCINE ANTIGEN VECTOR FOR MUCOSAL IMMUNITY STIMULATION

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Developing a safe and effective preventive HIV-1 vaccine remains the hope for controlling the global AIDS epidemic. A mucosal vaccine offers several advantages including non-invasive application, elicitation of both systemic and mucosal immune responses. Currently, the problem with the use of proteins in HIV vaccines is that they are generally far less immunogenic than live or killed whole organism vaccines. Therefore there are major needs for a new HIV antigen delivery platform to enhance these immunogenicity of proteins. Luna has developed a needle free carbon nanotube HIV-1 vaccine (CNTVac) delivery platform that is capable of delivering antigens mucosally to prevent HIV-1 infections. The needle-shape CNTs demonstrated faster entry into antigen-presenting cells (APCs) compared with other spherical nanoparticles, which significantly increased the utility of antigens and shorten the time to effect. CNTVac was bio-mimetic to the dimension of HIV particle (length and diameter). The modified gp120 surface protein coating density on CNTs was fully characterized to mimic HIV. Luna's CNTVac has experimentally demonstrated to be easily recognized and taken up by APCs for induction of much greater (10 times) immune responses compared to protein alone. Luna's biocompatible nasal vaccine formulation has also exhibit increased epithelial permeability (~ 6 times higher than control) using a surrogate marker TEER and flux assay for measuring the integrity of intracellular junction dynamics. CNTVac has multiple copies of antigens (same or different) without changing their immunogenicity and enables the intracellular multiple antigens uptake, which reduces the ratio of the delivery vehicle and maximizes the effective dose of antigens and the delivery efficiency.

1979

IMPLEMENTATION OF AN EXTERNAL QUALITY CONTROL PROGRAM FOR HIV RAPID TEST IN THE PERUVIAN ARMED FORCES

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While HIV rapid test and WHO-approved diagnosis algorithms have greatly improved the field diagnosis and linkage to care, External Quality Control (EQC) of HIV rapid test is critical to monitor and improve the quality of testing. Unfortunately, the EQC programs are expensive and often lacking in resource-limited settings. Proficiency testing (PT) using dried tube specimen (DTS) is a simple, low-cost and practical method developed by the Centers for Disease Control and Prevention (CDC) to prepare and distribute PT panels and implement an EQC program attainable in a wider variety of HIV testing settings. The aim of our effort with COPRECOS, NAMRU-6, DHAPP and UNMSM was to improve the quality of HIV rapid testing processes in military health facilities in Peru through an EQC program using the DTS method. Two PT training workshops using DTS were conducted for 29 health workers who perform HIV rapid test. These were followed by the implementation of an EQC program for HIV rapid test using DTS in 10 military health facilities in 2016 and then 18 in 2017. Three PT panels were sent to participating sites, 10 in Lima and 08 in other provinces every 3-4 months during both years. Test results were collected and entered into a customized Microsoft Excel spreadsheet. PT performance was evaluated according to the panel and documentation scores, and appropriate corrective actions were taken following the CDC decision tree. Among the health facilities assessed, the baseline average PT performance was 75% in 2016 and 94% in 2017. The main reasons for unsatisfactory results in the 2016 PT were the misinterpretation of final results and incomplete final report. The assessments also showed that the national HIV testing algorithm was not followed. In 2017, only minor errors were observed during the PT. At the end of the 6 PT assessments, both the 2016 and 2017 testing groups scored 100%. Implementing an EQC program dramatically improved performance for HIV rapid test process and the accuracy of results across facilities. Extension of the program to include other military health units performing HIV testing and planning for long-term sustainability are ongoing.

1980

CHAGAS DISEASE. A SYSTEMATIC REVIEW OF CASE REPORTS THROUGH THE XXI CENTURY

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Chagas disease is a parasitic disease caused by *Trypanosoma cruzi*, a protozoan which is transmitted to humans primarily vector-borne. During its early stage patients may remain asymptomatic or may present unspecific manifestations, meanwhile during the late stage they may develop life-threatening complications. It frequently goes underdiagnosed and therefore remains untreated. The objective of our review was to describe the epidemiology of published cases to aid with

the characterization of the disease. A systematic review was conducted following the "*PRISMA*" guideline. PubMed database was searched using the search terms "*Chagas disease*" or "*American trypanosomiasis*" and limited to case reports published in English or Spanish, but without time frame. Full text articles were assessed for relevance and data extraction was performed as an iterative process. During the initial search 250 articles were obtained, from which 136, containing 205 cases were included. Males were more affected than females (111 vs 94) and the medium age of presentation was 35.78 years (+/-SD 19.47). Non-vector-borne transmission was reported in 32% of the cases and vector-borne in only 7%. The route of transmission remained unknown in 61% of the cases. Fever (39%), headache (13%) and malaise (11%) were the most common initial clinical manifestations, meanwhile dyspnea (25%), chest pain (22%) and edema (21%) were the most common late ones. Cardiomegaly was detected in 35% of the cases and gastrointestinal megasyndromes in 7%. Benznidazole was the treatment of choice in 44% of the cases. The mortality rate was of 27%. The conducted systematic review provides valuable information about Chagas disease published cases. From our knowledge, we are the firsts to report how its trends and behaviors have been presenting around the world through the last century, as most of its previous data came from specific regions. We consider that case reports represent a great opportunity for clinicians to learn from others experience, and that by understanding the past and current epidemiology of the disease we can develop a better approach to handle it.

1981

CHAGAS DISEASE AND MEXICAN IMMIGRANTS LIVING IN SOUTHERN CALIFORNIA

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Chagas disease (CD) affects the Mexican population on both sides of the Mexican-U.S. border. Mexicans are the largest Latino population in the U.S., among whom are an estimated 360,000-plus cases of CD. The persistence of CD is linked to sociocultural, economic, and political processes in immigrant's origin countries but in the United States as well. This study examines the experience of Mexican CD patients' living in Southern California regarding CD health programs, coping with diagnostic and treatment in the U.S., and retrospectively while living in Mexico. Thus far, 4 women, and 3 men adult Mexican born immigrants with a positive diagnostic for *Trypanosoma cruzi* have been interviewed while data collection is ongoing. Patients live in LA area, and attend the Center of Excellence for CD. I expect to include the analysis of another 10-20 interviews with patients by the time of the presentation. Preliminary qualitative analysis utilized MAXQDA 12. Participants in Mexico were prevented from any information, testing and treatment for CD. Those living in rural communities in Mexico confronted major barriers to health care due to the limited health infrastructure, poverty, and absence of health transportation. They infection status was unbeknown at the moment of the migration, and they have little or no knowledge about the disease. In the U.S., health care access substantially varies by insured and uninsured patients being more vulnerable the undocumented due to their additional low-income, and lack of knowledge of health programs. All patients confront barriers for CD that are transversal through the entire health care system in both countries. CD's low political priority in both Mexico and the U.S. is experience by patients thought the lack of knowledge about CD among physicians and health providers, complete absence of testing and treatment, bureaucratic obstacles, and long wait periods to access those procedures. In the interest of supporting the prosperity of both nations is important to address a binational program for CD that enhance health not only as an individual human right but also as a collective wellbeing that oversteps political borders.

1982

GEOSPATIAL-TEMPORAL DISTRIBUTION OF TEGUMENTARY LEISHMANIASIS IN COLOMBIA (2007 - 2016)

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Tegumentary Leishmaniasis (TL) is a neglected disease with worldwide distribution and considered a public health problem, especially in Latin America. In Colombia, the governmental epidemiological surveillance system (SIVIGILA) is responsible for collecting information on the presentation of cases of TL from each of the municipalities and departments. In absence of a study compiling and analyzing currently available metadata of TL in Colombia, this study evaluates the geospatial-temporal distribution of TL and identifies the regions of the country on which prevention measures should be established in order to control the disease. This is an exploratory descriptive analysis of the distribution of TL in Colombia. Information was collected on new cases of the disease during the years 2007-2016 from the Colombian reporting system (SIVIGILA). Incidence calculations were made based on population estimates by departments and biogeographical regions. Time evolution is shown in biennial maps. A 10-year series was analyzed, showing that the Amazon region is the most affected in terms of incidence, while the Andean region has the highest number of cases with a high variability among the departments that make it up. In those departments where there is a greater reported diversity of vector species, a large number of cases was observed. Transmission dynamics of TL in Colombia in the past 10 years have been variable, with a greater concentration of cases in the central and southern departments. The present study contributes to improve the understanding of the patterns of distribution of TL in Colombia and can be a basis for future studies of impact evaluation of Health policies in the country and the region.

1983

THE PROBLEM OF HUMAN AND EQUINE AFRICAN TRYPANOSOMIASIS: THE ARMY MEDICAL VETERINARY CORPS AND THE ROYAL ARMY MEDICAL CORPS IN THE AFRICAN CAMPAIGNS DURING THE GREAT WAR

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The Boer War provided a training ground for the use of veterinarians during the Great War a decade and a half later. During the war in South Africa, only about 130 civilian veterinary contractees were responsible for more than 300,000 horses. Indeed, the experience there provided an important impetus in the creation of the Army Veterinary Medical Corps (renamed the Royal Army Veterinary Corps in November 1918) during the interwar years. Drawing on their experiences in the Boer War, human and veterinary medical officers in the African campaigns of 1914-1918 once again faced trypanosomiasis, one that struck humans and horses alike. The need for qualified veterinarians was obvious: Cavalry without healthy light-draught horses or at-the-ready heavy-draught horses to haul supply wagons and artillery found their battle-readiness significantly compromised. Moreover, stricken cavalrymen were of little use against the enemy. This presentation, based on archival materials and contemporary accounts from the Great War, examines the devastating impact of African trypanosomiasis on African- Campaign horses and men and the efforts of practitioners to control the disease.

1984

CARRIAGE RATES OF *TRYPANOSOMA CRUZI* AMONG KISSING BUGS (*TRIATOMA* SPP.) IN SOUTHERN ARIZONA

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The three predominant triatomine species found in southern Arizona (*Triatoma rubida*, *T. protracta*, *T. recurva*) are known to harbor *Trypanosoma cruzi*, the causative agent of Chagas disease. We analyzed 219 triatomines for carriage of *T. cruzi* (*T. rubida*, N=114; *T. recurva*, N=87; *T. protracta*, N=18). These triatomine specimens were provided from our citizen science program and field collections from Tucson, Arizona and surrounding regions in southern Arizona. The distal two-thirds of the intestinal tract of each insect were isolated during hindgut dissection and DNA extraction was performed according to the manufacturer's protocol (Qiagen DNeasy® Blood & Tissue Kit). The DNA was then amplified by RT-PCR using the *T. cruzi*–specific primers TCZ1 and TCZ2. *Triatoma rubida* and *T. protracta* needed annealing temperatures of 57°C for 15 to 30 seconds and *T. recurva* required an annealing temperature 58°C for 30 seconds for amplification of these sequences. All PCRs were run with a positive control, which consisted of purified *T. cruzi* DNA and a negative control of H_2O. *Trypansoma cruzi* was present in 28.8% (63/219) of the total tested triatomines (41.2% (47/114) *T. rubida*; 22.2% (4/18) *T. protracta*; 13.8% (12/87) *T. recurva*). Among those triatomines that we could sex, 34.3% (24/70) of male and 29.3% (27/92) of female specimens were positive; nymphal stages were 21.1% (12/57) positive. Interestingly, 54.7% (29/53) of *T. rubida* collected from the most populated region of southern Arizona (Tucson) were positive. *Trypansoma cruzi* is common among the triatomines that naturally inhabit southern Arizona. Carrier rates were nearly two-fold higher among *T. rubida* compared to *T. protracta* and *T. recurva*, but not significantly different among male, female, and nymphal stages. This is the largest analysis to date utilizing molecular techniques to assess the incidence of *T. cruzi* carriage among triatomines in southern Arizona. Further studies are needed to better understand the risk and potential transmission of *T. cruzi* to humans in this region.

1985

A CROSS-SECTIONAL STUDY OF CHAGAS DISEASE AND VECTOR EXPOSURE IN A HIGH-RISK POPULATION OF TEXAS HUNTERS

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Chagas disease, caused by the protozoan parasite *Tyrpanosoma cruzi*, remains one of the most significant neglected tropical diseases affecting the Americas. It is estimated that 6 to 8 million people are infected with this parasite worldwide, with 300,000 living in the United States. This disease causes progressive cardiac damage in about 30% of infected people, resulting in significant morbidity and mortality. Symptomatic cardiac disease does not usually develop until decades after the initial infection. Initial stages of Chagas disease are typically asymptomatic, making it difficult to detect cases early when the treatment is most effective. Therefore, identifying high-risk populations is important for understanding Chagas disease transmission and directing public health resources. We have recently identified that Texas hunters may be at elevated risk for contracting Chagas disease due to their extended amount of time spent outdoors and frequent contact with wildlife. To assess their exposure to *T. cruzi* and the arthropod vectors we began a state-wide screening program. Through our recruitment efforts at public hunting areas, private hunting organizations, and hunting expos we have been able to enroll over 1,000 hunters for this study. For each participant, we have conducted a risk-factor interview and collect a blood sample to assess disease status and exposure frequency to wildlife reservoirs and vector species. This large-scale screening program represents a novel approach to better understanding Chagas disease transmission and vector exposure in this high-risk population in the southern United States.

1986

TRYPANOSOMA CRUZI IN DOMESTIC AND WILD RESERVOIRS OF THE DEPARTMENT OF CÓRDOBA

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Introduction: T. cruzi reservoirs can act as a source of infection for animals, vectors, and humans contributing to the establishment of Chagas disease (CD) in a particular area. The department of Córdoba was not considered as a transmission area for CD; however, during the last years the report of several acute cases of the disease have motivated the study of a new transmission scenario for this region. In consequence, this study aimed to detect T. cruzi in domestic and wild reservoirs of the department of Córdoba. Materials and methods: During September 2016 and July 2017, a descriptive cross-sectional study was conducted in 4 villages of the municipality of San Andrés de Sotavento and two villages of the municipality of Sahagún. Blood samples from canines living in the zone were collected in EDTA vacutainer tubes after obtaining an informed consent from their owners. Wild reservoirs were capture using Sherman, Tomahawks traps and bats mist nets. T. cruzi DNA was detected using kDNA and the tandem repeat region as molecular targets. SL-IR was used as a molecular marker for detection of the lineage. Results: 168 canines and 146 wild mammals were included in the study, 114 individuals of the order Chiroptera, 12 individuals of the order Rodentia, 20 individuals of the order Didelphimorphia. The most abundant species was G. soricina with 17,1 % of the total individuals captured, followed by the species D. marsupialis with 13,0%. T. cruzi DNA was detected in one canine from Villa Lucía and in 13.0% of wild animals; 16 D. marsupialis, 2 H. anomalous and one A. planirostris. The TcI lineage was found in 12 D. marsupialis, 2 H. anomalous and one canine. One specimen of D. marsupialis with TcI and TcII lineages was also identified. Conclusions: T. cruzi DNA was detected in domestic and wild animals in the studied municipalities, indicating the circulation of the parasite in peridomiciliary environments. D. marsupialis presented the highest percentage parasites, representing an important reservoir candidate in the maintenance of the wild and domestic cycle in this geographical area.

1987

PREVALENCE AND RISK FACTORS OF CHAGAS DISEASE AMONG AGRICULTURAL WORKERS IN NORTHWESTERN NICARAGUA

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Chagas disease is an infection caused by the parasite, *Trypanosoma cruzi*, and is primarily transmitted to humans by Triatomine insects. This disease is endemic throughout the Americas, and it is estimated that 6-8 million persons are infected. Thirty percent of those infected are likely to develop

Chronic Chagas Cardiomyopathy (CCC), a rapidly, progressive form of cardiomyopathy. CCC is the most common cause of heart failure in Latin America, and sudden cardiac death is the most common presenting symptom. Nicaragua is a country that historically has had a high burden of Chagas disease. Despite the formation of vector control programs, there is evidence of sustained transmission among its inhabitants, but there is limited published literature available on the current prevalence of Chagas disease in northwestern Nicaragua. Given concerns for continued transmission and vector exposure, the prevalence and risk factors among northwestern agricultural workers was assessed. Using rapid diagnostics (chromatographic immunoassays), the prevalence of Chagas disease was found to be 1.6%. There was no statistical significance between those infected and uninfected based on age (≤21yo and ≥38yo), gender, or working in an indoor or outdoor environment. However, given the recent vector control programs, the lack of statistical significance of infection between the younger and older groups raises concern of ongoing transmission among this population. Further prevalence studies and risk factor assessment is needed to determine the current risk of Chagas disease among different populations in northwestern Nicaragua.

1988

DECREASING THE IMPACT OF CHAGAS DISEASE THROUGH MODELLING: THE DICTUM FRAMEWORK FOR RETRIEVING, COLLATING, AND ANALYSING SEROSURVEY DATA FOR CHAGAS DISEASE ACROSS LATIN AMERICA

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In order to assess progress towards interruption of intra-domiciliary transmission of *Trypanosoma cruzi* in the 21 endemic countries of Latin America, understanding the historical exposure to infection is paramount. Serological surveys can provide useful insight: if the data are age-structured, the force-of-infection (FoI) can be estimated retrospectively according to the ages of the serosurvey participants. Following from previous analyses of data from Colombia, which allowed understanding of the spatio-temporal profiles of the FoI, we expand this work across Latin America to support the endemic countries' goals. The first stage was to search published literature and construct a framework for collating and harmonising serological survey data and preparing these for modelling the FoI. We constructed a comprehensive search for PubMed, Embase, LILACS, Global Health, CAB Abstracts and Web of Science, to find published data for the endemic countries. Gaps in the published, retrievable literature were identified. We constructed standardised data extraction forms and collated data for a subset of these countries. The serosurvey results were harmonised and stored, with survey and source meta-data, in a relational database. The total number of serosurveys stored after screening and elimination of literature as of March 2018 is 241. This includes, from 3421 search results for these countries: from Brazil: 27; Argentina: 48; Mexico: 31; Bolivia: 19; Central America: 7 (Guatemala: 6; Costa Rica: 1), alongside 109 datasets from the Colombia work, which were collated from published and unpublished literature. Of note, Central American countries have a paucity of published data. There are 116 datasets from rural areas, 44 from urban, 32 from mixed settings or setting not ascertained from the reference. The database facilitates the retrieval of data for use in catalytic models to reconstruct estimates of the FoI through time and space. Model outputs, primarily the FoI estimates, are also stored in the database ready for further analyses such as obtaining estimates of disease burden.

1989

COMPARISON OF COSTS BETWEEN TWO STRATEGIES OF ACTIVE CASE FINDING OF HUMAN AFRICAN TRYPANOSOMIASIS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Human African Trypanosomiasis (HAT) is a parasitic vectorborne disease that remains a major problem in Sub-Saharan Africa. WHO's goal is the elimination of HAT as a public health problem by 2020. Current control and surveillance activities include case finding, treatment, and tsetse control. These strategies are implemented at different levels and intensity according to the epidemiological situation, the financial resources and the capacity available. Since many years active screening campaigns through truck-based mobile teams are the main control strategy for endemic areas. A more intense deployment of the right mix of strategies and sustained funding will be needed to achieve HAT elimination. Today only limited information is available to estimate the related costs. In 2015 a project started to eliminate HAT in two endemic health zones in the Democratic Republic of the Congo. One of the main activities was the expansion of the active screening capacity through the introduction of motorcycle based "mini" teams using an adapted screening algorithm. Both the classical and the mini-teams have a screening capacity of 60,000 to 70,000 people annually. The aim of this study is to estimate the costs of both strategies from a healthcare provider perspective. We combine several costing methods, including bottom-up costing and gross-costing. Sensitivity analysis is conducted on key parameters which could affect the results, such as the price of inputs and screening capacity to examine the robustness of the results. The preliminary results show that the average cost per person screened and per person diagnosed for a mini-team is lower (1.12$/person screened) than for a classical team (1.40$/person screened) excluding programmatic costs. We will present a detailed breakdown of the factors that influence these costs and discuss their importance under different scenarios, such as the type of screening algorithm, varying disease prevalence and inefficiencies in resource use for diagnosis. Our data allow for estimating the economic costs of HAT screening campaigns and will contribute to rational decision making in HAT control and elimination programs.

1990

THE VALUE OF HAT STAGING BEYOND TREATMENT

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Gambiense Human African Trypanosomiasis (gHAT) is rapidly approaching elimination as a public health problem in many of its endemic areas. As active screening efforts are reduced, control programmes are compelled to extract more information from a smaller set of epidemiological data. gHAT infections progress through two biologically distinct stages, with the transition from the first to the second marked by trypanosome infiltration of the cerebrospinal fluid. While stage information has traditionally only been used for treatment purposes, the incidence ratio of the two stages has been conjectured to contain useful information regarding undetected cases, the impact of interventions, and proximity to disease elimination. However, there remains much work to be done in bridging the gap between conjecture and application. In order to bring the stage ratio into the operational space for gHAT control, elimination, and surveillance, a detailed analysis of stage ratio behavior is needed. Through mathematical models of gHAT epidemiology, we explored how differences in near-

elimination scenarios would be borne out in measurements of the stage ratio, with attention given to potential uncertainties such as importation rates and the presence of asymptomatic cases. Based on the simulations, we determined what epidemiological conclusions can be reliably drawn from a given observation of the stage ratio, and identify any additional data sources that might narrow the range of transmission intensities consistent with the measurements. These results form the basis for recommendations to assist national control programmes in their ability to assess the need for sustained, decreased, or increased interventions. The value of this framework will be demonstrated by applying it to traditionally under-screened, HAT-endemic areas in Eastern Democratic Republic of the Congo that are thought to be near elimination.

1991

INVESTIGATING THE GENETIC DIVERSITY OF *TRYPANOSOMA CRUZI* FROM *PROCYON LOTOR* IN LOUISIANA USING A NEXT-GENERATION SEQUENCING APPROACH

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Trypanosoma cruzi, the causative agent of Chagas disease, is a protozoan parasite with complex transmission patterns and considerable genetic diversity represented by seven stable genetic lineages, known as discrete typing units (DTUs) named TcI-TcVI and TcBat. As *T. cruzi* can infect a broad range of mammalian hosts, enzootic cycles involving peridomestic animals may pose a spillover risk to humans. Local transmission has been demonstrated in the southern United States in recent years, highlighting the need for investigation into prevalence and transmission patterns to fully assess risk and plan control measures. Raccoons (*Procyon lotor*) are a particularly important reservoir to investigate in terms of *T. cruzi* infection in the United States due to their close association to human habitats, relatively high parasite prevalence, high parasitemia, and high infectiousness index. Previous investigations have reported the circulation of TcI and TcIV in raccoons in the US, but have faced genotyping limits inherent to Sanger sequencing methods, which fail to detect mixed infections and accurately capture the scope of parasite diversity. We tested heart and colon samples from 71 raccoons trapped across six parishes in Louisiana via polymerase chain reaction using primers targeting *T. cruzi* satellite DNA, resulting in a prevalence of 23.9% (17/71). Primers targeting a 500-bp mini exon region were then used to amplify DNA for next-generation sequencing and phylogenetic analysis. We predominantly identified TcI followed by TcIV in these raccoons. However, multiple haplotypes were found in most animals, including mixed infections with TcI and TcIV, highlighting the sensitivity of our methods to detect *T. cruzi* diversity in hosts and suggesting polyclonal infections. These results confirm the high infection rate in raccoons in the region, and programs to control *P. lotor* reservoirs may prove important to human health.

1992

COMPANION DOGS LIVING IN UNDERSERVED COMMUNITIES ALONG THE US MEXICO-BORDER SERVE AS RESERVOIR HOSTS FOR *TRYPANOSOMA CRUZI*, AN INCREASING PUBLIC HEALTH THREAT

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Chagas disease is caused by the zoonotic parasite *Trypanosoma cruzi* and transmitted by triatomine vectors; the parasite affects 8 million people worldwide. While high quality housing in the southern US generally prohibits colonization by triatomines, there are focal areas in which domestic infestations can occur, including in impoverished and underserved communities along the Texas-Mexico border (colonias). We previously detected a low prevalence of autochthonous human infections in colonias. In the current study, we expanded surveillance for *T. cruzi* infection in companion dog populations living in Texas colonias. We hypothesized that these dogs are highly exposed given that animals in colonias receive little to no veterinary care and typically live a free-range lifestyle. In summer and fall of 2016-2017, a cross-sectional study was conducted to quantify the prevalence of *T. cruzi* exposure among owned dogs. Through the aid of a promotora (a culturally-competent community health worker), we conducted door-to-door outreach and collected blood samples from dogs with informed owner consent. Through serological testing of 231 canines, we identified 82 (35.5%) positive individuals confirmed by at least two independent antibody detection platforms; this seroprevalence is markedly higher than that which we previously found in shelter (18%) and working (19%) dogs across the state. Dogs sampled in the fall were twice as likely to be seropositive than those in summer ($P<0.01$), likely due to time needed to develop antibodies following exposure during the peak summer activity period of vectors. Parasite DNA was detected in 6 (2.6%) of the dogs, indicative of a circulating infection. The subset of these dogs from which we determined parasite strain were infected with 'TcI', the *T. cruzi* strain associated with autochthonous human disease in the US. Domestic dogs living in colonias may serve as reservoirs to infect vectors in proximity to colonia residents, potentially increasing the risk of vector-borne transmission. Under a one-health framework, the identification of local reservoirs is a key component in developing public health interventions.

1993

ASSESSING BEHAVIORAL RISK FACTORS FOR DISEASE TRANSMISSION AT THE HUMAN-ANIMAL INTERFACE IN LAIKIPIA COUNTY, KENYA - 2017

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Human behaviors can drastically affect the risk of disease transmission between humans and animals such as anthrax, brucellosis and rabies. Understanding the cultural and behavioral risk factors in a community can therefore inform effective educational and intervention campaigns to minimize zoonotic disease transmission. We interviewed 156 persons living, working, or visiting two areas with high levels of human-animal contact in Laikipia County, Kenya, using cluster sampling from community-based recruiting. We interviewed 100 (64%) persons at Lekiji and 56 (36%) persons at Mpala, both villages with human-livestock-wildlife interfaces. We asked questions about demographic characteristics, indirect human-animal contact through water and food sources, human-animal interactions, and health status. Interviewed persons ranged in age from 11 to 84 years (median 28 years); 63 (40%) were male, and 151 (97%) reported handling live animals within the last year. Of 121 adults interviewed, 48 (40%) had received no formal education. The majority (67%) of respondents sourced drinking water from unimproved water sources, and 115 (74%) shared their drinking water with animals. Individuals who shared their water with animals were less likely to treat their drinking water (OR = 0.29, $p<0.05$) than those who did not share their water. Fifty-two percent of respondents reported eating a sick animal in the previous year, and 44% reported consuming meat from an animal found dead. Males were more likely than females to have slaughtered animals (OR = 2.4, $p<0.05$) and to have eaten raw meat (OR=2.5, $p<0.05$) in the last year. Ninety percent of respondents were worried about disease and disease outbreaks in live animals at local markets. Although respondents were concerned about disease transmission, safe practices in water, food, and animal handling to decrease risk were not commonly used. Educational messaging through trusted community leaders and community groups should target individuals at higher risk for disease transmission.

1994

DO SPOROTRICHOSIS ETIOLOGICAL AGENTS EQUALLY DISPLAY POTENTIAL VIRULENCE ATTRIBUTES?

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Since the mid-90s, Rio de Janeiro, Brazil, faces an epidemic scenario of sporotrichosis, a neglected zoonosis caused by pathogenic dimorphic fungi of the *Sporothrix* genus. *Sporothrix* adaptation to both saprophytic and parasitic lifestyles requires a set of survival skills in extremely different conditions. To describe and compare *in vitro* virulence attributes of culture collection as well as of clinical strains obtained from infected cats in the State of Rio de Janeiro, Brazil, yeasts, conidia and mycelia of three reference strains (*S. schenckii stricto sensu*- Ssch; *S. globosa* - Sglo and *S. brasiliensis*- Sbra) as well as eight *S. brasiliensis* clinical isolates (SbraC) were investigated. *S.glo* was not able to display hemolytic activity or phospholipase production. All other species/strains were able to produce protease and hemolityc activity. All *S. brasiliensis* produced phospholipase, while esterase was produced solely for those obtained from feline infection. Phytase production was not detected. *S. brasiliensis* hydrophobicity of conidial and yeast forms were higher than *S. schenckii*. Conidia size did not differ among *Sporothrix* species, while granularity showed great variation within species. As for the parasitic phase, both parameters were different among the three species. Yeasts and mycelia from all the studied strains formed biofilm on polystyrene in a typical time-dependent process. The capacity of yeast biofilm formation was different among the species concerning biomass and cell survival. Comparatively, SbraC biomass was smaller than obtained for the Sbra strain on polystyrene, however clinical isolates proven to be more able to survive on the same conditions. *SbraC* showed wider diversity in enzymatic activity, suggesting a potential role for these enzymes in *S. brasiliensis*-host interaction. In addition, although composed of lower biomass, clinical isolates of this species formed biofilms in polystyrene with higher cell survival rates. Our results add data to the understanding of *S. brasiliensis,* the most prevalent species in the Brazilian feline sporotrichosis epidemics, major adaptations to the vertebrate hosts.

1995

ZOONOTIC THREATS OF MONGOLIAN HERDING HOUSEHOLDS: A ONE HEALTH APPROACH TO IDENTIFYING RISK FACTORS AND DISEASE PERCEPTIONS

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Mongolian herding families live in close proximity to livestock and rely on animals for meat, milk, fur, hide, transportation, sport, culture, and income. However, this contact puts herders and their families at risk for zoonotic disease exposure. In order to understand the this herder-livestock relationship within a larger One Health framework, a comprehensive survey was conducted in 250 rural, peri-urban, and urban households across Selenge, Zavkhan, Dundgovi and Tov provinces to identify household animal contact, water, sanitation, and hygiene behaviors, gender roles and responsibilities related to animal husbandry and herding, animal and human diarrheal disease history, and zoonotic knowledge, attitudes, and risk perception. From our results, we found that animal contact at households occurs frequently (n=149) and almost all rural and many peri-urban households reported animal ownership, particularly with dogs (n=166), sheep (n=149), goats (n=148), cows (n=137), and horses (n=135). Animal husbandry practices were shared among men and women for herding, feeding livestock, assisting with sick animals, and tending to births. However, gender roles were apparent in other chores with males performing home slaughter and butcher and women in charge of the milking and cooking. Most study households lived in a ger (n=167) and the majority reported having a dedicated hand washing area at their home (n=147). Over 90% of households wash their hands after animal contact (n=136). And despite an overall knowledge (83%) of disease transmission from animals to humans (n=207), there was a gap in recognizing that humans can transmit disease to animals, or reverse zoonosis (n=21). The majority (82%) of households believe that animal contact presents a disease risk for humans (n=204) but most do not believe that it is possible to avoid animal contact as a zoonotic prevention method (n= 39). The relationship between Mongolian nomads and their animals is unique and future One Health education and interventions to make their human-animal interface healthy and safe should be tailored to the distinct needs of each herding community.

1996

COMPARING SURVEYS ON TICK BITE HISTORY TO EVIDENCE OF TICK-BORNE DISEASE EXPOSURE AMONG NOMADIC MONGOLIAN HERDERS

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Twenty-six percent of Mongolians live pastoral lifestyles, increasing their likelihood of exposure to ticks and placing them at a higher risk for contracting tick-borne diseases (TBDs). *Anaplasma* spp. and *Rickettsia* spp. have been identified in ticks, wildlife, and humans in Mongolia, but no known qualitative research has been conducted investigating the association between nomadic herder characteristics, tick bite history, and exposure to TBDs. To better understand the association between self-reported tick bites and symptoms versus actual exposure to TBDs, this study paired serological data with 335 administered surveys from 2014 to 2015. Results identified some similar trends in reporting of tick bites and actual exposure to TBDs, with significantly more reports of tick bites among men than women ($\chi^2 = 4.77$, $p = 0.029$), and increased exposure to either *Anaplasma* spp. or *Rickettsia* spp. among men ($\chi^2 = 5.25$, $p = 0.022$). However, results also identified inconsistencies in reporting and seroprevalence among different age groups, with children having the highest reporting and treatment seeking rates but low levels of exposure in comparison to other groups. While survey results showed that individuals were aware of peak tick seasons and tick species that inhabit specific areas, 58% of heads of households (49/84) were unaware that ticks can cause disease in livestock or dogs. This study suggests that herders are an at-risk population in Mongolia with gaps in awareness of TBD risk. Increased surveillance paired with focused outreach to prevent TBDs targeted to the herder population is encouraged.

1997

THE COEVOLUTION EFFECT: A HYPOTHESIS TO EXPLAIN ZOONOTIC PATHOGEN SPILLOVER

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Global habitat loss has led to the emergence of infectious diseases (EIDs) of wildlife origins into human. Despite this well accepted narrative, the mechanisms driving the association between habitat degradation and disease emergence remain unclear. A novel hypothesis, the Coevolution Effect, is proposed here to explain the underlying eco-evolutionary mechanisms at the population level driving EIDs. Evoking theories of island

biogeography, specifically, testing whether loss of habitat connectivity creates multiple coevolutionary engines that generate increased pathogen diversity across the landscape. The engines are isolated within habitat fragments with independent evolutionary trajectories comprising wildlife hosts, obligate parasite vectors (e.g., lice, batflies), and their coevolving microparasites. When combined with bridge vectors (e.g. mosquitoes, ticks) this coevolutionary feedback loop will cause increased opportunity for spillover of unique pathogens into human communities, and the potential for any one of them to induce an outbreak. To test the Coevolution Effect hypothesis, it is ideal to work in paired regions of high and low forest connectivity, the researchers will (1) integrate landscape genomics and demographic modeling to determine the effect of forest connectivity on the population structure of wildlife host and obligate parasite vectors; (2) characterize pathogen diversity in species across the landscape and target pathogen lineages within hosts and parasites to test for a relationship between the degree of habitat connectivity and pathogen genetic diversity using phylogenetic models; and (3) use targeted pathogen metagenomics to test the Coevolution Effect, specifically, if mobile bridge vectors like mosquitoes can mobilize the new pathogen genetic variants from the wildlife- parasite coevolutionary engines into human communities.

1998

ANTIMICROBIAL SUSCEPTIBILITY OF *ARCOBACTER*, AN EMERGING ZOONOTIC PATHOGEN ISOLATED FROM FECAL HUMAN AND ANIMAL SAMPLES (CATTLE AND PIGS)

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Few reports of evidence of antimicrobial susceptibility of *Arcobacter spp.* have been published worldwide and no reports yet exist for Peru. To know the antimicrobial susceptibility of *Arcobacter*, an emerging zoonotic pathogen isolated from fecal human and animal samples (pigs and cattle). Antimicrobial susceptibility tests were carried out with 20 strains of human fecal samples and animals, using E-test for the determination of the minimum inhibitory concentration (MIC) and the disc-diffusion method with 90 strains. *Arcobacter* was isolated 7 fecal samples of children from the National Institute of Child Health and the Maternal and Child Hospital of San Bartolomé. 83 strains were isolated from fecal samples of animals (pigs and cattle) from the La Colonial SAC refrigerator. In the antimicrobial susceptibility tests with E-test, we worked with 20 isolates of *Arcobacter*: 7 human fecal samples and 13 animals, with 5 antimicrobials: erythromycin, ciprofloxacin, tetracycline, ampicillin and nitrofurantoin; we found with erythromycin MIC_{50} and MIC_{90}, 2ug / ml and 32ug / ml respectively (15% resistance); with ciproploxacin, CIM_{50} and CIM_{90}, 0.75ug / ml and 16ug / ml respectively (20% resistance); with tetracycline: CIM_{50} and CIM_{90}, 8ug / ml and 32ug / ml respectively (35% resistance); with ampicillin, CIM_{50} and CIM_{90}, 2ug / ml and 38ug / ml respectively (25% resistance); with nitrofurantoin CIM_{50} and CIM_{90}, 8ug / ml and 96ug / ml respectively (25% resistance). In the disk-diffusion antibiogram of 90 isolates resistance was found: erythromycin 3.3%, ciprofloxacin, 11.1% tetracycline 58.8%, ampicillin 45.5% and with nitrofurantoin 18.8%. For the first time in Peru, results of antimicrobial susceptibility tests of *Arcobacter* isolated from human fecal samples and animals are presented here. These results show a high resistance of *Arcobacter* with ampicillin and tetracycline and low percentage resistance with erythromycin and ciprofloxacin 3.3% and 11.1% respectively; and with nitrofurantoin 18.86%; these are useful data to improve its control and adoption of preventive measures to infections in humans and in animals of popular consumption.

1999

COXIELLA BURNETII WITHIN SMALL LIVESTOCK HERDS IN AN AREA OF SEDENTARY PASTORALISTS IN LAIKIPIA, KENYA: HERDING BEHAVIORS, SOCIOECONOMICS AND SEROPREVALENCE

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Coxiella burnetii is an obligate intracellular bacterial pathogen and the causative agent in Q fever, a disease of livestock and humans. Little is known about how livestock management behaviors among small holder herders might impact risk for disease in East Africa. This research aims to describe the seroprevalence of C. burnetii in goats, sheep and cattle in a small community of sedentary pastoralists in a semi-arid area of Kenya. Researchers were introduced to eligible households through a local intermediary. Teams visited households and obtained permission to collect blood samples from livestock and to administer a survey instrument on livestock management, recent births, deaths and sales of animals, animal health and demographics. Animals were chosen at random with a target number of 15% of the herd or a minimum of 10 animals of each species, whichever was greater. GPS locations of each herd were taken at each location. Blood samples were then tested for presence of IgG antibodies using a commercially available ELISA kit. 28 individual households were surveyed, comprising 426 animals representing goats (262), sheep (91), cattle (55) and camels (18). Of these, 66 (17.5%) were seropositive, with seroprevalence varying by animal species. Within herds, seroprevalence varied, ranging from no animals being found to be seropositive to 40% or all animals being seropositive. Male animals were found to be less likely to be seropositive than females (OR .36 (.24, .95)). Among goats and sheep, risk for seropositivity was lower among households which reported selling animals to buy medications (OR .24 (.09, .63)). Weak, though protective associations were found for calling a veterinarian when animals are ill (OR .42 (.16, 1.09)) and monthly dipping for ticks (OR .53 (.27, 1.04)). Risk for seropositivity was not associated with increased proportions of animal losses (OR 0.28 (.00, 28.6)). Proactive herd management by smallholder livestock herders is associated with decreased risk for herd level infection in goats. Strategies to encourage or support protective practices such as spraying for ectoparasites might help reduce incidence of Q fever in livestock.

2000

CHARACTERIZING THE RISK OF BAT-BORNE VIRUS EXPOSURE AT POPULAR CAVE DESTINATIONS IN SOUTHEAST ASIA FOR PREVENTION AND RESPONSE

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Bat-human exposure is a driver of viral disease spillover. Bats were the documented source of Marburg Virus Disease (MVD) contracted by two tourists in 2008 after visiting the Python Cave in Uganda. In Southeast Asia, tourism is increasing steadily, exceeding 100 million visitors in 2015, and cave tours are a popular visitor activity. This study utilized robust surveillance data available for the region to identify risk factors for viral exposure to humans at top cave-tourism destinations. Bat species

that likely roost at these highly-visited caves were determined using range data and local species checklists. Species-specific viral detections proximal to these caves were inventoried from literature and using the Database of Bat-associated Viruses (DBatVir). Detection features relevant to transmission (sample prevalence, seasonality, media presence, and phylogenetic lineages) were identified. Relative risk between the caves was compared using visitation data, roosting ecology and population size, and the prevalence and proximity of bat-virus detections. Over the last 20 years, viruses have been detected in nearly 200 chiropteran species in 40 genra across the target region. The diversity of potential reservoir species present locally at heavily-visited caves were identified. Meta-analysis of transmission-related characteristics related to the bats and viruses enriched the cave-specific EID risk profiles. Disease modelling and localized surveillance of emerging infectious diseases (EID) are advancing the science of global biosecurity and One Health. On a regional scale, location-based risk information that is "actionable" by policy-makers can help advance prevention and response activities locally, travel-related disease surveillance, and for use in attracting innovative private investments that target climate resilience and pandemic prevention.

2001

ONE HEALTH APPROACH AND MOLECULAR EPIDEMIOLOGY OF MELIOIDOSIS IN SOUTHERN THAILAND

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It is well-recognized that melioidosis is endemic in most parts of Thailand. However, the prevalence of melioidosis in humans and animals, and the occurrence of its pathogen, *Burkholderia pseudomallei*, in natural environment of southern Thailand has not been updated for long time. We used "One Health" approach and multidisciplinary research to investigate epidemiology of melioidosis in southern Thailand. We have been collecting *B. pseudomallei* isolates from human and animal cases, and soils in Songkhla Province since January 2014. All culture-confirmed *B. pseudomallei* isolates from patients admitted to tertiary care hospitals in the region were further tested by real-time PCR targeting TTS-1, BTFC/YLF, and LPS genes. We have also investigated the presence of *B. pseudomallei* in soils especially in farming areas and a local zoo where animal cases have been reported. We used standard soil culturing techniques with Ashdown's agar. Suspected *B. pseudomallei* colonies were subjected to further identification by latex agglutination and real-time PCR. We have confirmed at least 209 melioidosis cases from humans, as well as, the presence of *B. pseudomallei* in soils in Songkhla. The infections were most likely seasonal and associated with rainfall. Genetic analysis using multi-locus sequencing typing (MLST) has indicated that most of recent isolates had same STs with those from the Finkelstein's historic collection from southern Thailand a half century ago. Specifically, strains with STs 288 and 84 were frequently found in Songkhla. Interestingly, at least 6 patients were confirmed to be infected by multiple STs suggesting a high genetic diversity of *B. pseudomallei* in natural source of the infections. Strains with ST3 were found in human and animal cases, as well as in the environment. We believe that implementing the "One Health" approach would provide a current situation of *B. pseudomallei* infections in humans and animals, as well as its occurrence in the environment in southern Thailand that forms an integral part of regional threat assessment of Thailand and Southeast Asia.

2002

UNDERDIAGNOSES OF RODENT-BORNE DISEASES IN PATIENTS HOSPITALIZED WITH ACUTE FEVER IN INDONESIA

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Leptospira spp, *Rickettsia spp*, and Seoul virus are prevalent in rodents in Indonesia. However, the prevalence in humans is not well understood. Few reports on *Rickettsia* and Seoul were based only on serological results. No cases were reported in the annual reports of the Ministry of Health. Clinical presentations of these three diseases vary, from mild to severe or even fatal. Mortality rates range from 1% in Seoul Virus to 5% in *Leptospira* infections. To have a better estimate of the burden of these diseases, an observational study on the etiologies of acute fever requiring hospitalization was conducted at 8 top-referral hospitals in 7 large cities from 2013 to 2016. All patients were monitored during enrollment and follow-up visits (14-28 days and 3 months after enrollment). Demographic, clinical data, and blood were collected during each visit. Molecular and serological assays to each pathogen were performed. From 1,486 enrolled subjects, we found *Rickettsia(R) typhi in 102 (6.9%), Leptospira spp* in 44 (2.9%), Seoul virus in 2(0.1%), and *R felis* in 1 (0.07%) subjects. *R typhi* was observed at all sites, most prevalent in Surabaya (11.8% of all subjects) and least prevalent in Yogyakarta (3.6%). *Leptospira* was most frequent in Semarang (5.4%), least in Jakarta (1.9%), and no cases in Denpasar, Bali. Seoul virus was only observed in one subject from Jakarta and Surabaya. Subjects with *Rickettsia* and *Leptospira* infections presented with multiple organ involvement, whereas liver involvement was prominent in subjects with Seoul virus infections. Fatalities in *Rickettsia* and *Leptospira* infections were 6.8%, and 2.3%, respectively, while two Seoul virus cases survived. All *R typhi* and Seoul virus cases, and 57% of *Leptospira* cases were diagnosed as other diseases. These findings highlight the importance to develop appropriate diagnostic criteria, to enhance laboratory capacities, to improve clinical management, and to initiate public health intervention.

2003

USE OF AN INDUCIBLE EXPRESSION SYSTEM TO EVALUATE THE EFFECT OF MUTATIONS IN *PNCA* ON THE PYRAZINAMIDE SUSCEPTIBILITY IN *MYCOBACTERIUM TUBERCULOSIS*

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Tuberculosis (TB) is a bacterial infection caused by *Mycobacterium tuberculosis (MTB*). TB is in epidemiologic vigilance to global level. The TB patients may have active or latent TB. The treatment of TB includes drugs as pyrazinamide (PZA), which is effective against dormant MTB. Often, mutations in the *pncA* gene cause resistance to PZA. These mutations may promote the total or partial loss of the pyrazinamidase (PZAse) activity. Previously, we identified *MTB* isolates resistant to PZA with *pncA* mutation. Mutant PZAses that showed a variety of PZAse activity according to the mutation: D49N and H51R affected directly the metal-binding site and T135P is near to the active site, neither showed activity. D12A, F94L,

and K48T are close to the metal-binding site or active site and showed middle activity, but for K48T was high. G78C is far from the active site and showed high activity. We want to evaluate the effect of *pncA* mutations on the PZA susceptibility in a *pncA knockout MTB* strain (*pncA KO MTB*). The *pncA KO MTB* strain was complemented with the mutated *pncA* using an inducible expression system. The wild-type (WT) and mutant *pncAs* were cloned in pNIT. The recombinant plasmids were transformed into *E. coli* and then in *pncA KO MTB* strain by electroporation. The *pncA* expression was induced in the complemented-*pncA KO MTB* strains and the PZAse activity was measured using the Wayne colorimetric test. In addition, the PZA minimum inhibitory concentration (MIC) was estimated. The association between PZAse activity and PZA susceptibility will be evaluated by Spearman correlation. In our first results, the complemented-*pncA KO MTB* strains with WT and G78C *pncA* gene restored its PZAse activity, but the color intensity was more for WT *pncA* complemented strain than with G78C, and PZA MIC was at 100 ug/mL for both strains. Likewise, the complemented strain with D49N did not restore its PZAse activity and was highly PZA resistant (MIC: 1000 ug/mL). We have planned to evaluate the other complemented strains. The inducible expression system will allow us to determine the specific level of PZAse activity and susceptibility to PZA of each mutant *pncA* in *pncA KO MTB* strain.

2004

WILLINGNESS TO VACCINATE AGAINST INFLUENZA A (H1N1) PDM09 AMONG UNIVERSITY EMPLOYEES IN BRAZIL

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In Brazil, a vaccination campaign against a new subtype of influenza A (H1N1) human virus of swine origin started in 2010 reaching almost 90 million people. Coverage among adults between 20 to 39 years of age reached 81%, varying from 63% to 91% across the Brazilian states. Why so many people did not get a widely available and free of charge vaccine during a severe outbreak being broadcasted in the news is intriguing. In 2012 we investigated, among university employees, factors associated with willingness to uptake pandemic influenza vaccination if a new campaign was to be carried out in the future. This is cross-sectional study among nonfaculty civil servants at university campi located in Rio de Janeiro, Brazil. The main outcome binary variable was "willingness to uptake a pandemic influenza vaccine in the future". Exposure variables were gender, age at the 2010 influenza epidemic, race, educational status, and occupation. Associations between variables were expressed as odds ratios (OR) with respective 95% confidence intervals (95%CI) estimated by logistic regression. Among the 2828 participants, 40.9% did not take the influenza A (H1N1)pdm09 vaccine and 15.9% would not be willing to take the vaccine in the future. Not willing to vaccinate was strongly associated with not taking previously the influenza A (H1N1) pdm09 vaccine (OR=11.6, 95%CI:8.77-15.4). Among those who had not taken the influenza vaccine, those aged >60 years and employed in non-health related occupations were significantly less willing to vaccinate in the future. For those who had previously taken the influenza vaccine, willingness to vaccinate was lower among females. Vaccine hesitancy is a recognized public health concern in developed countries but has not been considered as such in Brazil until recently when the recent outbreaks of yellow fever have shed light on the problem. Since a first experience of vaccination is strongly associated with willingness to vaccinate in the future, efforts should be made to identify population characteristics and their reasons to refuse vaccination and develop focused strategies to break the chain of vaccine hesitancy.

2005

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF CHILDREN WITH INVASIVE PNEUMOCOCCAL DISEASE IN LIMA, PERU

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Invasive pneumococcal disease (IPD) and vaccine serotypes have decreased since the introduction of pneumococcal conjugate vaccines (PCV). However, serotype 19A IPD is currently one of the most common causes of replacement disease after PCV7 and PCV10 introduction. In Peru, PCV10 was introduced into the childhood immunization schedule on 2011, and PCV13 on 2015. The aim of this report is to describe the demographic and clinical characteristics of children with IPD due to serotype 19A in Lima, Peru; to better understand the current trends. We are conducting a multicenter, passive surveillance study (Nov-2016 to Dec-2019) to determine the changes in serotypes and antimicrobial resistance in patients with IPD after PCV introduction in Lima. We are evaluating antibiotic susceptibility by E- test (penicillin and ceftriaxone) and by disc diffusion (azithromycin, clindamycin, and tetracycline). Strains are serotyped by a sequential multiplex PCR. As to date (Nov-2016 to Feb-2018), we have identified 45 S. pneumoniae isolates in children; 49% are serotype 19A (n=22), all isolated from children >1 year old; 7 isolated in children 1-2 years, 11 in children 2-5 years, and 4 in children >5 years. 19 children had pneumonia as the main diagnosis, 2 meningitis, and 1 peritonitis. The most common comorbidities were: asthma (n=2), malnutrition (n=2) and prematurity (n=2). Regarding vaccination status, 1 patient received PCV7, 8 received PCV10, 5 received either PCV10 or PCV13 (transition period), 4 received PCV13 (2 patients with complete 2+1 schedule), 2 had no PCV vaccination, and 2 have no data available. Both meningitis strains were resistant to penicillin and ceftriaxone. Among non-meningitis strains (n=20), penicillin resistance was 15%, azithromycin and tetracycline 100%, and clindamycin 95%. Current trends show serotype 19A as the main serotype circulating in Lima, associated with high resistance rates to commonly used antibiotics. This is and ongoing study; therefore, we will continue to monitor trends over time.

2006

GENOTYPING AND DRUG RESISTANT PREVALENCE OF *MYCOBACTERIUM TUBERCULOSIS* IN SOUTHERN PENINSULA OF HAITI

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Tuberculosis (TB) has been a major public health problem with 10.4 million of new TB cases with 1.3 million deaths in 2016. Haiti is one of the poorest countries in the Western Hemisphere with 16,431 of TB cases and a high incidence rate in 2015. A hospital based study was conducted in Haiti during 2014 and 2015. Sputum specimens obtained from 664 patients with suspected pulmonary TB were further tested by culture and fluorescent microscopy. Sixty-seven (10.1%) were smear positive and 119 (17.9%) were culture positive in liquid or solid media. Drug susceptibility tests were performed in randomly selected 102 isolates using solid media with isoniazid (INH; 0.2 µg/mL and 1.0 µg/mL) and rifampin (RIF; 1.0 µg/mL). Twenty (19.6%) were mono-resistant and 6 (5.9%) were multi drug resistant (MDR). Line probe assays, a molecular technique, were used to identify mutations in 81 base-pair of the Rifampicin Resistance Determining Region (RRDR) of *rpoB* gene for RIF resistance and *inhA* and *katG* gene for INH resistance. Seventy-five out of 119 isolates were tested. Eight (10.7%) isolates were mono-resistant and 4 (5.3%) strains were MDR. Two known mutation patterns, D513V and S531L, were identified in these resistance strains, while two strains had no known mutations in any of these genes. Sequence analysis of the RRDR in both strains showed that they had a novel amino acid substitution mutation S531G. Spoligotyping was conducted on 119 positive isolates. Of these, 31 known shared international types (SITs) were identified, while the other 32 spoligotypes were novel. Among these known SITs, the SIT2 (13.1%), SIT764 (11.1%) and SIT42 (7.4%) were the dominant. Phylogenetically, our study has identified that majority of the Haitian isolates are grouped with 3 major clades: H3 (24.7%), H2 (12.4%), and T1 (12.4%) which are also found in four broad geographic areas including Africa, the Americas, Europe, and Asia. This would suggest that the transmission of TB to Haiti was through the European colonization. Our study has shown that TB remains a public health problem in Haiti, and a better case management and pathogen characterization for the MDR-TB are greatly needed.

2007

COMPARISON OF TWO DECONTAMINATION METHODS OF SPUTUM SAMPLES FOR THE DIAGNOSIS OF TUBERCULOSIS BY MODS SYSTEM

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The early diagnosis of tuberculosis by microbiological methods, as well as the detection of susceptibility to drugs, have special importance in developing countries. In addition to conventional culture media based on eggs, the systems BACTEC MGIT® and MODS, based on liquid culture, are currently aviable but the contamination being a fundamental problem. The objective of this study is to compare two decontamination methods of sputum samples, based on the Petroff method, using a mixture of NaOH (0.5M), sodium citrate (0.65M) and N-acetyl-L-cysteine (NALC) (0.015M), in proportion 1:1 with the sputum sample for 15 minutes at room temperature. The standard method (A) store the mixture of NaOH and Sodium Citrate at 4°C and combine this mixture with the NALC the day of the process while the modified method (B) store the individual solutions (2X) and combine this and the NALC the same day of the process. MODS was the diagnostic methodology. PANTA ® was used as a suplement of Middlebrock 7H9 culture media. Clinical samples were used. The percentage of contamination issued with each method, based on the total of processes without conssidering repetitions, was calculated. The inverse of this was interpreted as a percentage of effectiveness. The result was that the "A" method showed an effectiveness of 62.5% (111/296) while the "B" method showed an effectiveness of 98% (5/255), using the MODS as diagnosis method. It is recommended the implementation of the method "B" of decontamination for TB diagnosis, using the MODS methodology, in hospitals and laboratories in developing countries for a cheaper and effective microbiological diagnosis of tuberculosis.

2008

SMS FOR PHARMACIES AS AN EDUCATIONAL TOOL TO IMPROVE THE KNOWLEDGE ON PREVENTION AND EARLY DETECTION OF MULTIDRUG-RESISTANT TUBERCULOSIS IN A DISTRICT OF LIMA, PERU

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In spite of the slow decrease of Tuberculosis (TB) in the world, Peru still registers one of the highest figures of sensitive and MDR TB of the Americas. Widespread private pharmacies are the first point of contact for many symptomatic respiratory patients, pharmacy workers can play a role in early detection and referral of cases to the TB programs. To evaluate the change in the baseline level of knowledge that pharmacy workers have before an educational intervention based on the sending of SMS (Short Messages Service). A pre-intervention knowledge survey was conducted on a random sample of 63 pharmacies in Lima and Chiclayo Peru. Previously, a pilot survey was carried out to adapt the SMS, pre-testing and evaluation of their content validity. That helped to consider preferences such as using the acronym "TBC" in the SMS instead of openly using the stigma related word tuberculosis. Later for the intervention one participant for each pharmacy received 25 SMS. 63 respondents 76.2% were women, the average age was 27 CI: 19- 72 years. They reported that the most frequent symptom of tuberculosis was cough (92.1%) and the most useful diagnostic test was the sputum test (71.4%). The mean score of knowledge before the intervention was 11.6 ± 1.7 points out of 17, having 9 or more points was deemed as pass. Regarding knowledge about TB, 98.4% answered that TB is transmitted among people coughing, 49.2% answered that patients with tuberculosis can almost always be cured; 9.5% responded that patients with MDR-TB are those who show resistance to isoniazid and pyrazinamide, and 33.3% of the respondents answered that the consequence of incomplete treatment is resistant TB. Conclusions: Workers in private pharmacies have adequate knowledge with better results in TB prevention and diagnosis, important gaps persist in knowledge about treatment and MDR-TB. Most of participants reported acceptance with the SMS intervention. This study served as a baseline for the subsequent evaluation of the impact of the intervention.

2009

DETERMINATION OF RESISTANCE TO PYRAZINAMIDE BY QUANTITATIVE WAYNE FROM SPUTUM SAMPLES FROM PATIENTS WITH TUBERCULOSIS

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Tuberculosis (TB) is considered the ninth cause of death worldwide. The effort to eradicate it has made possible to reduce deaths in patient sensitive to drugs, however, the biggest problem remains the multidrug-resistant (MDR). In 2015, in Peru, 1,365 cases of MDR TB were recorded. Therefore, establishing truthful methods of diagnosis of resistance is important for the prompt and adequate treatment. The objective of this work is to determine the resistance to pyrazinamide (PZA), one of the first line drugs, which is converted to pyrazinoic acid (POA) by the bacteria. Diagnosis of PZA resistance, is not routinely performed in part due to subjectivity of the Wayne test and long periods of culture. MGIT tests were used to evaluate PZA resistance in sputum samples to reduce time to detection; although the efficiency was high, the uninterpretable results

was 41%. Preliminary results showed that the Direct Quantitative Wayne allows objective measurement of resistance to PZA, by quantifying POA produced by mycobacteria in samples of sputum culture supernatants. The samples (n= 146) were cultured in 7H9 medium for 7-10 days, then PZA was added and incubated for 3 days. Finally, 10% ferrous ammonium sulfate was added. The supernatant was used to measure optical densities by a spectrophotometer at 450nm. The concentrations of POA (µM), were calculated from a standard curve whose minimum concentration was 31.25 µM (O.D 0.0072±0.003) and maximum 4000 µM (O.D 0.5841±0.015) with an r^2= 0.9999. Preliminarily, these results were compared with values obtained from visual evaluation of the color, being 0 (resistant n=14), 1, 2 and 3 (intensities of sensitivity, n1=49, n2=59 y n3=24) to see if there was correspondence between the ODs and this values. The (0) samples had an average concentration of 65.63 ± 18.04 (SEM) µM of POA, the samples weakly positive (1) samples 234.3 ± 28.83 µM, the (2) samples 530.4 ± 80.06 µM and the (3) samples 1657 ± 235.6 µM, there were significant difference between groups (p<0.0001). Now we are evaluating the strains with gold standard (MGIT), we expect results that promises an inexpensive and automated assay to detect PZA resistance from sputum.

2010

EVALUATION OF THE ROLE OF MSMEG_0232 GENE IN THE EFFLUX OF PIRAZINOIC ACID IN MYCOBACTERIUM SMEGMATIS

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Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis (MTB), currently considered a global emergency by the World Health Organization (WHO), and that mostly affects the developing countries. Pyrazinamide (PZA), a first line drug against TB, is known for being capable of eliminating MTB in their non-replicating state. This drug plays a key role in shortening the duration of treatment against MTB and its inclusion is crucial in the treatment of patients infected with strains of multidrug-resistant MTB (MTB-MDR). Therefore, the growing occurrence of PZA resistant isolates is a serious threat to public health. In view of this problem, many efforts have been made to understand the mechanisms of resistance to this drug. One of the key findings has been to find that the presence of mutations in the pncA gene, which codes for pyrazinamidase and converts PZA to its active form, pyrazinoic acid (POA), is the main mechanism of resistance to PZA. These mutations are found in more than 90% of the clinical isolates resistant to PZA, but do not explain all cases of resistance in MTB. It has been suggested that the other cases could be explained by mutations at the level of the POA efflux pump. However, despite being of great relevance, the identity of this protein is not clear. Around this, recently an analysis of the proteome of MTB has identified 4 possible candidate genes and that have also been found to harbor non synonymous mutations in resistant strains. The present project therefore aims to evaluate the role of one of these candidate homologous genes in the strain naturally resistant to PZA Mycobacterium smegmatis (Msm). For this, a recombinant strain was generated with the CRISPR interference system (CRISPRi) for the orthologous gene Msm_0232. Subsequently, the level of transcriptional repression by RTqPCR and the efflux rate of POA was evaluated. Our results show that preliminarly, after the induction of the CRISPRi, there are no significant decreases in the POA efflux rate. Thus, if confirmed, Msmeg_0232 would be discarded as a POA efflux pump in Msm.

2011

HIGH PREVALENCE OF RESPIRATORY VIRUS AND ATYPICAL BACTERIUM AMONG CHILDREN WITH A PROBABLE DIAGNOSIS OF PERTUSSIS

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Acute respiratory infections (ARIs) as the most common cause of morbidity and mortality in children, remains a major concern, especially affecting children under 5 years old from low-income countries. Unfortunately, information regarding their epidemiology is still limited in Peru. A secondary data analysis was performed from a previous cross-sectional study conducted in children with a probable diagnosis of Pertussis from January 2010 to July 2012. All samples were analyzed via PCR for the following etiologies: Influenza-A, Influenza-B, RSV-A, RSV-B, Adenovirus, Parainfluenza 1 virus, Parainfluenza 2 virus, Parainfluenza 3 virus, Mycoplasma pneumoniae and Chlamydia pneumoniae. A total of 288 patients were included. The most common pathogen isolated was Adenovirus (49%), followed by Bordetella Pertussis (41%) from our previous investigation, Mycoplasma pneumonia (26%) and Influenza-B (19.8%). Coinfections were reported in 53.5% of samples and the M. pneumoniae and Adenovirus (9%) was the most common association. There was a high prevalence of Adenovirus, Mycoplasma pneumoniae and other etiologies in patients with a probable diagnosis of pertussis. Despite the presence of whooping and other clinical characteristics highly suspicious of pertussis, secondary etiologies should be considered in children under 5 years-old in order to give a proper treatment.

2012

BORDETELLA PERTUSSIS IN CHILDREN HOSPITALIZED WITH A RESPIRATORY INFECTION. CLINICAL CHARACTERISTICS AND PATHOGEN DETECTION IN HOUSEHOLD CONTACTS

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Latin America is experiencing a resurgence of *B. Pertussis* infections affecting primarily infants under 6 months old. In Peru, the number of Pertussis cases doubled by the first half of 2017 compared to the previous year. In addition, limited laboratory resources in the rural setting represent an impediment to establishing the diagnosis in this vulnerable population. Methods: A consecutive cross-sectional study was conducted in Cajamarca, Peru from April 2016 to September 2017. A total of 88 children under 5 years old admitted with clinical diagnoses of 'whooping cough' were tested via polymerase chain reaction (PCR) for detection of *B. Pertussis*. Household contacts with respiratory symptoms were also analyzed for *B. Pertussis*. Results: A positive PCR result for *B. Pertussis* was observed in 20.5% of our samples (18/88), one-third of them were

from infants between 2 - 3 months old. The most common symptoms were paroxysms of coughing (88.9%), difficulty breathing (72.2%), cyanosis (77.8%) and fever (50%). The mother was the most common symptomatic carrier (27.8%), followed by uncles/ aunts (22.2%) among children with Pertussis. Conclusions: *B. Pertussis* is an important cause of respiratory tract infection in children under 5 years, primarily affecting infants to young to be immunized in whom the diagnosis is not always suspected. In rural areas of Peru, limited laboratory resources may be responsible for Pertussis underdiagnoses and increased antibiotics prescriptions.

2013

MECHANISM OF POST-DISCHARGE DEATH AND "SECOND HIT" INFECTIONS: THE EFFECT OF INTESTINAL INFECTIONS ON LUNG IMMUNITY

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Up to 15% of children die during the first 6 months after hospital discharge in resource-limited settings, often exceeding in-hospital mortality. Verbal autopsy studies have shown that deaths are often preceded by new symptoms of cough or fever, suggesting that "second hit" respiratory infections may be major contributors. However, the mechanisms behind "second hit" infections remain unclear. Using a mouse model of intestinal infection by *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium), we investigated how infection in the gut compartment can modulate immunity in the lungs. We first orally infected mice with a relatively low dose of *S*. Typhimurium (10^4 cfu) by gavage and six day's post infection we harvested lungs and assessed for antigen presenting cells (APCs) and innate lymphocytes by flow cytometry. We found higher percentage of plasmacytoid dendritic cells (pDCs), gamma-delta T cells and lower frequencies of monocytic dendritic cells (moDCs) in lungs of *S*. Typhimurium infected mice compared to uninfected mice. Furthermore, on subsequent challenge with *Klebsiella pneumoniae* we found that mice with prior intestinal infection responded with lower percentage of pDCs, moDCs and gamma-delta T cells, compared to mice without prior intestinal infection. Together these results suggest that *S*. Typhimurium infection in the gut effects lung innate lymphocyte and APC responses, potentially resulting in increased susceptibility to *K. pneumonia*e. At time of conference presentation, we will have complete data on survival and bacterial outcomes of mice receiving "second-hit" *K. pneumoniae* infections. In conclusion, this study reveals potential mechanisms of crosstalk between the lung and the gut during enteric infection that may affect susceptibility to subsequent respiratory infection. Our findings have the potential to uncover novel therapeutic strategies targeting the gut-lung axis during intestinal infections.

2014

TRANSCRIPTIONAL RESPONSES OF THE VECTOR SNAIL *BIOMPHALARIA GLABRATA* TO *SCHISTOSOMA MANSONI* AND TWO ADDITIONAL RELEVANT PARASITES: A COMPARATIVE APPROACH TO UNDERSTANDING SNAIL IMMUNITY

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Biomphalaria glabrata is an important Neotropical snail vector of human schistosomiasis, a neglected tropical disease afflicting 206.5 million people. As part of an ongoing effort to characterize and eventually exploit the immune system of the snail for schistosomiasis control, this research aimed to reveal the full extent of the transcriptional response of *B. glabrata* when exposed to *Schistosoma mansoni* or to two other unrelated parasites it might readily encounter in nature, the echinostome fluke *Echinostoma paraensei* and the snail-infecting nematode *Daubaylia potomaca*. From this, we can identify common or unique features in the *B. glabrata* response to different parasites. M-line strain snails were individually exposed to each parasite and at 2, 8 and 40 days post exposure (dpe), 7-8 snails/group were collected. Unexposed control snails (matched at 2 and 40 dpe) were also sampled. Snails were extracted for RNA, PCR assays run to check for parasite presence, and cDNA libraries (3 snails/ group/time point) were paired-end sequenced on an Illumina NextSeq500 instrument. The published *B. glabrata* genome was used as a reference for mapping with STAR. Bioinformatics tools were used for differential expression (DE) gene analysis and Gene Ontology (GO) term enrichment analysis. On average, 12 million raw reads/snail were sequenced. Each parasite provoked a distinctive overall pattern of responses at all time points, but in general the responses provoked by the two trematodes, the sporocyst-producing *S. mansoni* and rediae-producing *E. paraensei* were more similar to each other (persistent patterns of overall down-regulation) than what was noted with the nematode (early down-regulation followed by dramatic late up-regulation). The results are consistent with the need for trematodes to establish stable long-term infections in which progeny are continually produced, relative to the nematode which overwhelms the snails and is transmitted only when the snail is about to die. The snail genes responsive in common to all three parasites, and those expressed in a parasite-specific way will be discussed and quantified in the current research.

2015

DIFFERENTIAL GENE EXPRESSION OF PIWI IN *BIOMPHALARIA GLABRATA* SNAILS WITH VARYING SUSCEPTIBILITY TO *SCHISTOSOMA MANSONI*

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B. glabrata is an important intermediate host for the human parasite *S. mansoni*. The Piwi (P-element induced wimpy testis in *Drosophila*) family of proteins maintains the integrity of the genome by silencing transposable elements in germ cells by interacting with small non-coding piRNAs. It acts as guardians of the genome, protecting it from harmful mutations. Under conditions of stress and other cellular perturbations, piwi in combination with heat shock protein Hsp90 and the Heat Shock Organizing Protein (HOP) are involved in canalization, a process that maintains the phenotype in the face of genotypic variation. This buffering system is conserved allowing organisms to adapt to stressful conditions. We hypothesized that piwi, HOP, and Hsp90 are expressed in a fashion that maintains the regulation of the *B. glabrata* endogenous retrotransposable element during parasite infection. To test this hypothesis, expression of transcripts encoding piwi and HOP were examined in juvenile resistant (BS90) and susceptible (BB02) snails to *S. mansoni* infection by real-time qPCR after exposure to miracidia. Results showed that during early infection of resistant BS90 snails, the transcript encoding piwi was upregulated post- exposure unlike in susceptible BB02 snail where the piwi transcript was downregulated in the same time frame post-exposure. Expression of the transcript encoding HOP was conversely upregulated within 30min after infection in susceptible but not in similarly exposed resistant snails. The expression of transcripts encoding *nimbus* reverse transcriptase and Hsp90 have previously been examined and was found to be upregulated only in early infected susceptible snails. Previous data suggest that the early upregulation of transcripts encoding HOP and Hsp90 in the infected susceptible snail, and downregulation of piwi in early-infected susceptible snails, results in less canalization that leads to a more stressful environment for schistosomes to survive and develop. On the other hand, resistant snails, consistently showing elevated piwi expression after infection are better canalized to ward off infection.

2016

TRANSCRIPTS ENCODING HUMAN SIGLEC HOMOLOGS IN *BIOMPHALARIA GLABRATA* SNAILS ARE REGULATED IN RESPONSE TO *SCHISTOSOMA MANSON*I INFECTION: A MODEL SYSTEM TO STUDY EVASION OF INNATE IMMUNITY IN CANCER

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Siglecs are sialic acid-binding, immunoglobulin-like lectins that attach to glycans on cell surfaces. They are important in immune cell signaling and are part of an organism's innate immunity. When the miracidia of *Schistosoma mansoni* infect freshwater snail, *Biomphalaria glabrata*, stress proteins are elevated. This deploys an immune response that upregulates the expression of various siglec homologs. The developing miracidia (sporocysts) evade lysis by disguising themselves as glycan structures that are similar to those found on their host's immune cell surface, which binds to the host siglecs, providing effective mimicry to prevent attack from the snail innate defense system. This evasive mechanism utilized by schistosomes to escape recognition and destruction in the snail host is remarkably similar to cancer cells that disguise themselves from the body's innate immune system, and are consequently, capable of surviving in a hostile environment. To determine the mechanism(s) that snails use, like cancer cells, to escape the innate immune response, we hypothesized that blocking siglecs homologs by RNA interference (RNAi) will prevent schistosomiasis in snails which mirrors the effect of blocking siglecs on several cancer cells. To test this hypothesis, we identified several human siglec homologs such as nuclear receptor, peroxidasin, and C1q by interrogating the *B. glabrata* genome, and examined the temporal regulation of their expression by real-time qPCR. Results showed that after early infection of susceptible *B. glabrata*, the siglec homologs were upregulated following parasite infection. Double-stranded RNA will be synthesized by *in vitro* transcription to knockdown the corresponding siglec transcript by transfection of newly infected snails. Blocking transmission of schistosomiasis by this approach will offer proof of principle that the same process can be used to interfere with cancer since they utilize same evasion pathway as *S. mansoni* in infected snails. Both schistosomes and cancer camouflage themselves by using siglec ligands to bind host siglecs, to prevent recognition and destruction by the host immune system.

2017

EXPLORING THE ROLES OF SMJNK AND SMP38 IN *SCHISTOSOMA MANSONI* AND ITS POTENTIAL AS THERAPEUTIC TARGETS AGAINST SCHISTOSOMIASIS

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MAPKs are involved in *S. mansoni* development, reproduction and/or survival; and have been considered potential therapeutic targets. Due to the availability of a single treatment for schistosomiasis, this work aims at identifying drug targets, in which SmJNK and Smp38 are candidates themselves or specific parasitic genes regulated by them. First, we performed SmJNK and Smp38 knockdown in schistosomula by RNAi and assessed their transcriptional profile through RNASeq. Differentially expressed genes (DEGs) were identified by comparing each MAPK knockdown against untreated parasites. We identified 606 and 1154 DEGs in SmJNK and Smp38 knockdown schistosomula, respectively. A considerable proportion of DEGs (505) encodes proteins with unknown function and there is a significant crosstalk between both pathways. The SmJNK and Smp38 knockdown promotes decrease in gene expression related to antioxidant defense, structural composition of ribosomes, spliceosomes, cytoskeleton and purine metabolism pathway. Also, SmJNK and Smp38 knockdown was performed in adult worms, resulting in ~65% reduction in transcription levels. After knockdown, there was up to 86% reduction in oviposition in both treatments. As for SmJNK, oviposition was interrupted in the 6th day. Using the WormAssay software, knockdown male parasites for SmJNK presented an 80% movement reduction in the 8th day. To search for specific compounds, the structure of SmJNK is to be solved by crystallography. Thus, the synthetic SmJNK gene was cloned into the pCold-GST vector for recombinant protein production and expressed in *Escherichia coli*. The recombinant protein was obtained with the expected size and sequence was confirmed by mass spectrometry. A protein activity assay based on the interrogation of the ATP binding site using BODIPYFL ATP-γ-S fluorescent nucleotide which detects polarization was validated to test the activity of expressed protein. Evaluation of recombinant SmJNK activity is underway for further directed compound screening *in vitro*. This work allowed a better understanding of MAPKs signaling pathways, elucidating functional roles and targets they regulate.

2018

STUDY OF CHANGES IN THE TRANSCRIPTOME OF MOUSE BLADDER FOLLOWING BLADDER WALL INJECTION OF *SCHISTOSOMA HAEMATOBIUM* EGGS

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Background: Infection with *Schistosoma haematobium* leads to urogenital schistosomiasis (UGS), which afflicts over 100 million people. UGS can cause hematuria, calcification of the bladder, and increased risk of secondary infections by bacteria or viruses, and it is also linked to bladder cancer. Previous studies have used a mouse model that involves injection of *S. haematobium* eggs into the bladder wall to examine the effect of parasite eggs on host bladder biology, and they have identified changes in genome-wide methylation, as well as changes in the transcript level of some genes by microarray analysis. Here, we perform RNA-Seq on egg injected bladders to expand the detection of changes in gene transcript level to the scale of the whole transcriptome. Methods: We followed a combined infection/injection animal model in which female BALB/c mice were infected with *S. haematobium* cercariae and then, after 5 weeks, administered either parasite eggs or hamster liver extract by bladder wall injection. Another group of control mice did not receive surgery. RNA-Seq was performed on the RNA isolated from the bladders 4 days after bladder wall injection. Results/Conclusions: RNA-Seq analysis of egg-injected bladders and controls will reveal host gene pathways activated by the presence of S. haematobium eggs. Of particular interest are urothelial cell-related genes, including cancer pathways, differentially expressed genes associated with infiltrating and resident leukocytes, and fibrosis-related gene pathways. RNA-Seq analysis of schistosome-specific genes highlight the challenges of performing the parasite side of dual RNA-Seq in the setting of low amounts of parasite RNA relative to host RNA.

2019

USE OF THE *BIOMPHALARIA GLABRATA*, *SCHISTOSOMA MANSONI* HOST- PATHOGEN MODEL SYSTEM AS SURROGATE TO STUDY METASTATIC CANCER AS A PARASITIC DISEASE

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Schistosoma mansoni is a parasite that causes the disease schistosomiasis in over 75 tropical and sub-tropical countries. In the lifecycle, human beings are the definitive host for parasites that sexually reproduce eggs that when released from human excreta into freshwater hatch to release the miracidia that infect the snail, *Biomphalaria glabrata.* By penetrating the head foot, migrate to the hepatopancreas, asexually

reproduce cercariae that infect human host where cercariae transform into schistosomula and develop into adult worms which survive many years in vasculature and avoid immune attack. Likewise, cancer develops without immune attack in host. Schistosomes mimic behavior of metastatic cancer by migrating to specific tissues. It shares another hallmark of cancer, there is no super-infection, a condition called as concomitant immunity. Due to these similarities in cancer and schistosomiasis, we hypothesize-metastatic cancer is a parasitic disease, hence molecular determinants that underscore metastatic cancer will also play a role in biology of parasitism. To test this, we examined expression of snail homologs of cancer related transcripts, E-cadherin, beta-Integrin, myc, p53 and Snail after early (0,30min,1hr,2hr,4hr,16hr) stages of *S.mansoni* infection of resistant or susceptible *B.glabrata* snails. Results show expression of matrix proteins E-cadherin and beta-integrin, as in cancer, downregulated upon early *S.mansoni* infection in *B.glabrata* susceptible but not resistant snails. Cells lose their adhering capacity and become free due to downregulation of these proteins.The extracellular matrix detaches due to contact-inhibition and transitions into motile mesenchymal cells that enter the bloodstream and other tissues-hallmark of metastatic cancer. Matrix protein downregulation is an important factor for Epithelial-Mesenchymal Transition. Ability to track signaling networks that control expression of homologs of cancer related transcripts in a snail responding to the parasite provides a convenient surrogate model system to identify pathways that can be blocked to treat both cancer and schistosomiasis.

2020

COMPARISON OF METHODS FOR EVALUATING THE MOTILITY, INFECTIVITY AND VIABILITY OF SCHISTOSOME *CERCARIAE* IN WATER

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Schistosome cercariae are the human-infectious stage of the Schistosoma parasite. They are shed by intermediate host snails living in freshwater, and penetrate the human host's skin to develop into schistosomes, resulting in schistosomiasis infection. One way of cutting disease transmission is through water treatment, which aims to kill the cercariae to provide safe water for people to use as an alternative to infested lakes or rivers. At present, there is no standard method for assessing the impact of water treatment process on cercariae or definition of the point at which water is considered safe. Instead, water treatment studies to-date have assessed one of three cercarial characteristics: motility (ability to move), infectivity (ability to penetrate host skin) or viability (ability to penetrate host skin and develop into schistosomes). Motility is assessed microscopically, and assumes that cercariae are dead when they stop moving. Infectivity is assessed by counting cercariae before and after being exposed to a skin sample, the difference assumed to have penetrated the skin. Viability tests often use animal testing to determine if cercariae develop into worms. Vital and non-vital dyes have also been used to stain cercariae. The accuracy of these methods is uncertain and significant sources of error have been identified. We evaluated the relationship between five commonly used methods - movement, skin attachment, Fluorescein Diacetate, Hoechst, and neutral red dye - using *S. mansoni* cercariae. This research crucially establishes which methods most accurately determine the point at which water can be considered safe from a schistosome cercaria standpoint, thereby defining standards for confirming the effectiveness of water treatment processes (e.g. using chlorination or filtration) both in field sampling and laboratory trials. This work is timely as we look towards elimination of schistosomiasis as a public health concern and the role of water treatment in integrated interventions alongside preventive chemotherapy.

2021

DETECTION OF *SCHISTOSOMA MANSONI* SPOROCYST STAGE IN *BIOMPHALARIA GLABRATA* MOLLUSK IN EXPERIMENTAL CONDITIONS

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Approximately 240 million people in the world are infected by the *Schistosoma mansoni.* In Brazil, the goal of the control program for this parasitosis is to reduce the risk of its geographic expansion. The main intermediate host of *Schistosoma mansoni* is *Biomphalaria glabrata.* The detection of larval stages of the parasite in intermediate hosts is an important challenge to public health. The objective of this study is to standardize the detection of *Schistosoma mansoni* as primary sporocysts developing in *B. glabrata* mollusks artificially infected with Belo Horizonte (BH) *Schistosoma mansoni* strain, using the Polymerase Chain Reaction (PCR) and Two Sequential PCR-amplification (Re-PCR). From a laboratory cycle of *S. mansoni* we obtained thirty miracidea. Twenty mollusks *B. glabrata* specie were infected, four sporocysts packages were dissected, and the head-foot part was removed, and tissues were used the DNA extraction using the DNAeasy Tissue Kit (Qiagen). DNA was quantified in Nanodrop and two molecular approaches were compared, cPCR and Re-PCR. DNA samples were amplified using primers targeting a tandem repeat sequence of 121pb and amplification products were evaluated in 2% agarose gels. The amplification products showed distinct profiles when comparing the different DNA sources. For the head-foot tissues, clear bands were visible for both cPCR and Re-PCR suggesting a lower concentration of PCR inhibitors. The *S. mansoni* sporocyst molecular detection from the head-foot part demonstrated a high sensitivity in early characterization of snails infection to *S. mansoni* in experimental conditions and so can represent a tool in schistosomiasis monitoring and control.

2022

CYTOKINES AS PREDICTORS OF *SCHISTOSOMA MANSONI* INFECTION INTENSITIES AND REINFECTION RATES

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Schistosomiasis is a commonly occurring neglected tropical disease with over 240 million people infected globally. Despite over a decade of mass drug administration (MDA) in Uganda, certain endemic hotspots continue to persist whereby infection intensities and prevalence are similar to those recorded before MDA was rolled out. Past studies show a potential association with various cytokines and reduction in reinfection rates post chemotherapeutic treatment. This study investigates changes in cytokine profiles post treatment in order to determine potential predictors of resistance to *Schistosoma mansoni* infections. Samples were obtained from 197 school children aged 6-14 in the Mayuge district of Uganda. Infection data was acquired using three days of duplicate Kato-Katz to obtain *S. mansoni* egg counts. Soil-transmitted helminth (STH) egg counts and urine for point-of-care circulating cathodic antigen tests (POC-CCA) for *S. mansoni were also obtained*. Finger prick blood samples were obtained at baseline, 24 hours, 3 weeks, 9 weeks, and 6 months post treatment with praziquantel for immunological analysis and stored on filter paper. Using a commercial fluorescent bead-based Luminex cytokine assay, cytokine

profiles were established at each time point and correlated with infection data. Potential cytokine predictors of resistance-to-reinfections as well as any differences between those with high and low infection intensities of *S. mansoni* will be presented. These results will contribute to a broader biostatistical study looking at host factors that influence *S. mansoni* clearance and reinfection rates. Implications will be discussed in relation to ongoing MDA programmes and how to maximise treatment success in individuals taking the drugs.

2023

PROTEOMIC ANALYSIS AND IDENTIFICATION OF *SCHISTOSOMA MANSONI* CERCARIAL TAIL PROTEINS

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Schistosomiasis, caused by *Schistosoma mansoni*, is responsible for infecting 200 million patients worldwide; it is a key neglected tropical parasitic disease. An infection is initiated when the cercariae is released from its intermediary host, a *Biomphalaria* snail, into the surrounding water. Cercariae are free swimming, highly motile forms with bifurcated tails and they penetrate the mammalian skin tail-first, thus infecting the human host. Post attachment, the cercariae sheds its tail and the resulting somule continues to develop inside the host circulatory system. Cercarial tail motility plays an essential role in host location and survival of the parasites outside the mammalian body, yet the proteins supporting this prolonged, vigorous movement are not understood. In this study, we have extracted and identified the proteins in the isolated cercarial tails. Using mechanical separation of tails and bodies and mass spectrometric analyses, we have identified a total of 945 proteins in the combined cercarial proteome from 4 independent samples: 791 proteins in the cercarial tails and 645 proteins from the somule bodies. Based on the results and the research conducted from the *Schistosoma mansoni* database, we have selected 12 proteins upregulated in cercarial tails for recombinant expression allowing for biochemical studies and antibody production to localize these uncharacterized schistosome proteins. These include: twitchin, glycogenin, glutamine synthetase, tegument-allergen like protein; taurocyamine kinase, lengsin and 3 hypothetical proteins. By determining the function and localization of these specific proteins in the cercariae, we hope to propose possible therapeutic mechanisms via which parasitic host skin invasion can be avoided.

2024

COMPARATIVE GENOMICS PROVIDES INSIGHT INTO THE EVOLUTION OF TREMATODES IN THE FAMILY *FASCIOLIDAE*

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The liver and intestinal flukes in the family Fasciolidae cause zoonotic food-borne infections that impact both agriculture and human health throughout the world. Here, we present a whole genome sequence-based comparative analysis of *Fasciola hepatica* (1.14 Gb), *Fasciola gigantica* (1.13 Gb), and *Fasciolopsis buski* (748 Mb) with the aim to better understand their evolutionary history and the genetic basis underlying their phenotypic and ecological divergence. The split between the genera *Fasciolopsis* and *Fasciola* took place around 90 Ma in the late Cretaceous period, and between 65 and 50 Ma an intermediate host switch and a shift from intestinal to hepatic habitats occurred in the *Fasciola* lineage. The rapid climatic and ecological changes during this period (e.g., K–Pg mass extinction and Paleocene–Eocene Thermal Maximum) may have contributed to the adaptive radiation of these flukes. Analysis of gene family dynamics indicates the expansion of cathepsins, fatty acid binding proteins, protein disulfide-isomerases and molecular chaperones in *Fasciola* spp. highlighting the significance of excretory-secretory proteins in lineage-specific adaptation. *F. hepatica* and *F. gigantica* diverged around 5 Ma near the Miocene-Pliocene boundary that coincides with a reduced faunal exchange between Africa and Eurasia. Estimates of historical *F. hepatica* population size indicate a severe decrease in the effective population size around ~10 Ka, consistent with its recent global spread associated with ruminant domestication. Genome-wide analysis of selection signatures reveals that G-protein-coupled receptors are under positive and/or relaxed purifying selection in *Fasciola*, suggesting their roles in adaptation of physiology and behavior to new ecological niches. Together, these findings deepen current understanding of the biology and evolution of these important but neglected pathogens, and the genomic resources provided by these studies will help support the development of improved interventions, and lay a solid foundation for genomic epidemiology to trace potential drug resistance or introduction of infection.

2025

EXPRESSION OF EMBRYONIC SOXB IN SCHISTOSOMES AFTER HOST INVASION

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Schistosome helminths are a major global problem and infect over 200 million people. Human infection occurs when transient and free-swimming schistosome larvae, cercariae, penetrate human skin and begin a complex developmental transition in which the larvae adapt from a freshwater environment to survival in saline blood. As the parasite develops in the host it expands its the primordial gut, develops the tegumental surface, elongates its body, and evades the host immune response. While we have made significant progress in our understanding of schistosome biology, we are still limited when it comes to defining some of the basic genetic pathways crucial for schistosome development after infection of the human host. Sox B proteins are transcriptional activators expressed prior to blastulation in mammals and necessary for embryogenesis. Sox B proteins are pioneering transcriptional activators that coordinates with several protein partners and is associated with pluripotency and stem cells, neuronal differentiation, gut development, and cancer. Here we describe a schistosome Sox B homolog. Although most mammalian studies on Sox proteins have been analyzed in cell culture, schistosomes express a Sox2-like gene after infecting a mammalian host, long after formation of a blastula, when schistosomes have about 1000 cells. We have characterized a schistosome SOXB (SM-SOXB) homolog whose transcript is developmentally regulated, and we show that it is indeed a conserved Sox protein. SM-SOXB functions as a transcriptional activator, and it binds SOX specific DNA elements. We show its localization in the cercariae and developing schistosomula. However, it is not clear that the schistosome SOXB functions in conjunction with some known coregulators of SoxB proteins, suggesting that it may play a distinct role or have a differing mechanism of action in developing schistosomes. This work demonstrates that schistosomes are an unexpected resource to expand a potential role for Sox2 function at the organismal level, rather than at the embryonic level.

2026

THE "GESHIARO" PROJECT PROTOCOL: DEVELOPING A SCALABLE AND COST-EFFECTIVE MODEL OF INTERVENTIONS FOR THE INTERRUPTION OF TRANSMISSION OF SOIL-TRANSMITTED HELMINTHS AND SCHISTOSOMIASIS IN ETHIOPIA

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National deworming programmes rely almost exclusively on the mass administration of deworming drugs to children to control morbidity caused by these parasitic infections. The provision of other interventions, such as water, hygiene and sanitation behaviour change, and expansion of drug coverage to treat the entire at-risk population—not just children—should allow disease reductions achieved through drug distribution to be sustained once transmission has been interrupted. Experience indicates that the transmission of some parasitic worms can be broken in some settings through provision of multiple interventions, but it has not been attempted at scale in a developing country through the existing health system. The Geshiaro project has the potential to define an 'End Game' for soil-transmitted helminths (STH) and schistosomiasis (SCH) programmes. Interrupting transmission of these infections would eliminate the need for long-term repeated mass drug administration, lead to sustained health improvements in children, and allow health systems to focus on other disease priorities. The investment will test the feasibility of interrupting transmission at a large scale within a health system, through mass drug administration as well as complementary Water, Sanitation and Hygiene (WaSH) Interventions to sustain disease reductions and develop a working model for scaling up transmission interruption in Ethiopia and other countries. Interrupting transmission will require four years of biannual treatment to drive disease prevalence below 2%, the threshold at which transmission is believed to be no longer possible (transmission interruption). At that point, the WaSH interventions will be fully scaled up to sustain reductions, and treatment can stop. Five years will be needed to accomplish these implementation components and drive prevalence down to levels that cannot sustain transmission.

2027

QUANTITATIVE BIAS ANALYSIS TO ACCOUNT FOR MISCLASSIFIED PEDIATRIC DIARRHEA IN A CLUSTER-RANDOMIZED WATER INTERVENTION TRIAL

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Diarrhea is a major contributor to global child morbidity and mortality. Most trials measure diarrhea using caregiver-reported symptoms, which are prone to substantial measurement error. Quantitative bias analysis can be used to estimate the direction, magnitude and uncertainty arising from misclassified outcomes. We conducted a cluster-randomized trial of a water improvement intervention in the Amhara region of Ethiopia. Fourteen communities were selected for the trial, with half randomized to a water point intervention and the other half to control. Caregivers were asked to report if children had three or more loose or watery stools in a 24-hour period anytime in the past seven days. We used logit-binomial mixed-effects models with a random effect for community to estimate the effect of the intervention on caregiver-reported diarrhea. We then used probabilistic bias analysis to calculate bias-corrected prevalence ratio (PR) with a specified uniform distribution. 28.6% (42/155) children in the water intervention arm and 24.8% (38/160) of children in the control arm had a reported event of diarrhea [PR = 1.15 (0.69, 1.7), p = 0.571]. The bias-corrected PR was 1.36 (95% CI: 0.87, 4.88) for non-differential misclassification and 1.19 (95% CI: 0.16, 9.98) for differential misclassification. In conclusion, we found no effect of a clean-water intervention on pediatric diarrhea using caregiver-reported symptoms. Probabilistic bias analysis revealed that corrected effect estimates are highly dependent on the specificity of reported diarrhea and are father from the null under differential assumptions compared to non-differential assumptions. Due to the small effect size and wide confidence intervals, probabilistic bias analysis did not reveal a corrected true effect of the water-improvement intervention. Larger trials with more precise effect estimates may be more informative targets form probabilistic misclassification bias analysis.

2028

E. COLI DETECTION, GROWTH, AND DIVERSITY IN HOUSEHOLD SOILS FROM HARARE, ZIMBABWE AND MIRZAPUR, BANGLADESH

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Food and environmental compartments (i.e., water, hands, soils) act as intermediates in the spread of *E. coli* pathotypes from infected to susceptible people and animals. Increasingly, *E. coli* - including pathotypes - are detected in soils within or near households. The source of *E. coli* is unclear, with evidence of contributions from both people and animal. However, an overlooked factor may be *E. coli* growth in the external environment. In two studies in Harare, Zimbabwe and Mirzapur, Bangladesh, we investigate *E. coli* in soil collected from household plots. We evaluated isolates from soil and feces (human, chicken, cattle) for growth using soil microcosms and explored phylogenetic relationships based on whole genome sequencing. We found *E. coli* in 63% (n = 142) of soil samples in Harare, significantly higher than the 44% (n = 52) in Mirzapur (p = 0.02). Pathogenic *E. coli* were detected at both sites, with enteroaggregative *E. coli* (2/45 isolates screened) in Harare and shigatoxigenic, enteropathogenic, enteraggregative, and enterotoxigenic *E. coli* (18/175 isolates screened) in Mirzapur. At both sites, there were no or weak associations between *E. coli* in soils and household factors (agricultural/ practices; infrastructure; water, sanitation, and hygiene). In contrast, we found moderate correlations between *E. coli* and soil characteristics (i.e., moisture (Spearman's rho = 0.48, $p < 0.01$ in Mirzapur and rho = 0.36, $p< 0.01$ in Harare) and clay (Spearman's rho = 0.47, p = 0.01 in Mirzapur, not tested in Harare). Soil microcosms showed *E. coli* growth for isolates from both sites, dependent on soil (10 soils tested) but not isolate (4 from Harare, 10 from Mirzapur). Finally, phylogenetic reconstruction revealed high diversity amongst *E. coli* isolates from soil, chicken, human, and cattle in Mirzapur. Specifically, of 60 *E. coli* sequenced, there were 36 sequence types, with no type significantly more prevalent in a particular reservoir. Overall these findings indicate that *E. coli* circulating in domestic settings from different reservoirs are remarkably diverse, capable of growth in soil, and not meaningfully associated with household-level factors.

2029

EFFECT OF A BEHAVIOR CHANGE INTERVENTION TO IMPROVE PERI-URBAN SANITATION QUALITY IN LUSAKA, ZAMBIA: A RANDOMIZED CONTROLLED TRIAL

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Despite gains in sanitation coverage globally, the prevalence of peri-urban shared sanitation is on the rise in sub-Saharan Africa, and the growth rate of such peri-urban areas is increasing. Only a few studies have rigorously evaluated how to improve sanitation quality, and none have examined improving structural quality in addition to cleaning practices. We did an individually randomized controlled trial in a peri-urban area in Lusaka, Zambia between August 8, 2017 and March 1, 2018. We enrolled 1085 adult landlords on plots where the landlord and at least one tenant household lived. The intervention consisted of a series of group meetings of landlords discussing motivating sanitation quality improvement as a way to build wealth and reduce conflict and the control received no intervention. We measured outcomes one month before the start of the intervention and four months post-intervention through repeated surveys of landlords and tenants. We compared intervention and control groups on an intention-to-treat basis using difference in differences. We found significant impacts on several of our primary outcomes measuring individual sanitation components as well as on a composite measure of overall sanitation quality. Ensuring that intervention landlords attended all meetings and low response rates were major challenges, though these difficulties of working in a peri-urban setting were anticipated, and several different imputation assumptions and exposure adjustments are tested to determine the robustness of the findings. Behavior-change messaging to create demand for improving peri-urban sanitation quality may be a cost-effective tool and should be evaluated in other contexts as a supplement to current widespread infrastructure improvement projects.

2030

DEVELOPMENT OF RECOMMENDATIONS FOR FOMITE DISINFECTION IN CHOLERA OUTBREAKS

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Fomites are inanimate objects that can become contaminated and transmit infectious agents. Guidelines on how to disinfect fomites in cholera outbreaks, while mostly chlorine-based, are inconsistent in terms of concentration, contact times and recommended practices. We are investigating fomite disinfection in three studies: 1) a systematic review; 2) laboratory experiments; 3) field-based evaluations. The initial database search for the systematic review yielded 9,484 references and data was extracted from 89 studies that met inclusion criteria. Only one study using the cholera agent Vibrio cholerae was identified. Analysis is in process and data relevance to low-resource outbreak settings will be discussed. In the laboratory, we are assessing the efficacy of chlorine against Vibrio cholerae and the indicator Escherichia coli on hard and porous surfaces. Expected results by fall include: 1) whether Escherichia coli is an appropriate surrogate for Vibrio cholerae; 2) whether spraying or pouring a 0.2% chlorine solution can inactivate Vibrio cholerae on surfaces within a given contact time (or what chlorine concentration and contact time should be recommended). Lastly, we have developed a protocol in partnership with Action Against Hunger, Médecins Sans Frontières (Operational Center Amsterdam), and the International Federation of the Red Cross and Red Crescent Societies to evaluate household spraying and household disinfection kits in cholera outbreaks in terms of effectiveness, as measured by surface sampling in situ, and appropriateness. The first evaluation will take place in the Democratic Republic of the Congo in May 2018 and we expect to have preliminary results from at least two evaluations by the time of the conference. In summary, the presentation would provide an overview of currently available data on fomite disinfection efficacy, laboratory data on chlorine efficacy against cholera bacteria, and preliminary results from field evaluations of household disinfection interventions, thus providing an example of a comprehensive strategy to develop evidence-based recommendations for low-resource outbreak settings.

2031

HUMAN WASTES MANAGEMENT IN THE MANGROVE AREAS OF EASTERN OBOLO LGA, NIGER-DELTA, NIGERIA

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A study was carried out on human wastes management in the mangrove areas of Okorombokho, Eastern Obolo LGA, Niger-Delta, Nigeria between the month of March and December, 2017. A total of 340 questionnaires were administered by systematic random sampling technique and all were retrieved. Water samples were also collected from rivers and a stream located in the area and were analyzed using standard laboratory methods, including microbiological analysis. Results show high total heterotrophic bacteria (THB) counts in all the five Stations where water samples were collected, with the lowest count (1×10^2) recorded in Station 1 during dry season and the highest count (103×10^1) observed in Station 5 during wet season. Enumeration of coliform counts revealed that water samples from Stations 1, 2, 3, 4 and 5 had 90MPN/100ml, 90MPN/100ml, 160MPN/100ml, 90MPN/100ml and 160MPN/100ml, respectively for total coliform count during the dry season and 180MPN/100ml for all the five Stations during the wet season. Statistical analysis show that there is significant difference ($P<0.05$) in bacteria count in the values obtained for THB, total coliform and faecal coliform in all the Stations during the study while there is no significant difference for the fungal isolate ($P>0.05$). Values obtained for THB and total coliform counts were above WHO permissible limits for drinking water. Bacteriological identification of 514 isolates obtained from the samples revealed the presence of these genera: *Pseudomonas auruginosa,* 47 (9.14%); *Escherichia coli,* 84(16.34%); *Streptococcus aureus,* 202 (39.30%); *Streptococcus pneumonia,* 165 (32.10%) and *Geotrichum spp, 1*6 (3.11%). This study indicates that this water source is highly polluted due to high presence of faecal coliform and is above WHO standard for drinking water and thus not safe for drinking or domestic use. It is therefore recommended that government should build Central Toilet Systems in this mangrove area and also formulate an effective waste segregation/management policy that will properly curb the unhygienic waste disposal practices in this fast environmentally challenged ecosystem.

2032

SANIPATH-TYPHOID AND ENVIRONMENTAL SURVEILLANCE FOR TYPHOID: A PROTOTYPE FOR LARGE SCALE DEPLOYMENT IN CITIES IN LOW- AND MIDDLE-INCOME COUNTRIES

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Enteric fever affects millions of people throughout Low- and Middle-Income Countries (LMIC). According to the most recent WHO estimates published in 2014, approximately 21 million typhoid cases and 222,000 typhoid-related deaths occur annually worldwide. The Indian subcontinent has the highest reported incidence of typhoid fever globally. It is vital to develop sensitive lab methods for detection of those pathogens in various environmental samples, build an in-depth understanding of typhoid transmission, and monitor the circulation of *S.* Typhi and *S.* Paratyphi A

in the environment. The SaniPath-Typhoid study (SPT) was conducted in five areas with demographic and geographic variability. Environmental samples from up to 10 sample types were analyzed for *E. coli*, human-specific phage, *S.* Typhi, and *S.* Paratyphi A. Positive samples for *S.* Typhi or *S.* Paratyphi A were archived for genomic characterization and future diversity analysis. A typhoid environmental surveillance (ES) strategy was designed based on results from pilot environmental sample testing and mathematical models. The models incorporated data collected from typhoid ES, data from the city's clinical surveillance, results from the study of *S.* Typhi and *S.* Paratyphi A persistence in different environments, information on population density and sewage system, recovery efficiency during sample processing, and detection sensitivity. The models provide guidance on the selection of ES sampling sites. The ES will be conducted in the same areas covered by active typhoid clinical surveillance (Ward 58 and Ward 59) as well as throughout the entire city of Kolkata. A total of 1250 and 6500 samples will be analyzed over a 2-year period during SPT and ES, respectively. The results of the SPT and ES will provide critical information about the risk of typhoid transmission through different environmental pathways and about *S.* Typhi and *S.* Paratyphi A circulation in the environment in endemic settings with vulnerable resident populations. This study will enable evidence-based decisions about vaccine and water, sanitation, and hygiene interventions.

2033

SUCCESSFUL APPLICATION OF MICROBIAL SOURCE TRACKING USING GB-124 BACTERIOPHAGE AS AN INDICATOR OF HUMAN FECAL CONTAMINATION IN ENVIRONMENTAL SAMPLES IN KOLKATA, INDIA

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Fecal indicator bacteria (FIB) have been used for over a century to connote potential fecal contamination. However, FIB may not correlate well with the presence of human enteric pathogens when nonhuman fecal sources are present. To address this need, microbial source tracking (MST) using bacteriophage of the *Bacteroides* spp. can serve as a reliable method to indicate human fecal contamination and be used to examine the risk of exposure to pathogens of human origin. The objective of this study was to investigate the presence and abundance of bacteriophage against a strain of *Bacteroides fragilis* (GB-124) in different environmental samples as well as in animal fecal samples in Kolkata, India. During the preliminary phase, we tested 28 sewage samples collected from different parts of Kolkata and evaluated them in 4 serial dilutions for the presence of phage against the GB-124 *Bacteroides* host. These samples ranged from very concentrated sewage collected from the manholes to drain water collected after heavy rain. Phage against the GB-124 *Bacteroides* host species were detected in 26 of the 28 samples. The phage concentration ranged from 200 to 50,000 plaques per mL (PFU/mL). The 2 samples that had no detectable phage against the GB-124 host were collected from an urban pig farm. This is the first report of a phage-based MST technique used successfully in India. Our results suggest that, although the host strain GB-124 is geographically constrained, it is present in high concentrations (up to 50,000 PFU/mL) in Kolkata and thus can be used as an effective technique to differentiate origin of fecal contamination. GB-124 phage can provide a sensitive, specific, quantitative, low-cost and rapid culture-based MST method to identify human fecal contamination in an urban setting with dense human and animal populations. Next steps include: 1), testing additional samples from a range of animal species commonly found in Kolkata to further confirm that GB-124 is human specific in this setting, and 2) examining 12 different types of environmental samples to detect the presence of human fecal contamination.

2034

IMPACT OF A LARGE HEALTH SYSTEMS STRENGTHENING PROJECT ON THE PREVALENCE OF DIARRHEAL DISEASE IN UNDER-FIVE CHILDREN IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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The Access to Primary Health Care (ASSP) project was implemented between 2014 and 2017 in order to improve reproductive, maternal, neonatal and child health, while strengthening the overall delivery and quality of health services in the Democratic Republic of the Congo. ASSP worked across five provinces in 52 health zones, targeting those where ASSP's predecessor project had been implemented and those with relatively weak health systems. ASSP strategies included enhancing health facility infrastructure and availability of services, providing community-based health interventions, revitalizing community health committees, improving health education, and increasing sustainable access to safe drinking water and improved sanitation. A quasi-experimental community level panel design with intervention and matched comparison health areas, utilizing population-based surveys conducted in 2014 and 2017, was used to evaluate the effects of the program on WASH related outcomes. The impact of exposure to ASSP on period prevalence of diarrheal disease among under-five children was tested using a difference-in-differences approach in a logistic regression model. Although, only 13.2% of intervention villages received a water, sanitation and hygiene (WASH) intervention, the period prevalence of diarrheal disease fell by 8.6 percentage points among under-five children in ASSP areas when compared to matched comparison areas (p=0.006). Project mechanisms that likely attributed to the decreased prevalence of diarrhea include increased use of safe drinking water sources and improved sanitation at the household level, enhancement of water and sanitation facilities at health centers and increased community participation in health services. Future work will include a sub-analysis of villages that received WASH specific interventions to measure any additional impact of the community-led total sanitation intervention.

2035

THE OROMIA TRACHOMA F AND E STRATEGY: A CASE EXAMPLE OF EMERGING PREFERRED PRACTICE

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Trachoma is a neglected tropical disease (NTD) and the leading infectious cause of blindness globally. The WHO-endorsed SAFE strategy (Surgery, Antibiotics, Facial Cleanliness and Environmental Improvement) provides a framework to guide trachoma elimination efforts with all four components necessary for effective disease control. Oromia in Ethiopia is the most trachoma-endemic region, in the most trachoma-endemic country in the world. In 2017, the need for a new F and E strategy for Oromia was recognised. Building upon the International Coalition for Trachoma Control's (ICTC) guiding principles for F and E and lessons learned from previous programming, and drawing upon theory and process frameworks common practice in health promotion and behavioural science, a new region wide approach to F and E programming was proposed. The approach seeks to find a balance between the importance of contextualised, locally relevant solutions and the need to deliver to scale an effective region wide F and E program. The Oromia trachoma F and E strategy comprises several components: 1) guiding principles; 2)

program elements; 3) outcome statements and associated indicators; and 4) a program planning approach. This paper provides an overview of the F and E strategy and its development, discusses its implications for other trachoma programs, and identifies next steps towards preferred practice in trachoma F and E programming.

2036

THE ASSOCIATION BETWEEN COMMUNITY-LEVEL SANITATION ACCESS AND ANEMIA IN WOMEN AGE 15-49 YEARS

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Lack of sanitation facilities enables fecal-oral transmission of pathogens that can lead to poor health outcomes, including anemia. Sanitation access is typically monitored at the household level, however recent evidence suggests that community level sanitation access (CSA) may be an important driver of health, even for those with household sanitation. In order to assess how community-level and household-level sanitation access affect women's anemia status, we conducted an individual-level meta-analysis of cross-sectional surveys. We retrieved information from all publically available demographic health surveys as of July of 2017 that measured anemia in women 15-49 years old (smoking and altitude adjusted hemoglobin <12g/dl in non-pregnant women, <11g/dl in pregnant women). We matched women within datasets on several individual and community covariates to account for selection bias. We estimated the association between anemia and household and CSA utilizing a generalized linear model with the matched group as a random intercept (adjusting for dataset). 619,016 women from 93 surveys from 42 countries from 1997-2016 had recorded anemia status. After matching, there were 578,995 women in 1,356 matched groups. The prevalence of anemia was lower among women in communities with higher access to sanitation, whether or not the woman had access to household sanitation (χ^2 p-value <0.001). For women without household sanitation, the odds of anemia were decreased in communities with higher CSA, OR: 1.09 (0% CSA), Ref. (1-30% CSA), 0.92 (31-60% CSA), 0.94 (61-99% CSA) (p-value <0.001 for all). The same pattern was seen in women with household sanitation although not statistically significant except for those living in communities with complete CSA, OR: Ref (1-30% CSA), 0.97 (31-60% CSA, p-value: 0.275), 0.98 (61-99% CSA, p-value: 0.296), 0.95 (100% CSA, p-value: 0.03). Women are more likely to be affected by anemia in communities with lower CSA, especially among women without household sanitation. When estimating the benefits of eliminating open defecation, it is important to consider both the community and household level impacts.

2037

WATER, SANITATION, AND HYGIENE ACCESS IN SOUTHERN SYRIA: ANALYSIS OF SURVEY DATA AND RECOMMENDATIONS FOR RESPONSE

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Water, sanitation, and hygiene (WASH) are immediate priorities for human survival and dignity in emergencies. In 2010, before the ongoing conflict, Syria had >90% access to safe drinking water. The WASH response in Syria aims to reduce disease transmission risk in this middle-income context by maintaining: 1) the functionality of preexisting WASH infrastructure and, 2) safety of alternative trucked water. The objective of this study was to assess the impact of these risk reduction programs. In 2016 and 2017, respectively, 1,281 and 1,360 household surveys were conducted in Southern Syria to collect information on demographics, WASH access, drinking water free chlorine residual (FCR), and reported <5 years old children diarrhea. We built regression models to study associations between demographic and WASH variables with FCR presence and diarrheal incidence. Piped water as the main source declined from >90% in 2010 to 22% in 2016 and 15% in 2017; private sector trucked water filled this supply gap. Households accessed 50-60 liters of water/ person daily and spent ~20% of income on water. FCR presence in stored water increased from 4% in 2016 to 28% in 2017, with receiving risk reduction programming strongly associated with FCR presence (OR 24, 95% CI 6-99). Access to sanitation and hygiene were also protective against childhood diarrhea. Based on these correlations between risk reduction programs and outcomes, we are conducting a follow-up study to understand the relative impact of risk reduction interventions i.e. chlorine distribution and training, awareness campaigns, chlorinators at wells, and trucker incentive. The data were collected from four intervention groups implementing different combinations of these activities and a control group. Analysis to compare relative impacts of these strategies is ongoing; both studies would be presented at the meeting. In summary, appropriate WASH intervention can improve water quality and reduce disease in emergencies. Lessons from this protracted emergency response in a middle-income context with preexisting WASH services will provide insights for future WASH response design in similar context.

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A SPATIALLY EXPLICIT RISK ASSESSMENT OF FACTORS IMPACTING DRINKING WATER QUALITY IN RURAL BANGLADESH

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Most rural Bangladeshis source their drinking water from shallow tubewells as an arsenic mitigation approach. However, these wells are found to have poorer water quality and are associated with higher incidence of diarrheal diseases. The alternative mitigation approach—sourcing from deep tubewells—has demonstrated protection against arsenic exposure and offers improved water quality. However, the health effects of transitioning to these sometimes more distant and less accessible wells have not been adequately investigated. Using a combination of household surveys, geospatial data, and environmental microbiological data, we follow a prospective cohort of 500 households (250 shallow and 250 deep tubewell users) over two years to examine under what conditions deep tubewells are most protective against microbial contamination and diarrheal disease. Preliminary baseline results suggest that despite procuring water from sources with very low contamination at source, 50% households drank contaminated water. We ran Mann-Whitney tests to confirm that deep tubewell users were at lower risk of water storage contamination compared to shallow tubewell users. However, among the 40% deep tubewell households drinking contaminated water, we found that on average, they stored water longer, and had significantly higher travel time and distances to their drinking water source, compared to other households. These results suggest that although deep tubewells may protect against poor household drinking water quality, impediments to access such as longer distance and lack of ownership modify handling and storage, subsequently increasing the possibility of microbial contamination. Next, we will incorporate other contextual factors such as perceived barriers to access, seasonality, household wealth, and the sanitation environment from baseline and follow-ups to examine the ecological context under which deep tubewells modify microbial contamination risk. With increasing interest in installing more deep tubewells as an arsenic mitigation intervention, our results offer considerations for policymakers in rural Bangladesh and beyond.

2039

QUALITATIVELY DIFFERENT ENTERIC PATHOGEN RESERVOIRS AND TRANSMISSION PATHWAYS ARE ASSOCIATED WITH CHILDHOOD GROWTH IN THE KENYAN AND GAMBIAN SITES; AN ANALYSES OF THE GLOBAL ENTERIC MULTICENTER STUDY

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Important reductions have occurred in childhood growth impairment in many Sub-Saharan countries but reductions are not uniform across the region. Further reductions may be possible through the development of targeted precision health interventions based on an understanding off causal relationships between household risk factors, enteric pathogen transmission and childhood growth. Associations of potential household pathogen transmission pathways with HAZ were tested using structural equation models (SEM) in the rural communities of Nyanza Province, Kenya and Basse, the Gambia that were part of Global Enteric Multicenter Study (GEMS). Models first tested whether household pathogen reservoirs had direct effect on height-for-age z-scores (HAZ) or were mediated by enteric infections. Models then tested whether hygiene behaviors and improved water sources reduced pathogen transmission and children's exposure and so moderated these indirect associations. A total of 3,359 children were enrolled in Kenya and 2,598 enrolled in the Gambia. In Kenya, unimproved toilet facilities was negatively associated with HAZ while water storage had a direct negative association and an indirect association mediated by *Giardia* infection. Sheep, goats and rodents in the compound had indirect negative associations with HAZ mediated by *Giardia* and Enteropathogenic *E. coil* (EPEC) infections. Associations were reduced through elimination of infections by specific handwashing behaviors and household improved water sources. In Gambia, unimproved toilet facilities had an indirect negative association with HAZ via ETEC, *Cryptosporidium* and *S. flexneri* infections but handwashing after handling animal reduced this effect through elimination of infections. These differences in household pathogen reservoirs and corresponding pathways between the sites may result from differences in community infrastructure and economies. Targeted health interventions may more effectively reduce childhood growth impairment in these two countries using household level estimates of pathogen transmission and exposure risk derived from these models.

2040

FINE-SCALE SPATIO-TEMPORAL VARIATION IN PNEUMOCOCCAL PNEUMONIA BURDEN IN CHILDREN IN AFRICA IN THE ERA OF THE PNEUMOCOCCAL CONJUGATE VACCINE

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In 2000, pneumococcal pneumonia was responsible for 281,200 deaths in children under the age of five in Africa. By 2016, this decreased to 177,700. This is even more impressive when considering population growth across the continent - the mortality rate decreased from 25 per 10,000 to 11 per 10,000. In spite of this gain, pneumococcal pneumonia remains the most common etiology of lower respiratory infections across Africa (and globally). There are numerous reasons pneumococcal pneumonia burden has declined in Africa. Along with improved nutritional indicators and lower exposure to indoor air pollution, the introduction of the pneumococcal conjugate vaccine (PCV) has had a substantial impact in reducing risk in multiple countries. Zambia, for example, saw their under-5 mortality rate associated with pneumococcal pneumonia drop from 64 per 10,000 to 22 per 10,000 (their 2016 PCV coverage was estimated to be 87%). Conversely, several countries in the central Sahel have not yet introduced PCV, and while they have experienced some decline in the past 16 years, it is considerably slower than their neighbors. Here, leveraging house-hold surveys on both lower respiratory infection prevalence and vaccine coverage, applying Beysian geostatistical techniques and integrating the Global Burden of Disease project, we produce continent-wide estimates of fine-scale variation in pneumococcal pneumonia burden from 2000 to 2016. In addition to identifying substantial sub-national variation in burden across numerous African countries, we identify risk factors associated with these variations. In general, areas with high indoor air pollution and poor nutritional indicators are more likely to have high pneumococcal pneumonia burden, but there are regions were these risk factors are mitigated with PCV. As with our work on overall lower respiratory infection burden in Africa, we identify a concentration of risk to a few countries in the Sahel. Fine-scale estimates of burden are a necessary tool for public health interventions. These results should be used to identify optimal targeting strategies to reduce the burden of this vaccine-preventable infection.

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A GENE EXPRESSION SIGNATURE ACCURATELY IDENTIFIES VIRAL ACUTE RESPIRATORY INFECTIONS IN A SRI LANKAN POPULATION

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Viral acute respiratory infections (ARI) are commonly treated with antibiotics due to limitations with pathogen-based diagnostics. A host-based, reverse transcription polymerase chain reaction (RT-PCR) gene expression signature identified viral ARI with 90% accuracy in a US population. We determined signature performance in a Sri Lankan population. We enrolled febrile patients in Sri Lanka from Jul '12-May '13 and collected nasopharygeal samples, serum, and blood in PAXgene RNA tubes. We confirmed influenza using multiplex PCR (Luminex NxTAG). We phenotyped patients with a non-infectious syndrome if testing excluded respiratory viruses, dengue, leptospirosis, and rickettsial illnesses and they had a non-infectious clinical diagnosis. We extracted total RNA and performed host RNA sequencing (Illumina). We aligned reads to hg38 reference genome using Bowtie2, quantified at isoform level using Express version 1.5.1, and normalized using trimmed-mean normalization. We mapped genes from the prior signature to RefSeq transcripts and summed isoforms to obtain gene-level expression estimates. We used the limma-voom framework to assess differential expression. We used the first principal component of all genes to generate a score and assessed difference using a t-test. Among 29 patients with influenza and 3 patients with non-infectious syndromes, median age was 35.2 years (IQR 21.4-55.8) and 50% were male. Median duration of fever was 4 days and 72% patients received antibacterial therapy. Twenty-five genes from the prior signature were identified; 20 were different in patients with viral vs non-infectious syndromes ($p<0.05$). The small sample limited our ability to fit

a model and assess resampling-based performance. The summary score (79% explained variance by first principal component) differed between groups (p<0.001) and high discrimination was observed (AUROC=0.95). A gene expression signature performed well at identifying viral ARI versus non-infectious illness in a Sri Lankan population. Host-based diagnostics may play an important role in identifying viral ARI and reducing inappropriate antibiotic use globally.

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MAPPING LOWER RESPIRATORY INFECTIONS IN SPACE AND TIME ACROSS AFRICA, 2000 - 2016

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Lower respiratory infections (LRI) are the second largest cause of disease burden for children under five in Africa, responsible for more than 327,000 deaths and 2,850,000 severe infections in 2016. Previous efforts to understand the spatiotemporal burden of LRIs lack either the spatial breadth or fine spatial resolution presented here. Annual estimates of LRI prevalence, incidence, and mortality among children under five years old were produced with high geographic detail (5-km^2) across Africa from 2000 to 2016. Estimates were created using geolocated survey clusters from 201 household surveys, a suite of environmental and socio-demographic covariates, Bayesian geostatistical techniques, and calibrated to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2016 results. In 2016, estimated deaths due to LRIs in first administrative subdivisions ranged from less than one in Al Kufrah, Libya to 8,070 (95% UI 7930–8230) deaths in Oromia in Ethiopia. The highest mortality rates were estimated to occur in Juba Hoose (11 per 1,000 (95% UI 9 – 12)) and Juba Dhexe (10 per 1000 (95% UI 9 – 12)) in Somalia and Mont de Lam (8 per 1,000 (95% UI 7 – 9)) and Barh Koh (8 per 1,000 (95% UI 7 – 9)) in Chad. These two countries, along with Central African Republic are the current *Foci* of LRI mortality in Africa. Juba Hoose and Juba Dhexe also feature highest estimated rates of severe incidence at 45 per 1,000 (95% UI 40 – 51) and 41 per 1,000 (95% UI 36 – 47) respectively. Our findings identify substantial spatial and temporal variation in the burden of LRI on the African continent. However, we also note the increasing concentration of disproportionate burden due to LRIs in the central part of the continent—just south of the Sahel. These results expose areas of high burden, stagnation, and uncertainty. They should be used to guide targeted interventions within and between countries, assess the effectiveness of current strategies and plan data collection efforts.

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CLIMATE VARIABILITY AND CHILDHOOD PNEUMONIA IN RURAL BANGLADESH: A TIME SERIES ANALYSIS

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Pneumonia is the leading cause of mortality and morbidity for children under the age of 5 years old in developing countries. Very few studies have assessed the association between climate factors and child pneumonia in a climate vulnerable country like Bangladesh. The research aimed to examine the relationship between climate variability and childhood pneumonia in rural Bangladesh and compare the difference of the climate effects on pneumococcal conjugate vaccines (PCV) intervention. Weekly data on pneumonia cases and climate variables (temperature and relative humidity) were collected from the Matlab hospital icddr,b and Bangladesh Meteorological Department, respectively during 1st January 2012 and 31st December 2016. Time series cross-correlation functions were applied to identify the time lags of the effect of each climate factor on pneumonia. Time series generalized linear regression model (GLM) with Poisson link was used to quantify the relationship between climate factors and childhood pneumonia. Moreover, the study was divided into two research periods by the introduction of PCV in national immunization program. A total of 2,655 pneumonia cases were admitted during the study period. Our results showed that before PCV intervention (average 717 cases per year), only temperature (relative risks (RR): 0.966, 95% Confidence Interval (CI): 0.955-0.976) was significantly associated with child pneumonia but after PCV intervention (average 252 cases per year) both temperature (RR: 0948, 95% CI: 0.916-0.980) and relative humidity (RR: 0.972, 95% CI: 0.956-0.990) had the significant association with child pneumonia after adjustment of seasonality in the model. The results of this study suggested that weather variability may have played a significant different role in children pneumonia before and after PCV intervention. This results will help to develop the framework of early warning model based on climate factors for pneumonia infection in under developed country like Bangladesh.

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DEVELOPING A NOVEL DEEP LEARNING BASED CAVITY DETECTION ALGORITHM FOR TUBERCULOSIS SCREENING ON CHEST XRAYS

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Tuberculosis is the number one killer among infections accounting for ~1.6 mn deaths in 2016. Chest Xray is one of the most important screening tools for both Pulmonary and Extrapulmonary TB. With scarcity in X ray reading skills in Resource limited settings, its high time that we had automated reporting aiding in early diagnosis. Deep learning, particularly Convolutional Neural Networks(CNN) have shown promising results in identifying abnormalities on images. Though any CXR abnormality needs to be screened for TB, Cavity being one of the most common finding, we developed a 3 stage pipeline for detecting cavity from CXRs. a) Extract overlapping patches in the lung roughly corresponding to the three zones by using a thorax segmentation model. b) Classification of extracted patches as normal/containing cavity. c) Combining patch level predictions with a random forest classifier to reach a scan level prediction. For a, 750 CXRs were manually annotated and a segmentation model(UNET) was trained to emit masks for left and right thoraces separately. These masks were used to extract 3 overlapping patches from either side generating six patches that cover the entire lung area in a CXR. For b, 884 images containing cavities were annotated on a pixel level to create a cavity mask. The patches generated in a were assigned 'cavity yes/no' ground truths based on these annotations. Similarly, patches were extracted from images that do not have cavity and assigned a 'cavity no' ground truth. The dataset thus created was used to train a CNN to detect the presence/absence of cavity in a particular lung patch. These patch level predictions are used as features to train a final random forest classifier to obtain scan level predictions for cavity detection in c. On a testset, the Area under the ROC curve is 0.90 for the detection of cavity whereas the sensitivity at a specificity of 90 was 81. This novel approach of segmenting thorax into overlapping patches improves upon the current black box classification approaches. This algorithm alongside automated detection for other abnormalities on CXR, could be a significant milestone in the early diagnosis strategy towards ending TB.

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INFLUENZA INCIDENCE, PREVALENCE, AND MORTALITY ONE HUNDRED YEARS AFTER THE 1918 GLOBAL PANDEMIC: RESULTS FROM THE GLOBAL BURDEN OF DISEASE 2017

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On the 100th anniversary of the 1918 global influenza pandemic, the virus still represents a significant burden of disease and a looming threat for another global epidemic. The Global Burden of Diseases, Injuries, and Risk Factors Study 2017 (GBD 2017) is a systematic, scientific effort to measure the burden of lower respiratory infections (LRIs) and their etiologies, including influenza. Influenza was attributed to episodes, hospitalizations, and deaths due to lower respiratory infections using a counterfactual approach wherein the modeled frequency of influenza in LRIs and the association between influenza and LRI were assessed to create a population attributable fraction. We estimated separate PAFs for episodes, hospitalizations, and deaths due to LRI attributable to influenza. The GBD study enforces internal consistency between incidence and mortality to create directly comparable estimates for cases, hospitalizations, and deaths due to influenza LRI. In 2017, influenza was responsible for 121,000 deaths (95% UI 96,000-142,000) and 39,547,000 episodes of LRI (95% UI 30,787,000-49,691,000). Over half of influenza LRI deaths occurred among adults over 70 years old (63,000 deaths, 95% UI 51,000-74,000) including nearly 3000 deaths among this age group in the United States (2,800, 95% UI 2,400-3,200). Influenza was less frequently associated with hospitalized episodes of LRI than non-hospitalized episodes and was responsible for 2,843,000 LRI hospitalizations (95% UI 1,569,000-4,908,000), a rate of 0.38 per 1,000 (95% UI 0.21-0.66) and for 24,506,000 hospital-days (95% UI 10,295,000-55,606,000). Influenza was responsible for about 12% of all LRI episodes and represented a greater share of episodes across all ages including nearly 15% in adults 15 to 49 years and 10% among adults over 65 years. Our results suggest that influenza is an important cause of lower respiratory infections globally and particularly among adults over 70. Expanded access to the seasonal influenza vaccine and the development of a universal influenza vaccine may help alleviate some of the significant burden of disease due to influenza LRI.

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HARNESSING MOBILE PHONES TO DEVELOP A RESPIRATORY DISEASES EVENT-BASED SURVEILLANCE SYSTEM IN RURAL BANGLADESH: A PILOT STUDY

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Community event-based surveillance (CEBS) is the organized and rapid capture of information from communities about events that pose potential public health risks. We piloted a CEBS to collect information on respiratory symptoms via mobile phone Short Message Service (SMS) to explore the functionality and feasibility of the system. Between March 2014 and October 2015, we recruited and trained 104 community members from 5 villages from 5 districts in Bangladesh, including drug sellers, religious leaders, school teachers, backyard and commercial poultry raisers and veterinarians to send information on respiratory symptoms and disease outcome in humans, as well as in birds with potential exposure risks to human. Each group had a target to send an SMS to icddrb server ranging from daily to weekly irrespective of occurrence of an event. SMS costs were reimbursed. We conducted a formative study and an end-line survey to identify key motivators and barriers for participation in the study. Of the 104 study participants, 99 (95%) sent a total of 2820 (65% of target) SMS about respiratory symptoms. No major technical difficulties were reported. Religious leaders were the most consistent, sending 78% (121/156) of all SMS, but required frequent reminders and/or extensive training, followed by drug sellers, who were the most enthusiastic participants, with 69% (1328/1936). Backyard poultry raisers were the lowest reporting group with 57% (810/1415). Participants with higher educational status were more likely to send SMS regularly (p=0.001). The study participants' motivation factors associated with sending SMS were positive encouragement from community (89%, 95% CI: 87-95%), well-being of the country (84%, 95% CI: 76-91%) and quick response during an outbreak (67%, 95% CI: 57-76%). Forgetfulness (76%) and difficulty in using SMS system (19%) and were found to be the most reported barrier, and interestingly, the SMS reimbursement scheme was not perceived as a barrier. The event-based surveillance system using mobile phone networks through voluntary participation is functional and feasible in Bangladeshi rural communities.

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PLASMODIUM FALCIPARUM GENOME DIVERSITY AND ECOLOGICAL DYNAMICS IN *ANOPHELES FUNESTUS* MOSQUITOES FROM NORTHERN ZAMBIA

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Plasmodium falciparum undergoes asexual reproduction within the human host but reproduces sexually within its vector host, the *Anopheles* mosquito. In regions of moderate to high malaria transmission, infectious mosquito bites occur frequently and human infection typically consists of multiple parasite clones. Under these conditions, cross-fertilization between genetically distinct parasite gametes may occur more frequently than inbreeding within the mosquito midgut, possibly contributing to high genetic diversity of the parasite population. Although generation of genetic diversity arises within mosquitoes, few empirical studies have characterized the proportion of mixed infections in natural mosquito populations and the degree of inbreeding of co-infecting parasite clones within single mosquitoes. Measuring these parameters is critical to understanding how malaria parasite diversity arises within mosquitoes and the ecological mechanisms that drive parasite diversification. This information has profound implications for predicting transmission outcomes and parasite evolution. We used a multiplexed hybrid capture technique to selectively enrich and deep sequence whole *P. falciparum* genomes directly from 28 field-caught *Anopheles funestus* mosquitoes from a high transmission setting in Nchelenge District, northern Zambia. Across the sequenced samples, we obtained a mean of 72% of *P. falciparum* genomes covered at > 5x. A high proportion of *P. falciparum* polygenomic infections was found in mosquitoes based on genome-wide signal. A negative correlation was observed between parasite load and complexity of infection, suggesting interactions between parasite clones. Some of the polyclonal infections were comprised of unrelated parasite genomes. Population genomic studies of mosquito-derived *P. falciparum* samples can greatly aid understanding of transmission dynamics by

clarifying the role of mosquitoes in modulating parasite diversity at the population level and by detecting potential cotransmission of unrelated parasites through a single mosquito blood meal.

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HAPLOTYPES OF AUTOPHAGY RELATED-GENE 10 (*ATG10*) PROMOTER POLYMORPHISMS INFLUENCE SUSCEPTIBILITY TO SEVERE MALARIAL ANEMIA IN KENYAN CHILDREN FROM A HOLOENDEMIC TRANSMISSION REGION

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Severe malarial anemia (SMA, hemoglobin<5.0 g/dL, with any density parasitemia) is a leading cause of global morbidity and mortality among children under 5 years of age residing in *Plasmodium falciparum* holoendemic transmission regions. The molecular pathways that promote SMA are only partially understood. To identify molecular pathways that mediate the development of SMA, we performed GWAS and global transcriptomics in a subset of children with polarized distributions of mild and severe malaria. The global genomic studies revealed Autophagy Related Gene (*ATG)-10* as an important target for validation. Although largely unexplored in human malaria, autophagy is a fundamental cellular process which maintains homeostasis, protein degradation, and production of inflammatory mediators. ATG10, an ubiquitin-conjugating enzyme (E2)-like protein, is crucial for autophagosome elongation. We explored the relationship between *ATG10* promoter polymorphisms [rs2406905 (G-7723T), rs4391141 (T-4322A), and rs1023969 (G-2442C)] and susceptibility to SMA in a pediatric malaria cohort (n=1,313, 2-49 mos.) from western Kenya (holoendemic). Logistic regression analyses (controlling for anemia-promoting cofactors) revealed that carriage of the TAG haplotype was associated with significant protection against SMA, whereas carriage of the GTC, GTG, and TTC haploytpes were associated with significant susceptibility to SMA. Additional experiments in cultured human PBMCs from malaria-naïve donors revealed that phagocytosis of hemozoin (*Pf*Hz) caused a dose- and time-dependent increase in *ATG10* gene expression. Collectively, these findings demonstrate that ATG10 promoter haplotypes influence susceptibility to SMA, and that phagocytosis of hemozoin appears to activate the autophagy pathway.

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PLASMODIUM EVOLUTION AND GENETIC DETERMINANTS OF HOST SPECIFICITY IN ESSENTIAL INVASION GENES

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The genus *Plasmodium* is comprised of highly species-specific protozoan parasites that cause malaria, and affects a wide range of terrestrial vertebrates including humans. The transition and adaptation to new hosts represents a major driving force for parasite evolution, yet little is known about the molecular factors that constrain individual *Plasmodium* species to infecting a single vertebrate host. It was recently shown that the deadliest human-infective *Plasmodium* species, *P. falciparum* evolved from an ape-infective *Plasmodium* ancestor and belongs to the subgenus *Laverania*, whose other members infect African apes. This ape-to-human host transition for the parasite signifies a defining moment in its evolutionary history, and in order to understand the context of this critical host-switching event, we are using both protein binding assays and experimental genetics to explore species-specificity within the PfRH5 complex across the *Laverania* subgenus. In a recent publication from our lab and others on the sequencing of cryptic ape *Plasmodium* species, it was found that horizontal gene transfer between ancestral *Laverania* species likely resulted in the transference of two essential invasion genes - CyRPA (cysteine-rich protective antigen) and RH5 (reticulocyte binding-like homologous protein 5), which could have conferred a fitness advantage that predisposed *P. praefalciparum* to infect humans. In order to evaluate the role of these two essential invasion genes in host specificity, we implemented a strategy that uses inducible diCre recombinase-mediated conditional deletion of *P. falciparum* CyRPA and attempted genetic complementation to rescue parasite lethality with *Laverania* CyRPA. Studying the functional impact of changes in *Laverania* genomes will tell us about the evolutionary origins of one of the deadliest human pathogens, and may shed light on potential targets for vaccine development.

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A NOVEL METHOD FOR IDENTIFYING PREDOMINANTLY EXPRESSED *VAR*S FROM WHOLE BLOOD CLINICAL SAMPLES

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The *var* gene family encodes *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1) antigens. These proteins locate to the surface of infected erythrocytes and play a critical role in immune evasion. Each *P. falciparum* genome includes 40-60 *var* genes, yet each parasitized erythrocyte displays only one PfEMP1. Studies of *var* expression using non-leukocyte-depleted blood are challenging due to the predominance of host genetic material and lack of *var* reference sequences to guide read recruitment and assembly. To address these barriers, we compared the following mRNA library preparation and *var* transcript enrichment methods to generate assembled *var* transcripts using RNA extracted from whole peripheral blood samples: (1) polyA selection, (2) depletion of globin transcripts and rRNA, (3) both methods combined, and (4) a *var* read capture array, with capture probes based on >1,000 full-length *var* gene sequences. cDNA was generated via reverse transcription and libraries were sequenced using an Illumina platform. To identify *var* gene reads from methods 1-3, we performed *in silico* read capture to identify reads that mapped to any of the *vars* from 3D7, *var* sequences from the VarDom database or >1,000 new *var* sequences. Using 15 samples collected from Malian children with severe or uncomplicated malaria infections, methods were compared based on the percentage of reads mapped to the human and *P. falciparum* genomes and the proportion of *var* reads in the dataset. To identify the best preparation method for assembling *var* transcripts, the completeness and length of assembled *var* transcripts was compared. *De novo* transcript assemblies were generated and *var*s as long as 10,000 bp were identified. To determine the sensitivity of these methods, we used 11 of the samples for which the *var* repertoire is known, and compared the *de novo var* transcripts to the known *var* repertoires as positive controls. The results of this analysis will facilitate *var* expression comparative analyses in severe malaria, uncomplicated malaria and asymptomatic parasitemia to illuminate if *var* expression is associated with different malaria syndromes.

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A NOVEL METHOD FOR IDENTIFYING PREDOMINANTLY EXPRESSED *VAR*S FROM WHOLE BLOOD CLINICAL SAMPLES

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EXTREME DIVERSITY AND POPULATION STRUCTURE OF *VAR* GENES CAN EXPLAIN WHY IMMUNITY TO THE BLOOD STAGES OF *PLASMODIUM FALCIPARUM* IS NON-STERILIZING

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*Pf*EMP1 is encoded by the *var* multi-gene family and is a major target of naturally acquired immunity. Variation in *Pf*EMP1 underlies parasite fitness, measured as the ability to evade the host immune response and establish a chronic infection to optimize transmission. We investigated the extent of *var* sequence diversity and population structure in the asymptomatic *P. falciparum* reservoir in an area with seasonal transmission in Bongo District (BD), Ghana. We sampled individuals across all ages with asymptomatic *P. falciparum* infections (both microscopic and, for the first time, submicroscopic infections) to examine the entire parasite reservoir. With the aim to define the transmission dynamics of *P. falciparum* through the lens of *var* genomics, we set out to describe seasonal and age-specific patterns of *var* diversity at the *var* type and repertoire level, within-host infection complexity, temporal *var* dynamics, and population structure. Our analysis of 1,099 *P. falciparum* isolates across two transmission seasons revealed 42,399 *var* types circulating in the population. Strikingly, this extensive diversity was uniquely structured into *var* repertoires that had minimal overlap regardless of season and infection complexity. Through the lens of *var* genes, we explain several key epidemiological features of *P. falciparum* malaria in endemic areas and demonstrate that the transmission system in BD is extremely complex. Despite this complexity, three key features of the molecular epidemiology emerge: (i) extremely high *var* sequence diversity, (ii) limited overlap of *var* repertoires in both seasons, and (iii) rapid turnover of repertoires but maintenance of certain *var* types between seasons. The absence of related parasites was demonstrated by pairwise type sharing and most isolate repertoires shared <10% of their *var* types. Using computational experiments, this highly diverse parasite population with essentially non-overlapping *var* repertoires was shown to explain age-specific patterns of immunity and the epidemiology of *P. falciparum* malaria in high transmission areas.

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MALARIA AND MARITIME TRAFFIC ON THE COLOMBIAN PACIFIC COAST

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Despite global gains in malaria control since 2010 and relatively low incidence compared with other regions worldwide, malaria cases in Colombia doubled between 2015 and 2016, with over 100,000 estimated cases in 2016. As in many regions nearing elimination, malaria transmission is very heterogeneous in Colombia, with a main hotspot of transmission in Choco close to the pacific coast. Given this heterogeneity, it is critical to understand the contribution of human mobility to parasite dispersal, and in particular the importation of parasites into low transmission settings that are susceptible to outbreaks. To explore human drivers of parasite dispersal in Colombia, we have compared measures of human and parasite connectivity between sites on the Colombian pacific coast. Specifically, we compare connectivity between *Plasmodium falciparum* parasite populations using genetic measures of relatedness based on identity by descent, a fundamental population genetic measure linking ancestry to patterns due to recombination that is sensitive to recent demographic events. We then compared measures of parasite connectivity to measures of human connectivity based on maritime traffic and road networks. We find that genetic measures of parasite relatedness are associated with measures of maritime traffic, suggesting travel via boat plays a substantial role in the dispersal of parasites in the region. This finding generates hypotheses about outbreaks across the wider region of South America, and could lead to malaria elimination policies preventing the spread of parasites via maritime routes.

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EXPLORATION OF *PLASMODIUM VIVAX* TRANSMISSION AND RECURRENT INFECTIONS IN THE PERUVIAN AMAZON

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Plasmodium vivax is responsible for more than 75% of all malaria cases in the Americas constituting an important public health threat. Efforts against vivax malaria have been challenged by its ability to generate long lasting dormant liver parasites that can become active weeks or months after the initial infection. We explored *P. vivax* whole genome sequences obtained from 46 patients residing in three villages of the Peruvian Amazon and demonstrated a novel method for distinguishing homologous relapses from recurrent infections. We obtained 69 high quality *P. vivax* whole genome sequences directly from patient samples by selective whole genome amplification (SWGA). Sequence comparisons resulted in 24,571 high quality single nucleotide polymorphisms in the core *P. vivax* genome. These SNPs were mostly located in regions with high SNP density on chromosomes 3, 6, 10 and 13 that covered virulence factors such as the plasmodium interspersed repeat (pir) gene family, merozoite surface protein 8 (msp8), variant interspersed repeat 21 (vir21), and a hypothetical protein located on chromosome 6. Genomic information allowed us to estimate five *P. vivax* subpopulations and evidence of a highly heterogeneous ancestry of some of the isolates. Pairwise comparison of recurrent infections and identity by descent analysis allowed us to differentiate 10 homologous relapses and 3 potential heterologous relapses with highly related parasites from the rest of the samples. Our study shows that Whole genome sequencing (WGS) is a high-resolution tool for distinguishing relapses from reinfections with increased sensitivity, while also providing information about drug resistance and parasite population genetics.

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GLYBURIDE, A NLRP3 INHIBITOR REDUCES INFLAMMATORY RESPONSE AND IMMUNOPATHOLOGY IN *LEISHMANIA BRAZILIENSIS* INFECTION

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Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is characterized by an exaggerated Th1 immune response and peripheral blood mononuclear cells (PBMC) from CL patients secrete high levels of TNF and IFN-gamma that contributes for the control of parasite multiplication but also is associated with tissue damage and ulcer formation. NLRP3 Inflammasome is an intracellular protein complex which be activated by danger-associated molecular patterns, promoting activation of caspase 1 and release of IL-1β. Both NLRP3 inflammasome activation and IL-1β have been linked with disease severity in leishmaniasis. Glyburide is a ATP-sensitive K^+channels inhibitor used for the treatment of type 2 diabetes. Furthermore, glyburide have anti-inflammatory effect specially by prevention the formation of the NLRP3 inflammasome. In the present work we investigated the ability of glyburide to modulate the *in vitro* inflammatory response in CL patients. PBMC and skin lesions biopsies from CL patients were obtained and cultured with soluble *Leishmania* antigen (SLA) in the presence or absence of glyburide and cytokines levels were evaluated by ELISA. The levels of *Leishmania*-specific IL-1β, IL-17 and TNF produced by PBMC from CL patients significantly decreased after *in vitro* treatment with glyburide, without toxic effects to host cells. Biopsies were divided into two, with one half acting as a control, and the other treated with glyburide. While untreated skin lesion biopsies produced high levels of IL-1β, IL-17 and TNF, treatment with glyburide significantly decreased the release of these cytokines in culture. Moreover, levels of IFN-gamma, IL-6 and IL-10 did not change. A positive correlation among IL-1β levels in biopsies and lesion size was found, and this association was lacked after treatment with glyburide. Leishmanicidal effect of glyburide against *L. braziliensis* promastigotes and intracellular amastigotes was not found. Our data pointed out that glyburide reduces inflammatory cytokines in *L. braziliensis* infection and should be considered as adjuvant therapy for CL.

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TYPE I INTERFERONS SUPPRESSES ANTI-PARASITIC $CD4^+$T CELL RESPONSES IN VISCERAL LEISHMANIASIS

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Many pathogens, including viruses, bacteria, and protozoan parasites, suppress cell mediated immune responses through activation of type I Interferon (IFN-1) signalling. However, the role of IFN-1 during *Leishmania donovani* infection causing visceral leishmaniasis (VL) is not well known. Here we report that IFN-1 plays an important role in the pathogenesis of VL by impairing parasite clearance and suppressing pro-inflammatory cytokine production. Mice lacking type-1 IFN signalling (B6.IFNαR1$^{-/-}$ mice) and wild type (WT) C57BL/6 mice were infected intra-venously with $2x10^7$ *L. donovani* amastigotes. Parasite burden was measured at day 14, 28 and 56 post infection. To further study the impact of IFN-I on the immune response during experimental VL, we next analyzed serum cytokine levels in naive and infected WT and IFNαR1$^{-/-}$ mice. Intracellular cytokine staining and flow cytometry was performed to detect the $CD4^+$ T cell-derived IFN-γ production. Peripheral blood mononuclear cells (PBMCs) from VL patients (before and after treatment) and endemic healthy controls were also collected to measure mRNA encoding IFN-1 related genes and a whole blood assay was employed to measure the antigen specific immune response after IFN-1 signalling blockade. B6.IFNαR1$^{-/-}$ mice showed enhanced pro-inflammatory cytokine production and better control of parasite burden in liver as well as spleen, compared to wild type C57BL/6 mice. IFN-1 signalling suppressed $CD4^+$ T cell-derived IFN-γ production and prevented Th1 response from controlling parasite replication. Studies in VL patients supported these findings and showed enhanced accumulation of mRNA encoding type I IFN signature genes in PBMCs that were reduced following successful drug therapy. Critically, we also showed, using a whole blood assay, that blockade of type-1 IFN signalling enhanced antigen specific IFN-γ production, and that this response was HLA-II restricted. Together, these results identify the type-1 IFN signalling pathways as a potential therapeutic target to treat VL by enhancing anti-parasitic $CD4^+$ T cell responses.

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response during experimental VL, we next analyzed serum cytokine levels in naive and infected WT and IFNαR1$^{-/-}$ mice. Intracellular cytokine staining and flow cytometry was performed to detect the CD4$^+$ T cell-derived IFN-γ production. Peripheral blood mononuclear cells (PBMCs) from VL patients (before and after treatment) and endemic healthy controls were also collected to measure mRNA encoding IFN-1 related genes and a whole blood assay was employed to measure the antigen specific immune response after IFN-1 signalling blockade. B6.IFNαR1$^{-/-}$ mice showed enhanced pro-inflammatory cytokine production and better control of parasite burden in liver as well as spleen, compared to wild type C57BL/6 mice. IFN-1 signalling suppressed CD4$^+$ T cell-derived IFN-γ production and prevented Th1 response from controlling parasite replication. Studies in VL patients supported these findings and showed enhanced accumulation of mRNA encoding type I IFN signature genes in PBMCs that were reduced following successful drug therapy. Critically, we also showed, using a whole blood assay, that blockade of type-1 IFN signalling enhanced antigen specific IFN-γ production, and that this response was HLA-II restricted. Together, these results identify the type-1 IFN signalling pathways as a potential therapeutic target to treat VL by enhancing anti-parasitic CD4$^+$ T cell responses.

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ROLE OF EXOSOMAL MICRORNAS IN SHAPING PROTECTIVE IMMUNITY INDUCED BY LIVE ATTENUATED *LEISHMANIA* PARASITE VACCINES

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In our efforts for the development of an effective vaccine against visceral leishmaniasis, we have reported extensively on the immunogenicity of live attenuated Centrin gene deleted *Leishmania* mutants that are in use in ongoing preclinical studies. However, there is a need to develop biomarkers at early stages of vaccination in a human challenge model to measure vaccine efficacy with live attenuated parasites. Our *ex vivo* infection studies with human PBMCs obtained from U.S. blood donors demonstrated that the early gene expression profiles of several miRNAs (miRs) in virulent and attenuated *Leishmania* infections differ significantly. Towards identifying early biomarkers induced by infection with live attenuated *Leishmania* parasite, we performed *in vitro* infection of murine dendritic cells (DCs) with virulent and attenuated strains of *Leishmania* parasites. The exosomes from the infected DCs were isolated and the miR composition of the exosomes was determined by RNA sequencing. Results showed that several miRs were highly enriched in the exosomes derived from attenuated *Leishmania* infection compared to exosomes from uninfected or virulent infections. Based on the enrichment in attenuated *Leishmania* infection and the previously known roles in orchestrating host immunity, we have selected to investigate the expression of miR-Let-7e, miR-501, miR-16 and miR-361 in murine and *ex vivo* human infection experiments. qRT-PCR assays from the exosomes derived from human macrophages infected with virulent or live attenuated *Leishmania* parasites showed similar enrichment of miRs, as was observed in murine DC infection studies. Further analysis of the role of such miRs in immunity is being currently studied. Taken together, our data suggests that exosomal miRs could be used as biomarkers of protection in anti-leishmanial vaccines.

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CHAGAS DISEASE CARDIOMYOPATHY: ASSOCIATION WITH IL-17 AND IL-18 GENETIC POLYMORPHISMS

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Chagas disease is an important health problem in endemic and non-endemic areas on many continents, as consequence of immigration of infected people from Latin America. *Trypanosoma cruzi* infects around 7 million people nowadays. About 30% of chronic Chagas disease patients develop inflammatory cardiopathy. We investigated the association between the polymorphisms on inflammatory cytokine genes and the presence of cardiopathy in these patients. We analyzed 212 Chagas disease patients and 90 healthy controls. Trypanosomiasis diagnosis was defined by two positive tests (ELISA, Indirect Immunofluorescence or Hemagglutination). Patients were initially classified in clinical forms: Indeterminate form (without cardiopathy or digestive involvement), Cardiac, Digestive and Cardiac + Digestive forms. Additionally, they were analyzed according to the presence of cardiopathy. DNA was extracted by guanidine hydrochloride or by DTAB/CTAB (dodecyl trimethylammonium bromide/ cetyl trimethylammonium bromide) technique. Polymorphisms on IL1 511 G/A (rs1143627), IL6 174 C/G (rs1800795), IL17 152 G/A (rs2275913), IL18 137 C/G (rs187238), IL18 607 G/T (rs1946518) were analyzed by Real Time-PCR. The population was in Hardy-Weinberg equilibrium. There were no differences in genotypic and allelic frequencies between patients and healthy controls. Logistic regression analyses showed that IL17 152 AA and IL18 607 TT genotypes have protective effect on Chagas disease cardiopathy (p= 0.032, OR= 0.229, IC= 0.059-0.883 and p=0.026, OR=0.264, IC=0.081-0.854, respectively). In contrast to previous analyses, our study suggests a role of IL17 152 G/A and IL18 607 G/T polymorphisms in the presence of cardiopathy in chronic Chagas disease.

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INNATE AND ADAPTATIVE IMMUNE RESPONSE PLAY DIFFERENT ROLES IN THE PATHOGENESIS OF CUTANEOUS LEISHMANIASIS

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The cutaneous leishmaniasis (CL) caused by *L. braziliensis* is characterized by a well limited ulcer with raised borders. As only a few parasites are found at the lesion site, emphasis has been giving to the role of both CD4 and CD8 T cells exaggerated immune response in the pathogenesis of the disease. However cutaneous ulcers are documented in patients with a poor type 1 immune response as observed in patients co-infected with HIV and *L. braziliensis* and is also observed in CL patients with a negative delayed type hypersensitivity to the leishmania skin test (LST). Here we compare the immune response in CL patients with a negative and positive LST and correlate the immunologic response with pathology and response to therapy. Participants include 13 patients with CL with LST$^-$ (cases) and 26 CL patients with LST$^+$ (controls). The diagnosis was performed by the detection of DNA of *L. braziliensis* by PCR. Histopathological findings

were analyzed, cytokine levels were determined by ELISA in supernatants of mononuclear cells stimulated with SLA and the ability of macrophages to kill leishmania was determined by optical microscopy. There was no difference regarding the age, gender and illness duration between the two groups. All patients presented a typical CL ulcer and there was no difference in the size of the lesions in those with LST+ and LST- However while failure to antimony ($Sb^{5)}$ therapy was observed 9 of 13 (69,2%) of the cases it was found in 9 of 26 (34,6%) of controls (P< .05). There was no difference regarding the leishmania killing by macrophages. IFN gamma and TNF levels were lower (P<.01) in those with a negative LST. High levels of IL-1 beta, CXCL-9, and MMP-9 were detected in both groups. While there was no correlation between IFN gamma levels and size of the ulcers, there was a strong correlation between IL-1 beta levels and the size of the ulcers (R=0,78; P<001). In CL while high production of IL-1β is associated with pathology, impairment in Th1 immune response is associated with therapeutic failure.

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INSULIN-LIKE GROWTH FACTOR-I AS EFFECTOR ELEMENT OF IL-4 EFFECT LEADING TO SUSCEPTIBILITY TO *LEISHMANIA MAJOR* INFECTION

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In *Leishmania* infection IGF-I favors the parasite growth inducing an increase in arginase activity in infected macrophages. Since it is known that arginase is activated by IL-4, here we aimed to study the role of intrinsic IGF-I in conjunction with IL-4 in *Leishmania major* promastigote-infected RAW 264.7 cells through silencing *Igf-I* mRNA expression with addition of IL-4 (2ng/ml) in some experiments. After 48 hours *Igf-I* mRNA expression was quantified by Real Time (qPCR), and nitric oxide (*Nos2*) by qPCR and Griess method and, arginase (*Arg1*) by qPCR and arginase activation by urea production, and the parasitism by microscopy. In all groups treated with siRNA we observed a decrease in the arginase mRNA expression and arginase activity in macrophage and parasite, accompanied by a significant decrease in the parasitism from 149 (median) parasites per 100 cells to 93. Upon IL-4 stimulus *Igf-I* mRNA expression was increased as well as the parasitism that reached 171. However in siRNA-treated cells IL-4 stimulus did not induce any increase in *Igf-I* mRNA expression and the parasitism reduced to 87. Upon restoring IGF-I with 50 ng/ml recombinant IGF-I (rIGF-I) after knockdown an increase in the parasite arginase mRNA expression was seen accompanied by an increase in the parasite number to the level similar to the controls and further the effect of IL-4 stimulus was restored. Searching for intracellular signaling elements (by Westernblot) upon siRNA transfection, phosphorylated p44, p38 and Akt proteins were seen decreased affecting phosphatidylinositol-3-kinase (PI3K)/Akt pathway, effects that were reversed by the addition of rIGF-I. Further in *L. major*-infected C57BL6 resistant mice pre-incubation of parasites with rIGF-I that were subcutaneously injected in the footpad turned the infection profile similar to susceptible mice. These results suggest that IGF-I is directly related to expression and activation of arginase mainly on *Leishmania* in infected macrophages leading to increase in the parasitism and we conclude that IGF-I constitutes an effector element of IL-4 involving PI3K/Akt pathway during *L. major* infection.

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INVOLVEMENT OF TH17 RESPONSES IN HUMAN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a potentially fatal parasitic disease that impose a huge health problem on the vulnerable population claiming the lives of 0.2- 0.4 million worldwide. Better understandings of disease pathogenesis that dampen the immune response are critical for controlling the disease. We had previously shown the role of IL-27 and IL-21 in differentiation and expansion of antigen-specific IL-10 producing T cells and inhibition of Th17 differentiation in VL patients. Here, we investigated the IL-17 association with protection in human VL. We found elevated mRNA expression of indolamine 3-3-di-oxygenase in VL patients, the enzyme that negatively regulates T-cell effector function during infection. No up-regulation of IL-17 or RORγT mRNA expression in spleens cells and plasma IL-17 levels were detected in active VL, however significantly elevated mRNA levels of both subunits of IL-23 were detected in post treated VL splenic biopsies compared to active patients. IFN-γ or/and IL-10 neutralization does not affect IL-17 levels in whole blood cells of active VL and 6 months cured VL. However, plasma IL-17 and IFN-γ levels were elevated in 6 month cured VL which were further enhanced with SLA stimulation in whole blood. Analysis of Th17/Th1 cytokines response in subjects from a cohort of endemic healthy individuals who were protected against VL showed that IL-17 is strongly associated with protection against VL. These findings were further supported by enhance parasite clearance with recombinant IL-17 (rhIL-17) and rhIFN-γ by inducing TNF-α mRNA and down regulating IL-10 in monocyte derived macrophages infected with *L. donovani* amastigote.

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BUBBLE CONTINUOUS POSITIVE AIRWAY PRESSURE FOR CHILDREN WITH SEVERE PNEUMONIA AND SEVERE MALNUTRITION, HUMAN IMMUNODEFICIENCY VIRUS INFECTION OR EXPOSURE, OR SEVERE HYPOXEMIA IN MALAWI: AN OPEN LABEL RANDOMIZED CONTROLLED TRIAL

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Pneumonia is a leading cause of death among African children. Those children with the co-morbidities of severe malnutrition or human immunodeficiency virus (HIV) infection or exposure, or severe hypoxemia are at the highest risk of pneumonia mortality despite standard care with antibiotics and low-flow oxygen. Bubble continuous positive airway pressure (bCPAP) is a lower-cost non-invasive ventilation modality that may improve child pneumonia survival. This open label randomized controlled trial was conducted at Salima District Hospital, Malawi. We planned to enroll 900 children 1-59 months old with severe pneumonia and either severe malnutrition, HIV infection or exposure, or an oxygen saturation <90%. Children were randomly assigned 1:1 to low-flow nasal cannula oxygen (0.5 to 2 L/min) or bCPAP (5 to 8cm H_20). The primary outcome was hospital death. Safety analyses were done per protocol and primary analyses by intention to treat. The data safety and monitoring board stopped the trial early (ClinicalTrials.gov NCT02484183). We recruited 646 eligible children. A total of 324 children (50.2%) were randomized to low-flow oxygen and 322 (49.8%) to bCPAP. Overall 88 children died in the hospital (13.6%), 35 were allocated to low-flow oxygen (10.8%) and 53 to bCPAP (16.4%). Children receiving bCPAP had significantly higher rates of death compared to low-flow oxygen recipients (relative risk 1.52 (95% confidence interval, 1.02, 2.25, p=0.039). bCPAP significantly increased

hospital mortality among high-risk Malawian children with severe pneumonia, compared to low-flow oxygen. Based on these results bCPAP is not recommended for similar pediatric pneumonia populations.

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OUTCOME OF POST TRIAL IMPLEMENTATION OF BUBBLE CPAP IN TREATING CHILDHOOD SEVERE PNEUMONIA AND HYPOXEMIA IN BANGLADESH

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Despite of World Health Organization (WHO) recommended low flow (LF) oxygen therapy, antibiotics, and other standard care; deaths from childhood severe pneumonia exceed 10% in many hospitals of developing countries. A randomized clinical trial (RCT) conducted in icddr,b, Bangladesh from August 2011 to July 2013 showed bubble CPAP oxygen therapy was associated with significant reduction of deaths (4% vs. 15%, p=0.022) in treating childhood severe pneumonia and hypoxemia compared to those received LF therapy. Subsequently bubble CPAP was incorporated as the part of standard of care in treating such children. We evaluated outcome of post trial implementation of bubble CPAP in these children between August 2013 and December 2017. Children under-five with WHO defined severe pneumonia and hypoxemia were included in our analysis. Children who presented with features of respiratory failure (such as gasping respiration) or congenital heart disease on admission were not eligible for the bubble CPAP oxygen therapy. A total of 4101 children were admitted with pneumonia having different severity during the study period. Among them 2850 children had severe pneumonia, 1183 (29%) had hypoxemia, 742 (18%) were eligible for bubble CPAP oxygen therapy but the rest of the hypoxemic children (411) were not eligible to receive bubble CPAP therapy. The median age of the children who received bubble CPAP was 7.3 (IQR: 4.2, 11.4) months, 294 (40%) of them had severe acute malnutrition, 118 (16%) severe sepsis, 183 (25%) convulsion, 70 (9%) bacteremia, 72 (10%) abdominal distension, but none of them developed nasal bleeding or pneumothorax. A total of 211/4101 (5%) children died of pneumonia in all categories, 206/2850 (7%) died of severe pneumonia, and 42 (5.7%) died who received bubble CPAP. However, the pre-trial mortality was 21% in children with same eligibility criteria of bubble CPAP therapy. Thus, the outcome of implementation of bubble CPAP in treating childhood severe pneumonia and hypoxemia is consistent with the RCT in Bangladesh and has high potential to implement it in other developing countries having high mortality in childhood pneumonia.

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RESULTS FROM A RECENTLY CONCLUDED COMMUNITY BASED RANDOMIZED NON-INFERIORITY TRIAL OF AMOXICILLIN VERSUS PLACEBO IN FAST BREATHING PNEUMONIA IN LOW INCOME COMMUNITIES IN PAKISTAN

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World Health Organization (WHO) recommends use of amoxicillin in managing fast breathing pneumonia, diagnosed on clinical signs that classify children based on respiratory rates. Given non-specificity of case definition, there is equipoise regarding use of antibiotics in management of fast breathing pneumonia. We conducted a double blind randomized placebo controlled non inferiority trial with parallel assignment in high pneumonia mortality slums of Karachi, Pakistan. Children 2-59 months with fast breathing without any WHO defined danger signs and seeking care at primary health centre were randomized to receive either three days of placebo or amoxicillin. Primary outcome was cumulative treatment failure (new clinical sign based on pre-set definition indicating illness progression or mortality) on day 0, 1, 2 or 3 of therapy. Sample size was 4000 children assuming a treatment failure rate of 3.5% in amoxicillin arm, with one sided alpha of 0.05, power of 90%, non-inferiority margin of 1.75% and an expected 5% lost to follow-up/non per protocol. From November 2014 to November 2017, 98,334 children were triaged, 53,770 (54.7%) presented with cough or difficulty breathing. 7,887 (14.7%) met inclusion criteria, and 4003 (89.3 %) of them were randomized. In placebo arm, 2000 were randomized and 1927 were per protocol (96%), with 94 (4.9%) treatment failures and 40 (2.2%) relapses. In Amoxicillin arm, 2003 were randomized with 1929 per protocol (96%) with 51 (2.6%) treatment failures and 58 (3.1%) relapses. The difference of absolute treatment failure rates between two groups was 2.23% with one sided 95% CI (1.23-3.24). Based on preset non inferiority criteria, we failed to conclude non inferiority of placebo to amoxicillin. However, we found a high number needed to treat, 1 in 44 to prevent a fatal or non-fatal adverse event. Also, 90% of those who did receive antibiotics did not experience any clinical worsening. Findings are generalizable to low HIV and malaria setting with Hib/Pneumococcal vaccines in their national immunization plan and have implications for readdressing the respiratory rate cutoffs for defining fast breathing pneumonia.

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PROGNOSTIC BIOMARKERS IN UGANDAN CHILDREN WITH RESPIRATORY SYNCYTIAL VIRUS RESPIRATORY TRACT INFECTION

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Respiratory Syncytial Virus (RSV) is the leading viral cause of pediatric pneumonia worldwide. Optimizing outcomes from RSV pneumonia requires a determination of severity that, in resource-limited settings, is often based on clinical assessment alone. Here we describe levels of biomarkers of systemic inflammation, C-Reactive Protein (CRP), Chitinase-3-Like Protein 1 (CHI3L1) and Lipocalin-2 (LCN2), and a marker of pneumocyte injury, Surfactant Protein D (SP-D), and clinical outcomes of children hospitalized with RSV pneumonia in Uganda, as well as controls with rhinovirus and pneumococcus. 58 children hospitalized with clinical pneumonia (hypoxemia, tachypnea and/or chest in-drawing) were included. We compared 37 patients in whom RSV was detected in the NP swab, 10 control patients with rhinovirus, and 11 control patients with pneumococcal pneumonia. Diagnostically, patients in the RSV group had significantly lower levels of CHI3L1 and CRP than the pneumococcal group ($p<0.05$ for both comparisons) and similar levels to the rhinovirus group. In terms of prognosis within the RSV group, higher levels of CHI3L1 were associated with higher composite clinical severity scores at admission (RISC), $r= 0.41$, $p =0.019$, and predicted prolonged time to resolution of tachypnea and tachycardia, time to wean oxygen, and time to sit ($p<0.05$ for all comparisons). Higher levels of LCN2 were associated with prolonged time to resolution of tachypnea, tachycardia and fever, and time to feed. Higher levels of CRP were associated with prolonged time to resolution of tachypnea, tachycardia, and fever. Higher admission levels of CHI3L1, LCN2, CRP, and SP-D were predictive of a higher total volume of oxygen administered during hospitalization ($p<0.05$ for all comparisons). Of note, CHI3L1 and LCN2 appear to predict normalization of respiratory rate and supplemental oxygen requirement more accurately than CRP, the most widely-used host inflammatory biomarker in clinical practice. Our findings suggest that CHI3L1 and LCN2 may be clinically informative biomarkers of childhood RSV respiratory tract infection in low-resource settings.

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RISK OF TUBERCULOSIS IN HOSPITAL ENVIRONMENTS IN A COUNTRY WITH HIGH ENDEMICITY

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Health workers are one of the occupational groups with the highest risk of becoming infected with tuberculosis (TB) due to their regular occupational exposure. This study was aimed to estimate the TB risk at the hospital environments with the highest incidences of TB within the Peruvian Social Security System (EsSalud), and its associated factors. Using a cross-sectional study design, we estimated the TB risk at each of the hospital environments destined to provide care to the TB patients at the hospitals with the highest incidence of TB (upper quartile) across EsSalud regional health networks. We quantified the TB risk using the Wells-Riley airborne model and either by the CO2 concentration or the flow rate techniques, based on the feasibility for the total closure of the environment. Then, we assessed the characteristics of the environments, hospitals and health networks as associated factors by using a multilevel mixed-effects model. We assessed 480 environments, 22 (25%) hospitals (22±5 environments/hospital) and 12 (46%) regional health networks and estimated an average TB risk of 42% (95% confidence interval [CI]: 39% -45%). At the bivariate analysis, we found that the TB risk was significantly lower in the regions of Lambayeque (vs. Loreto, Huánuco, Tacna, and Arequipa) and Lima (vs. Loreto), in the TB Program environments (vs. emergencies, hospitalization, and doctor's offices), in those with natural ventilation (vs. air conditioning), and in those with the lower ratio of windows to doors, but significantly higher at level III hospitals (vs. levels I and II). However, in the multivariate analysis, we identified as associated factors the type of environment (TB Program [Reference]] vs. doctor's offices [β_1=12, IC95%:3-22], hospitalization [β_2=20:11-29] and emergencies [β_3=25: 16-34]), and the type of ventilation (natural [Ref.] vs. air conditioning [β_4=17:6-21]). To sum up, the TB risk at the environments from the hospitals with the highest incidence of TB at EsSalud is high and highly variable, being lower at the TB Program facilities and the naturally ventilated environments

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DIFFERENCES IN CLINICAL SEVERITY OF MONO AND MULTIPLE RESPIRATORY VIRAL INFECTIONS IN HOSPITALIZED CHILDREN IN NHA TRANG, VIETNAM

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It is a standing question whether clinical severity of an infection varies by pathogens or by infection with multiple pathogens. Using hospital-based surveillance in children, we investigate the range of clinical severity for patients singly, multiply and not infected with a group of 12 commonly circulating viruses in Nha Trang, Vietnam. We find no difference in severity between 0-, 1-, and 2-concurrent viruses and little differences in severity between specific viruses. We find that concurrent infection with 3 or more viruses was associated with nearly a 3-fold increase in the odds of being a severe case, as well as detection of Human metapneumovirus and respiratory syncytial virus -- either as a monoinfecton or as part of a coinfection -- is associated with increased risk of being severe. Finally, we find infection with adenovirus to be consistently associated with lower risk of severity. Clinically, based on the results here, if Human metapneumovirus and respiratory syncytial virus virus is suspected, PCR testing for confirmatory diagnosis and for detection of multiple coinfecting viruses would be fruitful to assess the probability of whether the patient's disease course is going to be severe.

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LOWER RESPIRATORY INFECTIONS ARE THE LEADING INFECTIOUS CAUSE OF DEATH GLOBALLY: INCIDENCE, HOSPITALIZATIONS, AND MORTALITY IN THE GLOBAL BURDEN OF DISEASE STUDY 2017

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Lower respiratory infections are the leading infectious cause of death globally, responsible for more deaths than tuberculosis and HIV/AIDS, combined. The Global Burden of Disease study 2017 (GBD 2017) is a systematic, scientific effort to quantify health for all ages, both sexes, every geography, and from 1980-2017. In this presentation we will share methodology and updated results for lower respiratory infections (LRIs) in GBD 2017, focusing on deaths, incidence, and hospitalizations. Deaths, incidence, and hospitalizations were modeled separately using vital registration, verbal autopsy, survey, health records, and scientific literature data and these regression models were strengthened by covariates and by hierarchical space-time trends. Overall, lower respiratory infections were the third leading cause of disability-adjusted life years (DALYs) globally in 2017, responsible for 91,844,000 DALYs, 2,558,200 deaths, and 336,461,000 incident episodes. The number of DALYs, deaths, and episodes has been increasing since 2000 (about 30% overall increase during this time), but the rate has remained about the same for episodes, and the mortality rate has decreased dramatically (mortality decreased 28% during this time). In 2017, we estimated that there were 32,990,000 hospitalizations due to LRIs. Most of these hospitalizations occurred among adults over age 65 (11,490,000 hospitalizations), accounting for a rate of 18.2 LRI hospitalizations per 1,000 people. The next highest rate of hospitalization due to LRIs was among children under 1 year (9.2 per 1,000) with lower rates in the 15 to 64 years age groups. Overall, about 10% of LRI episodes were hospitalized globally with the highest proportion hospitalized among elderly adults over 65 years (13.8%). The Global Burden of Disease study provides a timely, comprehensive, and detailed picture of the health loss associated with LRI and provides public health experts, policy makers, and health officials with evidence for decision making and a roadmap for reducing the burden of the disease.

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DRY SEASON *P. FALCIPARUM* RESERVOIR

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Dependent on Anopheles mosquito for transmission, *P. falciparum* faces a challenge during the dry season in the regions where rain seasonality limits vector availability for several months. While malaria cases are restricted to the wet season, clinically silent infections can persist through

the dry season and are an important reservoir for transmission. Chronic asymptomatic *P. falciparum* infections allow transmission to resume with every rainy season, and predict decreased clinical malaria risk during the next malaria season. However, asymptomatically infected Malian children appear to maintain only minimal immune activation during this time and *P. falciparum*-specific antibodies decline similarly in children who do or do not harbour asymptomatic *P. falciparum* infections during the dry season. We hypothesized that newly transmitted parasites are more virulent and induce stronger immune responses than parasites that persist during long periods of asexual replication in blood. We thus compared whole genome transcription of *P. falciparum* parasites maintained during the dry season, with parasites collected from children during acute febrile malaria episodes. Our data indicates that *P. falciparum* modulates its transcription during the dry season, while the host immune response seems to be minimally affected, suggesting that the parasite has the ability to adapt to a vector-free environment for long periods of time. *P. falciparum* signaling pathways related with metal ion transport, phospholipid biosynthesis, DNA replication and macromolecular membrane complexes were significantly altered during the dry season leading us to investigate exported proteins and adhesive properties of infected erythrocytes, as well as *P. falciparum* replicative ability during the dry and wet seasons. Understanding how the parasite adapts to a vector-free environment for long periods of time remaining undetectable to the immune system, and how it can restart transmission in the ensuing rainy season will reveal complex interactions between *P. falciparum,* its host, and the environment, and may inform malaria elimination strategies.

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TRANSCRIPTOME PROFILING UNRAVELS NOVEL LIGANDS REQUIRED FOR *PLASMODIUM VIVAX* INFECTIONS IN *SAIMIRI* MONKEY - IMPLICATIONS ON *PLASMODIUM VIVAX* INFECTIONS IN AFRICA

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Plasmodium vivax causes severe disease in humans in Asia and South America. The engagement of *P. vivax* Duffy binding protein 1 (DBP1) with Duffy blood group antigen (Duffy antigen) in the host erythrocytes is important for invasion. Most Africans lack Duffy antigen on the erythrocyte surface and hence *P. vivax* infection in Africa is remarkably rare or completely absent. However, recent cases of *P. vivax* infections have been reported in various parts of Africa in Duffy-negative Africans. This may suggest that *P. vivax* uses alternate invasion pathways to invade Duffy-negative erythrocytes. Evolution of *P. vivax* may, therefore, put Africans at risk of severe disease in the future. To identify the candidate ligands that are crucial for *P. vivax* infection in Africa, we study clinical isolates and in parallel, we use relevant primate models. DBP1, the key ligand for *P. vivax* Salvador I infection binds to *Aotus* monkey erythrocytes but not to *Saimiri* monkey erythrocytes. It is known that DBP1 binds to Duffy-positive erythrocytes but not to Duffy-negative erythrocytes. Thus, studying the difference in the expression of genes in Salvador I parasites from *Saimiri* and *Aotus* monkeys might unravel alternative invasion pathways that might provide insights on *P. vivax* infection in Duffy-negative erythrocytes. We have performed *P. vivax* Salvador I infection in three *Saimiri* and four *Aotus* monkeys. Trophozoites stage parasites were isolated by magnetic separation using MACS columns and allowed to mature to late schizont stage parasites *in vitro*. Next-generation sequencing using Illumina platform was performed to gain insights on the difference between the *P. vivax* transcriptomes in *Saimiri* and *Aotus* infected monkeys. Interestingly three novel genes showed up to a 30-fold increased gene expression in *Saimiri* infection when compared to *Aotus* infection. These proteins may potentially be involved in alternate invasion pathways and need to be examined in Duffy-negative *P. vivax* infection. Currently, we are elucidating the biological significance of these upregulated proteins which may be vital for understanding Duffy-negative *P. vivax* infections in Africa.

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CHARACTERIZATION OF *PLASMODIUM VIVAX* GENE EXPRESSION DURING CLINICAL INFECTIONS AND UPON CHLOROQUINE TREATMENT

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Studies of gene expression have the potential to provide unique insights on the biology of *P. vivax* parasites. However, due to the lack of continuous *in vitro* propagation system for *P. vivax*, most studies have to rely on patient samples, which complicates molecular investigations due to the polyclonality of most infections, the concurrent presence of different blood-stages (each with their own regulation and responses), and the abundance of host molecules. Here, we show that we can routinely generate robust RNA-seq profiles for blood-stage *P. vivax* parasites using blood from infected patients, without any pre-processing. Gene expression deconvolution analysis of 26 Cambodian patients confirms that most RNA molecules derive from trophozoites and, thus, that the asynchronicity of *P. vivax* infections *in vivo* is unlikely to confound gene expression studies. Despite this overwhelming signal from trophozoites, we show that known gametocyte genes are detectable in most infections, but form two distinct clusters of co-regulated genes (possibly reflecting different transcriptional regulations of male and female gametocytes). We also perform high-throughput sequencing of mRNA from selected genes after RT-PCR amplification and show that, in polyclonal infections, the proportion of parasites committed to gametocytogenesis is similar across clones. Finally, we analyze the changes in parasite gene expression induced by chloroquine treatment and show that, despite a large effect on parasitemia, chloroquine does not alter the gene expression profiles. We hypothesize that chloroquine efficiently clears most *P. vivax* parasites but does not affect trophozoites, leaving the overall patterns of gene expression unscathed. Lastly, we show that RNA-seq analyses of *P. vivax*-infected patient blood can be complemented with single-cell RNA-seq experiments in non-human primates. These experiments enable to rigorously study changes in stage composition and in the regulatory program of each development stage, although the cost and logistical difficulty associated with these experiments might limit their use.

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A SYSTEMS BIOLOGY APPROACH TO CHARACTERIZING *PLASMODIUM FALCIPARUM* RESPONSES TO HIGH PARASITE DENSITY

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The malaria parasite *Plasmodium falciparum* encounters diverse environments throughout its asexual lifecycle, including high parasite densities during sequestration of trophozoites and schizonts in the microvasculature. High parasite densities in the human host have been associated with severe malaria *in vivo*, while regulation of parasite populations below the fever-inducing threshold has been observed in asymptomatic children in Papua New Guinea. Such disparate behavior suggests a key role for parasite responses to density with respect to disease progression and severity. *In vitro* cultures of high density, asexual *P. falciparum* exhibited aberrant morphology during the late trophozoite and schizont stages. These were arrested development, failure of schizont

maturation and merozoite formation, and hallmarks of cell death such as loss of cell volume, mitochondrial membrane depolarization, and blebbing of the parasite plasma membrane with eventual release of aberrant parasites from infected erythrocytes. Density-dependent cell death was not a consequence of glucose or essential amino acid depletion or excess lactate. A multi-omics analysis was performed to characterize the transcriptomic, metabolic, and lipid profiles within the local environment of *P. falciparum in vitro* cultures at densities corresponding to severe malaria. Metabolic analysis of conditioned medium derived from high-density parasite cultures revealed perturbation of compounds involved in amino acid and carbohydrate metabolism, as well as depletion of glycerophospholipids. Individual omics platforms were integrated using a latent component discriminant analysis (DIABLO), identifying a subset of features that described the effects of density on parasite metabolism and were highly correlated across omics platforms. Gene ontology analysis of features selected by integrated analysis illustrated the relatedness of biological pathways perturbed under high-density conditions. Ultimately, this systems biology approach to characterizing parasite metabolism in high-density environments may highlight potential targets for intervention.

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REFINED SUB-STRUCTURE OF *PLASMODIUM FALCIPARUM* POPULATIONS IN SUB-SAHARAN AFRICA AND THE IMPLICATIONS FOR THE EMERGENCE AND SPREAD OF ARTEMISININ RESISTANCE

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In recent years, the emergence of artemisinin drug resistance in SEA has resulted in focused attention to this region because it is feared that resistance might spread to SSA. Recent studies have shown K13 mutations are mostly localized in small geographical area in SEA where they originate independently and have particular genetic background. Some of these mutations have also been identified in SSA parasites, but without the delayed clearance phenotype. NS K13 mutations are undergoing strong evolutionary selection in SEA but not in SSA where mutations originate locally. We evaluated the status of artemisinin resistance in SSA by analyzing the K13 mutations. Data reported here is from 10 countries representing east, west and central African nations with different transmission intensities and are geographically dispersed. More than 1300 Pf samples were whole genome sequenced on Illumina. Additional 315 samples from ACT efficacy studies in Kenya were sequenced on Sanger. Despite the presence of sustained high infection complexity, low linkage disequilibrium and high recombination in the isolates tested, NS K13 mutations were present in low frequencies and none of the WHO validated K13 resistance mutations were present. Population structure analysis revealed presence of distinct east African, Ethiopian and west African parasite populations. Ethiopian isolates were significantly divergent from all other populations including those from neighboring Kenya. Analysis of the drug resistance SNPs resulted in loss of the east-west geographical sub-division of the parasite population structure. There was no evidence of drug resistant gene flow from east to west Africa which is against the popular dogma of east to west gene flow, where resistant parasites migrate from SEA to SSA. K13 mutations in SSA are private, emerge locally, are not under positive pressure, and do not confer artemisinin resistance. The K13 mutations and frequency differ even inside each country, and K13 mutations were present in pre-ACTs parasites. There is no evidence that artemisinin resistant parasites from SEA are likely to flow to SSA, using the east to west route.

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LARGE-SCALE USE OF INSECTICIDE-IMPREGNATED 'TINY TARGETS' TO CONTROL TSETSE FLIES IN THE DEMOCRATIC REPUBLIC OF CONGO

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In 2014, half of all cases of gambiense-Human African Trypanosomiasis (gHAT) worldwide occurred in the former Bandundu Province of Democratic Republic of Congo (DRC). Efforts to eliminate the disease have been based largely on active screening and treatment of the human population. This approach has halved the incidence of gHAT but additional interventions are required if WHO elimination goals are to be achieved. Control of the vectors - tsetse flies - could accelerate progress but standard methods of controlling the important vectors of gHAT were too costly and complex to implement at scale. Insecticide-treated 'Tiny Targets' have been used successfully to control gHAT in Uganda, Guinea and Chad. We tested whether Tiny Targets could be used in the particular setting of DRC to control *Glossina fuscipes quanzensis* in an integrated program combining active and passive screening with vector control. Between 2015 and 2018, tsetse control has been scaled up from an area of 500km^2 to 2700km^2 across Yasa-Bonga and Masi-Manimba Health Zones. Between 3000 and 12000 Tiny Targets were deployed at six-month intervals in riverine areas where tsetse concentrate and the catch of tsetse from a network of 250 monitoring traps declined by over 90% within three months of targets being deployed. The operation was implemented by locally-recruited people who had no prior experience of tsetse control; after one day of training they deployed targets largely from dugout canoes navigating along major rivers. By 2018, planning, management and supervision of the operation was conducted by a single entomologist from the national control program ('Programme National de Lutte contre la Trypanosomiase Humaine Africaine', PNLTHA). Tiny Targets offer a simple and cost-effective method of tsetse control which can be readily implemented by local people with technical guidance from a national vector control program. Tsetse control operations are currently being expanded across Bandundu in what we expect will be the largest vector control operation against gHAT ever.

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PARATRANSGENIC MANIPULATION OF MICRORNA-275 EXPRESSION IN THE TSETSE FLY MIDGUT, AND THE DOWNSTREAM IMPACT ON TRYPANOSOME INFECTION OUTCOMES

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Tsetse flies (*Glossina* spp.) are prominent vectors of pathogenic African trypanosomes. To establish an infection in tsetse, trypanosomes must cross the fly's peritrophic matrix (PM), which is a chitinous barrier that lines the midgut's luminal surface. Upon entering tsetse's midgut, bloodstream form parasites shed their variant surface glycoproteins (VSG), which are then internalized by the fly's PM-producing cardia organ. This process results in down-regulated expression of a tsetse microRNA (miR-275) that regulates the production of proteinaceous components

of the fly's PM. The molecular mechanisms that govern this pathway are poorly understood. To better define the impact of this microRNA on PM formation processes, we developed a novel *in vivo* 'paratransgenic' system that employs tsetse's commensal endosymbiont, *Sodalis*, to constitutively express a miR-275 antagomir (ant-275) or a scrambled control miR (scr-275) in the fly's gut. We stably colonized tsetse with either ant-275 or scr-275-expressing *Sodalis*, and confirmed miR-275 knockdown in the cardia of treatment flies compared to control flies. Subsequently, we used RNA-seq to determine the impact of miR-275 depletion on downstream gene expression in cardia tissue, and we monitored trypanosome infection outcomes in both lines of paratransgenic tsetse. We found that knockdown of miR-275 expression in tsetse's cardia sequentially altered the expression of genes that encode PM associated proteins. Furthermore, treatment flies were significantly more susceptible to infection with trypanosomes than were control individuals. Our results demonstrate the efficacy of using paratransgenesis to express and study the function of microRNAs in an animal host-commensal symbiont model system. Using the tsetse fly in this capacity, we characterize in detail the role of miR-275 in regulating trypanosome infection outcomes in the fly. This powerful tool can be further developed for use in unraveling the molecular mechanisms that underlie trypanosome-tsetse interactions.

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CHAGAS DISEASE ECO-EPIDEMIOLOGY: VECTOR HOST-FEEDING PATTERNS SUGGEST AN EPIDEMIOLOGICAL RISK OF CHAGAS DISEASE ON THE CARIBBEAN ISLAND OF TRINIDAD

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Chagas disease, (etiological agent *Trypanosoma cruzi*), is not recognized as an epidemiological risk in the Caribbean, due in part to a belief that the triatomine bug vectors in the region do not feed on humans. Lending evidence to the contrary, three studies on the Caribbean island of Trinidad found *T. cruzi*-infected triatomine bugs and *T.* cruzi-seropositive humans with clinical features of Chagas disease. However, there have been no reports of direct contact between humans and triatomine bugs on the island, begging the question, are the bugs feeding on humans? To answer this question and gain a better understanding of the epidemiological risk of Chagas disease in Trinidad, we analyzed blood meals from triatomine bugs collected near human homes from five sites in northern and central Trinidad. For each bug, we diagnosed *T. cruzi* infection, and sequenced its DNA to determine the last host species from which it fed. Out of 55 bugs (54 *Panstrongylus geniculatus* and 1 *Rhodnius pictipes*), 46 (83.6%) were infected with *T. cruzi*. DNA sequencing yielded conclusive host identification for 48 (87.3%) of the 55 bugs. The most common hosts were humans (25 bugs; 52.1%), followed by chickens (10 bugs; 20.1%), sylvatic mammals (10 bugs; 20.1%), and sylvatic birds (3 bugs; 6.2%). Of the 25 bugs that fed on humans, 21 (84%) were infected with *T. cruzi.* At least one bug from each of the five collection sites was infected with *T. cruzi*, and at least one bug from each site had taken a human blood meal. *T. cruzi*-infected bugs with human blood meals were found at three of the sites. Ecologically, our findings suggest that in Trinidad, the triatomine bug species *P. geniculatus* feeds on a range of taxa across sylvatic and domestic habitats, a common characteristic of key Chagas disease vectors. Indeed, *P. geniculatus* is a competent vector of *T. cruzi,* and has been implicated in Chagas disease transmission in regions recognized as Chagas-endemic. Epidemiologically, our results suggest that humans are likely to be involved in a vector-borne *T. cruzi* transmission cycle in Trinidad, and that Chagas disease may be a higher epidemiological risk in Trinidad than previously believed.

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IMMUNE RESPONSE OF THE BED BUG TO SIMULATED TRAUMATIC INSEMINATION, STARVATION, AND INFECTION WITH ENTOMOPATHOGENIC BACTERIA

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The bed bug, *Cimex lectularius*, is a tenacious human pest. While bed bugs are thought to have evolved several immune-related adaptations to aid their remarkable lifestyle, the mechanisms involved in their innate immune response are poorly understood. Along with hematophagy, bed bugs are believed to undergo significant pathogen exposure during the process of traumatic insemination. During this process, male bugs pierce the female through her cuticle and inseminate the body cavity directly. As the bed bug genome is now available, we have the ability to measure gene regulation in response to immune challenge. Here, we used simulated traumatic insemination, or direct infection with a suite of entomopathogenic bacteria, followed by qRT-PCR-based analyses to measure the post-infection transcriptional response of conserved innate immunity-related genes. The primer sets used were designed and validated as part of this study and represent a novel immunity gene suite for this species. Current results suggest that antimicrobial peptides are generally upregulated in response to immune challenge in a pathogen and temperature dependent fashion, while several other immunity-related genes do not change significantly in response to infection or starvation. Current progress from an expanded suite of immune genes and pathogens will be presented along with a discussion of how this knowledge could be applied towards bed bug control.

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NOVEL VECTORS AND PARASITE OF CUTANEOUS LEISHMANIASIS TRANSMISSION IN GILGIL, NAKURU COUNTY, KENYA

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Cutaneous leishmaniasis (CL), a Neglected Tropical Diseases is endemic in parts of Kenya. Phlebotomine sandflies are the only proven vectors of the disease but knowledge of their distribution and diversity in Kenya is scarce. Accurate knowledge of this is fundamental in implementation of vector control strategies. We explored occurrence, distribution and diversity of sandflies in Gilgil, Nakuru County, Kenya. Vector sampling was done from five villages, CL hot spots. Trapping was done using CDC light traps and castor oil sticky papers. Morphological and molecular identification of vector species was done using taxonomic keys and sequencing of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. Midguts of sandflies suspected to harbor promastigotes were cultured in NNN media overlaid with complete Schneider's media or blood agar. Parasite identification was done using PCR-high resolution melt of the ITS1 gene and sequencing. Cytochrome b (*cyt-b*) gene was analyzed for host preference determination. A total of 1320 sandflies were collected: Sergentomyia (8.5%) and phelebotomine (91.5%). For phlebotomine, 5 species were identified: *P. guggisbergi (*69%), *P. saevus* (18%), *P. sergenti* (2%), *P. aculeatus* (7%). Vectors were differentially distributed with 70% and 30% accounting for outdoor and indoor collection respectively. Both *Leishmania tropica* and *Leishmania major* parasites were isolated from *P. guggisbergi* while *L. tropica* was isolated from *P. saevus.* Blood-meal sources included: humans (75%), rock hyraxes (19%), rats (2%), rabbits (2%) and pigs (2%). This is the first report of natural infection of *P. saevus* with *L. tropica* and the first evidence of *P. guggisbergi* as a vector of both *L. tropica* and *L. major*, implying that these species are involved in transmission of CL in Kenya. The possibility of *P. guggisbergi*

as a permissive vector remains to be determined. This study also reports occurrence for the first time of *P. sergenti* in the study site. Indoor and outdoor vector control activities need to be implemented in the study area. Targeted sandfly control strategies should be implemented.

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HUMAN ANTIBODY RESPONSES AGAINST SALIVARY PROTEINS OF *AMBLYOMMA AMERICANUM*

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Ticks are obligate blood-feeding ectoparasites and the most important arthropod vectors of human pathogens in the United States. The lone star tick, *Amblyomma americanum*, is widely distributed throughout the Midwest of the US, and it is a known vector of pathogens causing Rocky mountain spotted fever, tularemia, ehrlichiosis, STARI and a Lyme-like disease. There have been several studies about the immunologic response of the host when encountered with a tick-borne pathogen and host acquired immunity to ticks after tick feeding; however, little is known about other potential tick antigens that may affect the human host; whether it is bite associated or simply by contact. Nevertheless, it is important to elucidate which tick proteins and what mechanisms might be associated with a host immune response after exposure to ticks. Previous studies have shown the usefulness of antibodies against vector saliva as reliable markers for the human-vector contact and the disease transmission risk. In this study, we measured IgG, IgM and IgE antibodies against whole salivary gland extract of *A. americanum* in blood from individuals living in Kansas. We found a significant negative correlation between IgG and IgE antibody levels and age. Our analysis also shows that people using repellents have lower IgG antibodies against tick salivary proteins. We also found significantly lower antibodies in samples collected in the Fall respective to those collected during the Summer months. In addition, a western-blot test revealed three main immunogenic proteins identified by IgG antibodies (75, 45 and 37 kDa). Recombinant proteins will be used as biomarkers for human-tick exposure in an ELISA-based assay. Additionally, antibodies against pathogens can be correlated to the level of exposure to tick bites to calculate risk of disease. These results are the first report of immune responses to the lone start tick feeding in relationship to exposure in healthy individuals living in the US.

2079

EXPLORING THE SALIVARY GLYCOPROTEOME OF BLOODFEEDING ARTHROPODS AND THEIR RELEVANCE IN PATHOGEN TRANSMISSION

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The saliva of hematophagous arthropods is a powerful cocktail of substances meant to facilitate bloodfeeding, by counteracting the host's healing processes. Saliva can also stimulate significant immune responses, but while most research has focused on the proteins it contains, the glycans (sugars) that modify them remain overlooked. As glycans can determine a protein's biological role, they can be responsible for the saliva's effects on pathogens and their transmission. Therefore, in this work we set out to characterise the salivary glycans of ticks (*Amblyomma cajennense*), mosquitoes (*Anopheles gambiae, Aedes aegypti*), tsetse flies (*Glossina morsitans*), sandflies (*Lutzomyia longipalpis*) and triatomines (*Rhodnius prolixus*). To do this, we dissected and harvested saliva from each of these arthropods and characterized the sugar structures using a glycomics approach. This included enzymatic treatment, followed by analyses through high-performance liquid chromatography in combination with highly sensitive mass spectrometry. Our work shows that the salivary glycoproteins of these vectors are mostly composed of *N*-linked mannose-type sugars; the comparison between species shows variations mainly in the abundance of these structures. Interestingly, there were hybrid sugars specific to each organism, with mosquitoes and tick glycoproteins in particular displaying the most striking and potentially immunogenic structures. As some receptors on host immune cells are specific for mannose structures, we also performed overlay assays using recombinant fractions of human mannose receptors; salivary glycoproteins from all species were positively recognised, hinting at *in vivo* interactions with macrophages and dendritic cells. These interactions may be responsible for the saliva-specific immune responses that affect the process of pathogen infection; additionally, they can have a role in the host clearance (half-life) of the salivary glycoproteins themselves. Finally, the similarities of the sugars found indicate suggests the presence of conserved pathways of salivary protein glycosylation.

2080

CRYOPRESERVATION OF INFECTIOUS *CRYPTOSPORIDIUM PARVUMOO* CYSTS

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Cryptosporidiosis in an enteric infection caused by *Cryptosporidium* species and is a major cause of acute infant diarrhea in the developing world. The lack of methods to cryopreserve *Cryptosporidium* oocysts greatly hinders research on these parasites. Here, we report that ultra-fast cooling enables cryogenic storage of *C. parvum* oocysts. Cryopreserved oocysts exhibited high viability and robust *in vitro* excystation. Cryopreserved oocysts were infectious to interferon-γ knockout mice. The course of the infection was comparable to what we observed with unfrozen oocysts. Oocysts viability and infectivity was not visibly changed after several weeks of cryogenic storage. Cryopreservation will facilitate the sharing of oocysts from well characterized isolates among different laboratories.

2081

CHARACTERIZATION OF GENETIC VARIATION BETWEEN HOST-SPECIFIC *THEILERIA PARVA* POPULATIONS

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East Coast Fever (ECF), caused by the apicomplexan parasite *Theileria parva*, kills more than a million cattle each year, in the regions of sub-Saharan Africa where the disease is endemic. The African Cape buffalo is believed to be the natural reservoir of *T. parva* and rarely exhibits clinical symptoms when infected, but transmits the parasite to cattle via a tick vector. A vaccine for ECF exists, which is effective against cattle-transmissible strains but much less effective in protecting cattle from *T. parva* strains originating in buffalo. Previous studies based on a few genetic markers have shown that buffalo-derived *T. parva* populations contain greater antigenic diversity, possibly explaining the failure of the vaccine to protect against the more diverse buffalo-derived parasites. Interestingly, cattle is infected and killed by *T. parva* of buffalo origin, but cannot transmit those parasites, suggesting that a degree of host specificity has occurred. The characterization of genetic variation among

and between cattle- and buffalo-derived *T. parva* strains is critical to shed light on the evolutionary processes that have led to the host specificity, as well as for the design of next-generation vaccines against all strains. We generated whole genome sequence data from 25 cattle- and 28 buffalo-derived *T. parva* isolates following whole genome DNA sequence capture. Based on genome-wide SNP data, we identified genomic islands of divergence between the cattle- and buffalo-derived *T. parva* isolates. An F_{ST} analysis yielded a genome-wide value of 0.27 between the two sets of strains, revealing strong differentiation. Known antigens Tp8, Tp10, and p67 were among the genes with highest F_{ST} values, warranting further exploration for vaccine development. We are mining regions with strongest differentiation for genes with a potential role in infection and transmission, based on the function and properties of the encoded proteins.

2082

NOVEL ASSAYS FOR ANTICRYPTOSPORIDIAL *IN VITRO* EFFICACY

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Novel assays for anticryptosporidial *in vitro* efficacy Cryptosporidiosis is a diarrheal disease predominantly caused by *Cryptosporidium parvum* (*Cp*) and *Cryptosporidium hominis* (*Ch*), apicomplexan parasites which infect the intestinal epithelial cells of their human hosts. The only approved drug for cryptosporidiosis is nitazoxanide, which shows limited efficacy in immunocompromised children, the most vulnerable patient population. New therapeutics and *in vitro* infection models are urgently needed to address the current unmet medical need. Toward this aim, we have developed novel cytopathic effect (CPE)-based *Cp* and *Ch* assays in human colonic tumor (HCT-8) cells and compared them to traditional imaging formats. Further model validation was achieved through screening a collection of FDA-approved drugs and confirming many previously known anti-*Cryptosporidium* hits as well as identifying a few novel candidates. Collectively, our data reveals this model to be a simple, functional, and homogeneous gain of signal format amenable to high throughput screening, opening new avenues for the discovery of novel anticryptosporidials.

2083

EUKARYOTYPING: A NOVEL METHOD FOR AIDING OUTBREAK INVESTIGATIONS INVOLVING THE SEXUAL EUKARYOTIC PATHOGEN *CYCLOSPORA CAYETANENSIS*

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Cyclospora cayetanensis is an intestinal protozoan of humans responsible for seasonal outbreaks of foodborne illness in the United States (U.S.) due to importation of contaminated fresh produce from endemic countries. No independently validated molecular tools have been developed to aid cyclosporiasis outbreak investigations. *Cyclospora* reproduces sexually and possesses three discrete genomes, one nuclear and two organellar, resulting in a complex genetic structure and intra-isolate heterozygosity which complicates typing using traditional multi-locus-sequencing approaches. Additionally, the availability of only two published *C. cayetanensis* genomes hinders selection of informative typing markers. We sequenced 11 *C. cayetanensis* genomes and identified three Polymerase Chain Reaction (PCR) enrichment friendly markers, including two nuclear loci and one mitochondrial locus. We applied PCR enrichment and Sanger sequencing of these loci to 74 fecal specimens containing *C. cayetanensis*, 27 of which had been epidemiologically linked to U.S. cyclosporiasis outbreaks. Next, we developed a novel method, eukaryotyping, which incorporates machine learning approaches to identify genetic clusters that represent parasite familial relationships, and used it to analyze the sequence data. Eukaryotyping differs from other methods as it considers intra-isolate heterozygosity, a common feature of nuclear loci in sexual eukaryotes partly attributable to their mechanisms of nuclear inheritance. This is an important advancement, as heterozygosity confounds traditional approaches, sometimes resulting in exclusion of data. Eukaryotyping resolved the parasites within the 74 fecal specimens into 10 genetic clusters, represented as a 2D scatter-plot for ease of interpretation. These clusters were largely corroborated by the epidemiological data, supporting the utility of eukaryotyping for assisting epidemiological trace-back. While the markers examined here are specific to *C. cayetanensis*, the principals behind eukaryotyping will likely have broader implications that extend to other sexual eukaryotic pathogens.

2084

EVALUATION OF THREE COMMERCIAL DIAGNOSTIC TESTS FOR *CRYPTOSPORIDIUM* INFECTIONS IN HUMANS

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Diarrhea is the second greatest killer of small children worldwide, responsible for 800.000 deaths of under 4-year-olds yearly. Most lethal diarrhea in small children is caused by rotavirus, which is soon to be controlled by vaccination programs. This is closely followed by the eukaryote *Cryptosporidium,* which is one of the commonest, and at the same time most poorly understood, water-borne parasites of humans. To aid diagnosis and to support control programmes adequate diagnostic tests should be in place. Several rapid diagnostic tests (RDTs) for cryptosporidiosis are nowadays available and we have evaluated three different brands: RIDA QUICK for *Cryptosporidium/Giardia* Combi (R-Biopharm, Germany); CRYPTO/GIARDIA DUO-Strip (Coris BioConcept, Belgium); GIARDIA/CRYPTOSPORIDIUM QUIK CHEK (TechLab, USA). All three test are based on the lateral flow principle, able to detect both *Cryptosporidium* as well as *Giardia* and are sold as complete test kits. Stool samples of children from Malawi or Kenya suspected of having a protozoan infection causing diarrhea were used for diagnostic evaluation using PCR as reference standard. The Cryptosporidium incidence based on antigen detection was calculated to range from 9.5% to 18.1% in the samples from Malawi and from 15.0% to 25.0% in the Kenyan samples. The majority of stool samples from Malawi (71.4%), as well as Kenya (84%), was typed as "C. hominis". Infections with only "C. parvum" were less abundant (22.9% and 11.5% for Malawi and Kenya, respectively) and there were no mixed Cryptosporidium species infections. The Giardia incidence on the basis of RDTs ranged from 12.1% to 19.8% and from 6.7% to 12.5% in the Malawian and Kenyan samples, respectively. Using PCR as reference standard the sensitivity and specificity of the tests was respectively as follows: DUO 59.4% (95% CI: 47.9 - 70.4) and 95.5% (95% CI: 91.0 - 98.1%); RIDA: 67.1% (95% CI: 55.6 - 77.3%) and 83.4% (95% CI: 76.7 - 88.9%) and QUICK 76.0% (95% CI: 65.0 - 84.9%) and 96.2% (95% CI: 91.9 - 98.6%). The QUICK test had the best agreement with PCR (k-value: 0.760, SE of Kappa: 0.043, 95%CI: 0.676 - 0.845; agreement is considered: "good").

2085

POTENT AND SELECTIVE ANTI-*GIARDIA* COMPOUND SERIES: PROGRESS AND NEW DEVELOPMENTS

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Giardia duodenalis infects a wide array of hosts and is the most frequently reported human intestinal parasite. On an annual basis this parasite is responsible for ~1 billion human infections of which >200 million result in symptomatic disease. While infections are more prevalent in the

developing world, this parasite is ubiquitous. *Giardia* infection can result in severe and chronic disease, causing malabsorption, weight loss and failure to thrive in children. There is also evidence that infection is linked to post-infection disorders including irritable bowel syndrome. Despite growing evidence of *Giardia* associated morbidity, current treatment options are inadequate. The frontline drug, metronidazole (MTZ), is associated with side-effects and drug resistance. It is also incredibly distasteful and must be taken in multiple doses over 5-7 days, factors which result in poor compliance, treatment failure, rapid re-infection and parasite resistance. In addition there is increasing evidence that MTZ has a collateral effect on host microbiome. To improve giardiasis treatment options in the long-term we recently screened compounds from the Compounds Australia Open Access Scaffold Library for anti-*Giardia* activity (2451 compound subset; 2/scaffold; Z-factor 0.75). Rational selection based on activity, novelty, and chemical liabilities identified three compound series with potent (IC_{50}≤1μM) and selective activity for *G. duodenalis.* These compound series have been chosen as starting points for anti-*Giardia* drug development. The most promising compound identified to date is active against assemblage A, B and metronidazole resistant parasites (290-fold more potent than metronidazole) and has a *Giardia* vs human selectivity index of >9000. Preliminary *in vivo* studies with this compound suggest no toxicity at up to 10X the calculated therapeutic dose and ~75% efficacy at a dose of 0.7mg/kg daily for 3 days. Further *in vivo* and activity studies with this compound and other lead series candidates are now under way and will be presented.

2086

THE EFFECTS OF NITIDINE CHLORIDE AND CAMPTOTHECIN ON THE GROWTH OF *BABESIA* AND *THEILERIA* PARASITES

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The treatment of bovine and equine piroplasmosis is limited to diminazene aceturate (DA) and imidocarb dipropionate. To address this challenge, we need to explore novel drug compounds and targets. Topoisomerases are potential drug targets because they play a vital role in solving topological errors of DNA strands during replication. This study documented the effectiveness of topoisomerase inhibitors, nitidine chloride (NC) and camptothecin (Cpt), on the growth of *Babesia* and *Theileria* parasites. The half maximal inhibitory concentrations (IC_{50}s) against *B. bovis*, *B. bigemina*, *B. caballi*, and *T. equi* were 1.01 ± 0.2, 5.34 ± 1.0, 0.11 ± 0.03, and 2.05 ± 0.4 μM for NC and 11.67 ± 1.6, 4.00 ± 1.0, 2.07 ± 0.6, and 0.33 ± 0.02 μM for Cpt, respectively. The viability experiment showed that *B. bovis*, *B. bigemina*, and *B. caballi* did not regrow in 4, 10, and 4 μM or 48, 8, and 8 μM treatments of NC and Cpt, respectively. However, *T. equi* regrew in all of the concentrations used. Moreover, increasing the concentration of NC and Cpt to 16 μM and 1.2 μM (8×IC_{50}) did not eliminate *T. equi*. The micrographs of *B. bigemina* and *B. caballi* taken at 24 h and 72 h showed deformed merozoites and remnants of parasites within the RBC, respectively. The treatments of 25 mg/kg DA and 20 mg/kg NC administered intraperitoneally and 20 mg/kg NC given orally showed 93.7, 90.7, and 83.6% inhibition against *B. microti*, respectively, compared to the untreated group on day 8. In summary, NC and Cpt were effective against *Babesia* and *Theileria* parasites *in vitro*. Moreover, 20 mg/kg NC administered intraperitoneally was as effective as 25 mg/kg DA against *B. microti* in mice and showed no toxic symptoms in mice. The results indicate that NC may, after further evaluations, prove to be an alternative drug against bovine and equine piroplasmoses.

2087

A TRIAL OF A NOVEL TRIPLE DRUG TREATMENT FOR LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA

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A single dose of diethylcarbamazine (DEC) plus albendazole (ALB) given for multiple annual treatments are required for elimination of lymphatic filariasis outside sub-Saharan Africa since a single dose does not reduce blood microfilaria counts below the threshold required to interrupt transmission. This study tested the efficacy of single dose of ivermectin (IVM) combined with DEC/ALB and provides the final results of this clinical trial. We conducted a randomized clinical trial in which *Wuchereria bancrofti*-microfilaremic adults in Papua New Guinea were assigned to treatment with IVM/DEC/ALB once (n=60), DEC/ALB once (n=61), and DEC/ALB annually for 3 years (n=61). Clearance of blood microfilaria was measured at 12, 24 and 36 months. A single dose of IVM/DEC/ALB cleared microfilaremia in 55 of 57 (96%), 52 of 54 (96%) and 55 of 57 study participants (96%) at 12, 24 and 36 months, respectively. At the same time points one dose of DEC/ALB cleared microfilaremia in 18 of 56 participants (34%), 31 of 55 (56%), and 43 of 52 (83%) (relative risk to IVM/DEC/ALB at 36 months=0.20 P=0.035). 20 of 59 (32%), 42 of 56 (75%) and 51 of 52 participants (98%) treated with DEC/ALB annually for 3 years cleared microfilaremia over the same time period. Moderate adverse events were more common after treatment with the triple- than two-drug regimen (27% vs. 5%, P<0.001). There were no serious adverse events. A single dose of IVM/DEC/ALB induced rapid and sustained clearance of blood microfilaria in almost all individuals for 3 years and was superior to one dose or three annual doses of DEC/ALB.

2088

OXFENDAZOLE TREATMENT HAS A MACROFILARICIDAL EFFICACY AGAINST THE FILARIAL NEMATODE *LITOMOSOIDES SIGMODONTIS IN VIVO* AND INHIBITS *ONCHOCERCA GUTTUROSA* ADULT WORM MOTILITY *IN VITRO*

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Oxfendazole (OXF) is a promising drug candidate for the elimination of onchocerciasis that is closely related to the macrofilaricidal benzimidazole flubendazole (FBZ), but possesses an improved oral bioavailability. In the current study, we evaluated the *in vitro* efficacy of OXF against *Onchocerca gutturosa* adult filariae and *Onchocerca lienalis* microfilariae and compared the *in vivo* efficacy of OXF and FBZ in the *Litomosoides sigmodontis* rodent model of filariasis. OXF inhibited the motility of *O. gutturosa* adult worms in a dose dependent manner and had only marginal effects against *O. lienalis* microfilariae. Accordingly, OXF lacked a microfilaricidal efficacy in mice injected with *L. sigmodontis* microfilariae, whereas 5 days of subcutaneous administrations with OXF (25mg/kg) and FBZ (2, 6, 20mg/

kg) completely cleared the adult worm burden in *L. sigmodontis* infected mice. A complete clearance of the *L. sigmodontis* adult worm burden was also achieved by 5 days of oral treatments with OXF (2x12.5, 2x25mg/kg), but not FBZ (2, 6mg/kg). Oral OXF treatments (10 days, 2x5mg/kg) of patently *L. sigmodontis* infected jirds reduced the peripheral microfilaremia by >99% within 3 weeks post treatment start and a complete inhibition of the embryogenesis of remaining female adult worms was determined at necropsy, 16 weeks after treatment start. Pharmacokinetic analysis of OXF identified exposure time over threshold as driver of efficacy. These results indicate that OXF has an excellent macrofilaricidal efficacy following oral and subcutaneous administrations, but lacks a strong microfilarcidal efficacy, which reduces the risk of associated severe adverse events. Ongoing evaluations in a phase II study for trichuriasis and a multiple ascending dose phase I study will further help to consider the potential of OXF as clinical candidate for human filarial infections.

2089

CLINICAL PRESENTATIONS OF ONCHOCERCIASIS-ASSOCIATED EPILEPSY IN CAMEROON

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Onchocerciasis (oncho) is known to cause skin and eye disease. However, reports from oncho-meso and hyper-endemic areas reveal a high prevalence of epilepsy with a wide spectrum of seizure disorders constituting onchocerciasis-associated epilepsy (OAE). Data on the clinical features of OAE is currently scarce, whereas it is most likely the first cause of mortality due to oncho. Door-to-door surveys were carried out between July 2017 and January 2018 in 5 oncho-endemic villages in Cameroon. Epilepsy diagnosis was done in 2 steps: administration of a standard 5-items questionnaire and confirmation of suspected cases by a neurologist. OAE was defined as ≥2 seizures without an obvious cause, starting between the ages of 5-18 years in previously healthy persons having resided for at least 3 years in an oncho area. PWE were examined and relevant history including birth conditions, health during childhood, seizure types and ivermectin (IVM) use were noted. Rapid tests were done to qualitatively assess the presence of anti-Ov16 antibodies. 156 PWE were recruited; the frequent seizure types were generalized tonic-clonic seizures (89%) and absences (39%). 34 PWE (22%) with nodding seizures (NS) and 1 case with Nakalanga features were identified. Mixed seizure types were seen in 40%; moreover in certain PWE, the seizure spectrum varied over time. 93% of all PWE met the diagnostic criteria for OAE. 57% of PWE tested positive for Ov16, but no clinical difference was observed with those who were Ov16-negative. In one village, 56% of PWE had onchocercal skin lesions confirmed by a dermatologist. Only 28% of PWE had taken ivermectin prior to the onset of seizures. In oncho-endemic areas in Cameroon, OAE presents as a spectrum of seizure types including NS. Onchocercal skin lesions and anti-Ov16 antibodies may be associated. Such clinical pictures should evoke a potential link with oncho. Although OAE pathophysiology is still unclear, its onset may be favored by poor IVM prophylaxis.

2090

A TRIAL OF SINGLE DOSE IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE FOR TREATMENT OF LYMPHATIC FILARIASIS IN SUB-SAHARAN AFRICA

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The recommended drug regimen for elimination of lymphatic filariasis in sub-Saharan Africa is a single dose of ivermectin (IVM) plus albendazole (ALB). Multiple annual treatments are required for elimination since a single dose of this regimen does not reduce blood microfilaria counts below the threshold required to interrupt transmission. Recent studies in Papua New Guinea have shown that a single dose of co-administered IVM, diethylcarbamazine (DEC) and ALB can sustain complete clearance of microfilariae (Mf) in 96% of participants for 3 years. This study examined the efficacy of a single dose of IVM+DEC+ALB in sub-Saharan Africa. We conducted an open-labeled, single-blinded clinical trial in which *Wuchereria bancrofti*-microfilaremic adults from Cote d'Ivoire were randomized to treatment with either a single dose of IVM+DEC+ALB (IDA, N=42) or IVM+ALB annually for 3 years (IA, N=45). Clearance of blood microfilaria and inactivation of adult worm nests were measured at 6, 12, 24 and 36 months. 36 month data will be collected in July 2018. One dose of IDA completely cleared Mf in 34/35 (97%), 29/38 (76%) and 23/36 (64%) of individuals at 6, 12, and 24 months respectively. In contrast, IA given annually x 2 achieved clearance in 17/45 (38%), 11/45 (24%) and 22/40 (55%) at the same time points (risk relative [RR] to IDA=0.12, P=0.037 at 6 months, RR=0.31, P=0.0001 at 12 months, and RR=0.80, P=0.44 at 24 months). IDA also demonstrated greater inactivation of adult worm nests in participants with worm nests; 10/15 (67%) and 16/19 (84%) and 14/20 (70%) at 6, 12 and 24 months. By comparison IA inactivated worm nests in 6/22 (27%), 7/25 (28%) and 14/28 (50% risk relative to IDA=0.6, P=0.19 at 24 months) at the same time points. New worm nests were observed in 10, 3 and 1 men treated with IA at 6, 12 and 24 months respectively, compared with no men at 6 or 12 months and 2 men at 24 months treated with IDA (P=0.001, P=0.083, P=0.397 at 6, 12 and 24 months respectively). Treatment with a single dose of IDA is superior to IA at 12 months and non-inferior to two doses of IA at 24 months. Re-infection may contribute to the lack of sustained Mf clearance at 24 months.

2091

CORALLOPYRONIN A: AN EFFECTIVE ANTIWOLBACHIAL COMPOUND FOR THE TREATMENT OF FILARIAL INFECTIONS AND ANTIBIOTIC FOR ANTIMICROBIAL RESISTANT STIS

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Depleting essential *Wolbachia* endosymbionts from filarial nematodes (*Wuchereria bancrofti*, *Brugia* spp. and *Onchocerca volvulus*, causing lymphatic filariasis and onchocerciasis) with antibiotics blocks

development and kills adult worms more effectively than ivermectin or diethylcarbamazine, which are mainly microfilaricidal. Antiwolbachial therapy is a novel way to eliminate these poverty related diseases affecting >100 Mio people. Alternatives to doxycycline and rifampicin are required. Corallopyronin A (CorA) inhibits bacterial DNA dependent RNA polymerase. CorA MoA is different to rifamycins and is active against rifampicin-resistant *S. aureus*. Although not effective against most Gram-negative bacteria, CorA depletes *Wolbachia* >10-fold better than the gold standard doxycycline, resulting in >90% macrofilaricidal activity in jirds. Within the German Center for Infection Research (DZIF) we are developing CorA to treat filarial infections, and for the Global Antibiotic Research & Development Partnership (GARDP) priority program for gonorrhea. We conducted non-GLP ADME and *in vitro* toxicity studies showing that CorA: has similar PO and IP bioavailability, and is non-toxic and does not inhibit host cell proliferation. The expression of CYP450s is not altered and CP450 3A4 is minimally induced, limiting drug-drug interactions seen with rifampicin. It is stable in human, mouse and dog microsomes and is metabolized via phase I reactions. With heterologous expression of the biosynthetic genes in *Myxococcus xanthus*, we achieved gram-scale, cost-effective production of CorA. We also developed an efficient downstream process, guided by quantitative NMR and LC/MS, to yield >95% pure CorA. Using this CorA in the *Litomosoides sigmodontis* rodent infection model, we reduced the minimal effective *in vivo* dose 6-fold and treatment time to 7-10 days, fitting the macrofilaricidal TPP. The final production protocol will provide pure CorA for final non-GLP activities, including dose escalation tox, Ames and hERG tests. Partnering with a CMO to produce GMP CorA, we will conduct GLP-tox studies and plan a phase 1 trial with BfArM.

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DEVELOPMENT AND VALIDATION OF AN *ONCHOCERCA OCHENGI* ADULT MALE WORM IN GERBIL MODEL FOR MACROFILARICIDAL DRUG DEVELOPMENT

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Onchocerciasis currently afflicts an estimated 18 million people and is the second leading infectious cause of blindness world-wide. The development of a macrofilaricide to cure the disease has been hindered by the lack of appropriate small laboratory animal models. To overcome this challenge, we developed and validated a Mongolian gerbil model that supports the implantation of the closely related *Onchocerca ochengi* male worms from cattle in the peritoneum of the small animal. Animals were treated with drug or the vehicle three days after worm implantation. The implanted worms were recovered and analyzed for viability 35 days after. In control animals, the recovery of the worms was on average 36%, with 90% of them being 100% motile. During model validation, only 6% of the worms were recovered in animals treated with Flubendazole (FBZ), resulting in significant reduction of worm burden. Notably, the motility of the recovered male worms was 0% in FBZ group versus 91.1% in controls. FBZ was further tested in five different experiments of the model and the results were all similar. We used the validated model to test a related drug, Oxfendazole (OFZ) and found that it also significantly reduced worm burden by 52.7%. The motility of recovered worms in the OFZ group was 22.7% versus 95.0% in the controls. In conclusion, we have developed and validated a gerbil *O. ochengi* adult male worm model for testing new macrofilaricidal drugs *in vivo*, and have used it to show that Oxfendazole is a potential macrofilaricidal candidate. *Supported by grants from the Bill & Melinda Gates Foundation (OPP1098475 and OPP1017584)*

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PROGRESS IN THE DEVELOPMENT OF NEEM EXTRACT AS A NOVEL ANTHELMINTIC THERAPEUTIC

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Despite the large burden of disease caused by helminths, there are currently very few medications available to treat these parasitic infections. We demonstrate that extract from the Neem tree (*Azadirachta indica*), used in Indian Ayurvedic medicine, has *in vitro* activity against tissue-invasive filariae that cause diseases such as elephantiasis and river blindness. Additionally, we have preliminary *in vivo* evidence that shows Neem efficacy in reducing adult worm burdens in *L. sigmodontis* infected mice. Neem extract was produced by partitioning the aqueous phase of the tree's seed oil, and then precipitating the small molecules. The precipitate was dissolved in a working stock of 2.5% ethanol. *In vitro*, we placed single adult *Brugia malayi* or *Litomosoides sigmodontis* worms, or pairs of *Schistosoma mansoni* worms, in 1 ml of media containing Neem extract at various concentrations. Worms were monitored daily for motility, fecundity, and viability. The extract rapidly kills adult *B. malayi* and *L. sigmodontis worms* within 5 days at 25 mcg/ml and within 1 day at 100 mcg/ml. *Schistosoma mansoni* worms were killed within 2 days of exposure at 100 mcg/ml. *In vivo,* BALB/c mice were infected subcutaneously with 40 L3 stage *L. sigmodontis* worms and treated seven weeks post-infection with 25 or 50 mg/kg of Neem extract by intraperitoneal injection. Two weeks following treatment, animals were euthanized and adult worms enumerated. A single dose of Neem extract at 50 mg/kg decreased the adult worm burden in these mice by over 50%. Fractionation analysis is currently under way to identify the specific active molecules within the Neem extract that exert anthelmintic activity. Additionally, we are analyzing several commercially available components of Neem and have had some success in demonstrating possible repurposing efficacy. This study demonstrates that Neem extract has broad-based anthelmintic activity against the two filarial pathogens tested and against *Schistosoma mansoni*. Neem exhibits excellent potential as a novel anthelmintic agent.

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MODERNIZING OUTBREAK INVESTIGATION FOR EMERGING INFECTIONS: AN INTEGRATED PHYLOGENETIC AND EPIDEMIOLOGICAL APPROACH FROM THE WEST AFRICAN EBOLA OUTBREAK IN SIERRA LEONE DETECTS A POTENTIAL NOVEL MECHANISM OF TRANSMISSION AND VALIDATES THE BENEFIT OF INCORPORATING GENOMIC DATA

Cordelia Coltart[1], Daniel Cooper[2], Maria Calvo Cortes[3], Matthew Cotton[4], Ian Goodfellow[5], Anne Johnson[1], Stephane Hue[3]

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The West African Ebola epidemic was the first emerging outbreak in which near real-time sequencing was available. Novel phylogenetic methods can determine transmission events, but have not previously been used in emerging outbreaks. Thus, the value of such data for outbreak investigation remains unclear. We use data from the Ebola outbreak in Sierra Leone (SL) to illustrate how incorporating genomic data with traditional epidemiological analyses provides additional insight, with potential to inform interventions and limit cases. We used routinely collected outbreak data linked to Ebolavirus (EBOV) genome sequences. A phylogeny of 554 EBOV sequences from SL was reconstructed by maximum-likelihood inference from which transmission clusters were identified. We focused on an isolated outbreak of Ebola in a rural village

(64 cases, 41 sequences) and inferred a 'sequence based transmission tree' (SBTT) using Outbreaker package. The conclusions were compared to those drawn from the traditional epidemiological investigation. Sequence based analysis confirmed the traditional epidemiological findings with respect to the origins and sources of this outbreak- a single introduction via the same index case. However, the SBTT was inconsistent with the epidemiological hypothesis of parent to child transmission. Sequences for several children clustered together and were directly related to the index case despite no contact. Combined with field reports this suggested children may have been infected by playing in puddles contaminated with Ebolavirus after a traditional healing ceremony, a previously unrecognised transmission route. To our knowledge, this is the first time molecular methods have detected a potential novel mechanism of transmission, not identified via traditional epidemiological surveillance. Thus, sequence data, where possible, should be incorporated into standard outbreak investigation. This combined approach is likely to enhance understanding of transmission dynamics, which allows faster and more effectively targeted interventions, thus limiting the impact of infectious disease outbreaks.

2094

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2095

IMPROVING CROSS-BORDER AND REGIONAL COMMUNICABLE DISEASE SURVEILLANCE AND RESPONSE THROUGH A NOVEL MULTICOUNTRY COLLABORATION, TOGO, BENIN, NIGERIA, AND CAMEROON

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In environments with strong transborder community connectivity and population movement, local disease outbreaks can quickly impact two or more countries. Establishing and strengthening binational and regional public health collaboration is a critical step in improving preparedness and response capacity to mitigate international spread of a communicable disease. The Ministries of Health in Togo, Benin, Nigeria, and Cameroon initiated a collaboration, in partnership with the US CDC Division of Global Migration and Quarantine, to analyze national cholera surveillance data to inform evidence-based recommendations for collaborative national, binational, and regional surveillance policies and response strategies to mitigate cross-border spread of future cholera outbreaks. Collaborators created a data set of all 33,451 confirmed and probable cholera cases from 2010 and from Togo and Benin in 2011 (n=75 [Togo 2010], 30 [Togo 2011], 62 [Benin 2010], 779 [Benin 2011], 21,111 [Nigeria 2010], 11,394 [Cameroon 2010]) to identify consistencies in data management approaches and spatial patterns in outbreak progression. All countries reported case information by at least administrative levels 1 and 2, similar to US state and county, and reported or could calculate the aggregate number of cases and deaths by epidemiological week. Inconsistencies in epidemiologic week definitions were rectified through consensus and data cleaning. Collaborators applied the results to create data management strategies that will facilitate cross-border surveillance data sharing during response activities in future outbreaks. For example, they defined a common, minimum list of variables and their definitions to include in an investigation for an outbreak with risk of cross-border transmission. Through this innovative, multicountry partnership, which will expand to analyze cholera data from 2011 to the present, Ministries of Health are strengthening cross-border collaboration and contributing to improved global health security against communicable disease threats.

2096

MAKING PASTORALISTS COUNT: HEALTH SURVEILLANCE OF A NOMADIC POPULATION USING A GEOSPATIALLY DERIVED SAMPLING FRAME

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Nomadic pastoralists are among Sub-Saharan Africa's poorest, hardest-to-reach, and least-served populations. Pastoralist communities are notoriously difficult to survey due to factors including their high degree of mobility, the remote terrain they inhabit, fluid domestic arrangements, and cultural barriers. Current sampling methodologies do not adequately capture the demographic and health parameters of nomadic populations and as a result, mobile pastoralists are often "invisible" in population data such as national censuses and the Demographic and Health Surveys (DHS). We developed methodology combining remote sensing and geospatial analysis to enumerate seasonal pastoralist encampments in southwest Ethiopia with the goal of demonstrative the effective use of an alternative sampling frame to collect representative data on a mobile population. Using a sampling frame constructed based on geospatial data, we conducted a survey focused on Maternal and Child Health (MCH) - a domain highlighted in the Key Indicator Report of the 2016 Ethiopian DHS - using standardized instruments from the DHS questionnaires. Our field validation suggests that the methodology used in this study is a logistically feasible alternative to conventional census-based sampling frames. Surveying 347 women of reproductive age, we found substantial disparities between the nomadic pastoralist populations in our sample compared to DHS-derived country estimates in key maternal and child health indicators such as antenatal care, skilled birth attendance, and vaccination coverage. We draw comparisons between numerous MCH indicators among this population and national data, highlighting unique characteristics of the study population that hold key relevance to designing health interventions. We propose the use of alternative geospatial sampling frames in pastoralist regions to reduce under-coverage and prevent bias in national data. Implementing effective methodology for the health surveillance of mobile populations is a crucial step towards developing health systems for these underserved groups.

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COMMUNITY HEALTH VOLUNTEERS TRAINED VIA U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT (USAID)/ PRESIDENT'S MALARIA INITIATIVE (PMI) PARTNERS RAPIDLY RESPOND TO A PNEUMONIC PLAGUE OUTBREAK, MADAGASCAR, 2017

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Human plague is endemic in Madagascar; approximately 400 cases are reported annually, most of which are bubonic disease. In late August 2017, an outbreak of pneumonic disease began in the capital city and one other urban center and spread to surrounding districts. Following a Ministry of Health (MOH) request, Community Health Volunteers (CHV) previously trained by USAID and PMI partners to provide basic health services in 6,726 Fokontany (smallest administrative unit), rapidly responded. The outbreak, which eventually totaled 2,158 cases and 174 deaths, was declared over on November 27, 2017. We describe efforts of USAID/PMI's partners to improve community-based health services via trained CHVs, and how these CHVs were quickly trained and equipped for the plague response. Within two weeks of the MOH's declaration of an outbreak in early October, 2017, USAID/PMI partners mobilized the CHVs they support. Partners used existing channels to distribute educational messages and outbreak tools developed by the MOH and the World Health Organization. They trained 440 CHVs based in plague-affected areas using an existing cascade-training infrastructure to teach CHVs to perform response activities. CHVs detected and referred 457 suspected plague cases and traced and referred for post-exposure prophylaxis 3,924 of the 7,270 (54%) case contacts reported during the outbreak. CHVs also led community hygiene campaigns to decrease rat infestations, provided door-to-door information to residents regarding plague prevention and prompt care seeking, chaired community awareness meetings, and distributed 30,000 educational tool kits. CHVs reached an estimated 430,000 persons during the response. Training and supporting CHVs to provide basic health services via PMI and USAID partners in rural communities in Madagascar resulted in a cadre of providers capable of responding to a public health emergency. The efforts of Malagasy CHVs likely contributed to limiting the magnitude and duration of the 2017 pneumonic plague outbreak.

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IDENTIFICATION OF ENTERIC PATHOGEN RESERVOIRS AND TRANSMISSION PATHWAYS ASSOCIATED WITH CHILDHOOD GROWTH IN THE URBAN INDIAN, AND RURAL BANGLADESH SITES OF THE GLOBAL ENTERIC MULTICENTER STUDY

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The burden of childhood growth impairment remains high in many South Asian communities and the region as a whole. Identification of causal pathways linking enteric pathogen reservoirs and transmission with childhood growth in different community settings can aid in the development of more effective intervention strategies to reduce this burden. Structural equation models (SEM) were developed separately for the Bangladeshi, and Indian components of the Global Enteric Multicenter Study (GEMS) to model effects of potential household pathogen transmission pathways on growth measures in rural and urban communities. Models were developed to test whether pathogen reservoirs were associated directly with height-for-age z-scores (HAZ) or were indirectly mediated by enteric infections. Tests also determined whether hygiene behaviors and maternal education moderate associations of pathogen reservoirs with infections and subsequently HAZ. A total of 3,582 children were enrolled in Kolkata, India and 3,859 enrolled in Mirzapur, Bangladesh. In India, unimproved toilet facilities had an indirect negative association with HAZ mediated by *Giardia* infections. Handwashing before nursing reduced this negative effect through the elimination of the positive association of unimproved facilities with *Giardia*. Stored water was negatively associated with HAZ but treating water reversed this effect. In Bangladesh, fuel dung use, unimproved toilet facilities and unsafe child feces disposal had negative indirect associations with HAZ via both *Cryptosporidium* and *Giardia* infections. Specific handwashing behaviors and maternal education reduce this association via the elimination of these protozoan infections. In conclusion, household pathogen reservoirs and corresponding pathways incorporating *Giardia* and *Cryptosporidium* infections are qualitatively different between urban and rural sites. Greater reductions in growth impairment may be achieved in South Asia through the development of targeted public health interventions for rural and urban communities using indicators of the unique pathways found in each of these settings.

2099

DEVELOPING A PUBLIC HEALTH TOOL TO MONITOR THE TRANSMISSION POTENTIAL OF NIPAH VIRUS DURING OUTBREAKS

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In Bangladesh, human Nipah virus (NiV) infections have been reported almost every year since 2001 with regular person-to-person transmission events. With a case fatality of >70% and no available treatments or vaccines, NiV was identified by the World Health Organization as one of nine pathogens that pose the highest risk for large-scale outbreaks. Prompt identification of changes in the transmission potential or the clinical presentation of cases is important so that public health responses can be deployed efficiently. We present a tool that allows public health officials to identify significant changes in a set of key transmission and case characteristics including (i) the annual number of spillovers from the bat reservoir, (ii) the proportion of cases who transmit to others, (iii) the reproduction number and (iv) the case fatality rate. We investigated how these measures changed over time based on all cases identified since systematic hospital-based surveillance began in 2007 to 2014 and explored differences in outbreak patterns before 2007. From 2001 - 2007, 18 spillovers were detected with a mean of 3 per year (95%CI 0.8-11). From 2007-2014, after systematic surveillance began, 61 spillovers were detected. The mean number of annual detected spillovers increased from 2.7 (95%CI 1.3-5.4) in 2007-2009 to 10.6 (95%CI 7.9-14) in 2010-2014. By contrast, the proportion of cases who transmitted (11%, 95%CI 6-18), the reproduction number (0.2, 95%CI 0.1-0.5) and the case fatality rate among primary (91%, 95%CI 84-96) or secondary cases (46%, 95%CI 29-63) did not change significantly over the period of stable surveillance. The person-to-person transmission potential of NiV appears stable since start of systematic surveillance and public health officials should be able to detect significant departures from this baseline in future outbreaks using the tool. Increases in detected spillovers over time could represent year-to-year variation in infections in the reservoir host. Systematic surveillance of NiV has allowed the establishment of baseline trends and will be essential to monitor changes in transmissibility to prevent large outbreaks.

2100

RDHS: AN R PACKAGE TO INTERACT WITH THE DEMOGRAPHIC AND HEALTH SURVEYS (DHS) PROGRAM DATA SETS

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The Demographic and Health Surveys (DHS) Program has collected and disseminated population survey data from over 90 countries for over 30 years. In many countries, DHS provide the key data that mark progress towards targets such as the Sustainable Development Goals (SDGs) and inform health policy such as detailing trends in child mortality and characterising the distribution of use of insecticide-treated bed nets in Africa. Though standard health indicators are routinely published in survey final reports, much of the value of DHS is derived from the ability to download and analyse standardized microdata datasets for subgroup analysis, pooled multi-country analysis, and extended research studies. We have developed an open-source freely available R package '*rdhs*' to facilitate management and processing of DHS survey data. The package provides a suite of tools to (1) access standard survey indicators through the DHS Program API, (2) identify all survey datasets that include a particular topic or indicator relevant to a particular analysis, (3) directly download survey datasets from the DHS website, (4) load datasets and data dictionaries into R, and (5) extract variables and pool harmonized datasets for multi-survey analysis. The suite of tools within the package represents the output of conversations with numerous research groups globally, and serves to simplify commonly required analytical pipelines. The end result aims to increase the end user accessibility to the raw data and create a tool that supports reproducible global health research. We detail the core functionality of *rdhs* by demonstrating how the package can be used to identify heterogeneity in malaria rapid diagnostic test (RDT) false negative rates (FNR) in Africa, and the association between malaria prevalence and RDT FNR. Using *rdhs* we extracted paired microscopy and RDT results for 216,587 children under the age of 5 from 22 African countries from 2008-2016. We identified a significant association between RDT FNR and malaria prevalence (OR = 0.31, 95% CI = 0.23-0.42, p<0.001), which gives support for utilising high sensitivity RDTs in lower transmission settings.

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IMPACT OF INDOOR RESIDUAL SPRAYING IN REDUCING MALARIA CASES IN RWANDA, 2013-2017

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Indoor residual spraying (IRS) is a core malaria prevention tool along with insecticide treated nets (ITNs). In 2013, 83% of Rwanda households owned ≥1 ITN; and during 2013–2016, Rwanda conducted IRS twice yearly in 3–5 districts. From 2012 baseline to 2017, national malaria cases increased >8-fold, but malaria burden from 5 districts receiving IRS at least twice decreased from 57.6% of national confirmed malaria cases to 18.8%. To better understand IRS impact, we calculated percentage change in number of new confirmed malaria cases for sprayed districts in 12 months following each spray round compared with the prior 12 months. With resource constraints, districts were not always fully covered by IRS. For districts with full coverage, combined malaria cases decreased 31.4% in the 12 months after IRS (range: -79.5% to 137.3%; median: -26.5%). For spray rounds targeting <50% of a district, combined malaria cases increased 75.4% (range: -32.3% to 516.6%; median 63.8%), similar to the 87.7% median annual case increase among districts not receiving IRS. In one district, cases declined 79.1% (late 2014) and 6.2% (early 2015) after full-district IRS, but withdrawal of IRS from nearly 70% of the district late 2015 was followed by a 283.6% increase in cases; resumption of full-district IRS in 2016 slowed case increase to 24.0% from the 12 months prior. Delay implementing IRS by one month for the early 2016 rainy season in 2 districts was followed by a combined 70.8% increase in cases compared with a 43.4% decrease in cases for 2 districts using the same insecticide one month earlier. Malaria cases increased 50.1% after IRS using a pyrethroid class insecticide compared with reductions in malaria cases after IRS with carbamate and organophosphate of 8.0% and 56.3%, respectively. Impact of carbamate diminished over time from 18.1% decrease in malaria cases during initial 2 years of use to 3.5% decrease in cases thereafter, likely related to detected resistance. IRS appears to

decrease malaria burden in Rwanda, but IRS impact can be optimized by sustained full-district coverage, appropriate seasonal timing, and rotation of insecticide class to mitigate resistance.

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RAPID IMPROVEMENTS TO RURAL UGANDAN HOUSING AND THEIR ASSOCIATION WITH MALARIA FROM INTENSE TO REDUCED TRANSMISSION: FINDINGS FROM A COHORT STUDY

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Improving housing presents a promising opportunity for malaria control by reducing indoor exposure to mosquitoes. We measured recent changes in house design in rural Uganda and evaluated their association with malaria in relation to a mass scale-up of control efforts. This analysis was part of a cohort study designed to estimate longitudinal changes in key malaria metrics. All children aged 6 months to 10 years (n=384) living in 107 households in Nagongera sub-country, Tororo, Uganda, were given long-lasting insecticide-treated nets and followed between Aug 19, 2011, and June 30, 2017. Repeat rounds of indoor residual spraying (IRS) of insecticide were initiated on Dec 5, 2014. Socioeconomic data were collected at two timepoints (Sept 25-Oct 9, 2013 and June 21-July 11, 2016) and houses were classified as modern or traditional. Associations between house design and human biting; parasite prevalence; and malaria incidence were evaluated before and after the introduction of IRS: For all analyses, the P-values were less than 0.004. The implementation of IRS was associated with significant declines in human biting rate (33·5 vs 2·7 Anopheles per house per night after IRS), parasite prevalence (32·0% vs 14·0%), and malaria incidence (3·0 vs 0·5 episodes per person-year at risk). The prevalence of modern housing increased from 23·4% in 2013 to 45·4% in 2016. Compared with traditional houses, modern houses were associated with a 48% reduction in human biting rate before IRS (adjusted incidence rate ratio [aIRR] 0·52, 95% CI 0·36-0·73), and a 73% reduction after IRS (aIRR 0·27, 0·17-0·42). Before IRS, there was no association between house type and parasite prevalence, but after IRS there was a 57% reduction in the odds of parasitemia in modern houses compared with traditional houses, controlling for age, sex, and socioeconomic position (adjusted odds ratio 0·43, 95% CI 0·24-0·77). House type was not associated with malaria incidence before or after IRS. Improved housing in rural Uganda and was associated with additional reductions in mosquito density and parasite prevalence following the introduction of IRS.

2103

THE IMPACT OF MULTIPLE ROUNDS OF INDOOR RESIDUAL SPRAYING ON MALARIA INCIDENCE AND HEMOGLOBIN LEVELS IN A HIGH TRANSMISSION SETTING

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Malaria is the leading cause of morbidity and mortality in Uganda, with some of the highest levels of malaria transmission intensity in the world. Indoor residual spraying (IRS) is an important control intervention used in targeted areas in Uganda. The objective of this study was to estimate the effect of IRS activity, including the longevity of its effect, on malaria incidence and hemoglobin levels in a cohort of children in Nagongera, Uganda. A dynamic cohort of children was enrolled in Nagongera, Uganda starting in 2011. Household were randomly selection from enumeration surveys and all eligible children aged 0.5-10 year olds enrolled from 107 households. Malaria was diagnosed using passive surveillance and defined as a fever and the detection of parasites by microscopy. The first three rounds of IRS utilized a carbamate (bendiocarb) approximately every six months and a fourth round utilized an organophosphate (pirimphos-methyl) with plans to administer every 12 months. The analysis included data through June 20, 2017 and Poisson generalized linear model with a log link function. There were 384 children with 2,874 incident episodes of malaria and an average of 2.0 episodes per year per person over the entire study period. The number of incident malaria episodes per person year after the implementation of IRS was reduced by 79% with the age at first episode of malaria increasing to 6 years old from 4.8 years pre-IRS. Hemoglobin levels significantly increased by 11% by IRS round 4 and by over 1 g/dL, when compared to pre-IRS levels. The largest reductions in malaria occurred within the first two to seven weeks of IRS implementation, coinciding with high household coverage. A rebounding of cases occurred several weeks after the spraying round finished, with large variation between the rounds. We also found that larger households were associated with a larger reduction following IRS compared to smaller households. Our study supports the policy recommendation of IRS usage in a perennial transmission area to rapidly reduce the transmission and also that the most significant reductions in incidence were observed when high household coverage was obtained.

2104

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY AND BIRTH OUTCOMES: ASSESSMENT OF THE FIVE DOSE SULFADOXINEPYRIMETHAMINE POLICY IN RURAL NORTHERN GHANA

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Intermittent preventive treatment of malaria in pregnancy with sulfadoxine-pyrimethamine (IPTpSP) decreases placental parasitaemia and improves birth outcomes. Ghana based on the WHO current recommendation administers five doses of SP given during antenatal care to pregnant, spaced one month apart after 16 weeks of gestation till delivery. This study determined the level of uptake of the five-dose SP policy and its association with birth outcomes in rural northern Ghana. A hospital-based study was carried out among nursing mothers who had delivered within ten weeks and were seeking postnatal care at the district Hospital. Antenatal data on first ANC, number of visits, receipt of IPTp-SP and birth outcomes were documented. Mothers were interviewed on socio-demographic characteristics and obstetric history. Bivariate and multivariate analyses determined association between antenatal indicators, uptake of IPTpSP and birth outcomes. The proportion of uptake of three to five doses of SP were: IPT3 (76.4%), IPT4 (37.3%) and IPT5 (16.0%). The proportions of women who received first dose of SP at 16, 17-24 and 25-36 weeks gestation were: 16.9%, 56.7% and 26.4% respectively. Women who took the first dose of SP during the second trimester were more likely to receive ≥3 doses of SP than those who took the first dose during the third trimester ($\chi 2 = 60.1$, $p<0.001$). Women who made ≥4 visits were more likely to receive ≥3 doses of SP than those who made <4 visits ($\chi 2 = 87.6$, $p<0.001$). A higher proportion (82.5%, 95% CI: 76.4-87.5) of

mothers who took ≥3 doses of SP delivered at term compared to 61.7% (95% CI: 48.2-73.9) of those who took <3 doses (p = 0.001). Similarly, 88.1% (95% CI: 82.7-92.7) of mothers who took ≥3 doses of SP gave birth to babies weighing ≥2.5 kg compared to 61.7% (95% CI: 49.9-75.4) of those who took <3 doses of SP (p <0.001). Uptake of five doses of SP (new Ghana policy) was very low. Increased efforts towards improving early ANC attendance could increase uptake of SP and improve pregnancy outcomes.

2105

IMPACT OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY WITH MONTHLY DIHYDROARTEMISININ-PIPERAQUINE ON THE INCIDENCE OF MALARIA DURING INFANCY

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Malaria in pregnancy has been associated with an increased risk of malaria during infancy in observational studies. However, evidence from controlled trials on the impact of intermittent preventive treatment in pregnancy (IPTp) on malaria during infancy is limited. We compared the incidence of malaria among infants born to women enrolled in a double-blinded randomised controlled trial where monthly IPTp with dihydroartemisinin-piperaquine (DP) was found to be far superior to sulfadoxine-pyrimethamine (SP) for the prevention of malaria during pregnancy in a high transmission setting in Uganda. Children are being followed for all of their health care needs through 12 months of age with malaria diagnosed as fever and a positive blood smear by microscopy using passive surveillance. Of 674 live births with at least 1 day of follow-up, 336 were born to mothers randomised to monthly IPTp-SP and 338 born to mothers randomised to monthly IPTp-DP. Follow-up of infants is ongoing and here we present preliminary results through February 2018, which includes a total of 624 episodes of malaria and 440 person years of follow-up. Between birth and 3 months of age the incidence of malaria has been relatively low and similar between children born to mothers who received IPTp with DP compared to SP (0.64 vs 0.69 episodes PPY, p=0.71). After 3 months of age, the incidence of malaria is 20% lower among children born to born to mothers who received IPTp with DP compared to SP (1.64 vs 2.07 episodes PPY, p=0.048). This protective effect was greatest among children born to primigravid mothers where after 3 months of age, the incidence of malaria is 37% lower among children born to born to mothers who received IPTp with DP compared to SP (1.44 vs 2.29 episodes PPY, p=0.04). In summary, monthly IPTp with DP was superior to SP for the prevention of malaria during pregnancy in a high transmission setting of Uganda and appears to have the added benefit of reducing the incidence of malaria during infancy, especially among children born to mothers who were pregnant for the first time. It is anticipated that near final results of this study will be available at the time of the ASTMH meeting.

2106

SEASONAL MALARIA CHEMOPREVENTION, AN EFFECTIVE INTERVENTION FOR REDUCING MALARIA MORBIDITY AND MORTALITY

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Malaria remains the leading cause of morbidity (43.4% of reasons for consultation in 2016) and mortality (21.8% of deaths in 2016) in Burkina Faso; the heaviest burden being amongst children under 5 and pregnant women. Since 2013, Jhpiego, has implemented the Improving Malaria Care project (IMC) funded by PMI, in collaboration with the National Malaria Control Program of Burkina Faso. In 2017, IMC supported the implementation of the Seasonal Malaria Chemoprevention (SMC) campaign in Boromo and Dano health districts, as part of a larger national campaign carried out in 59 districts. SMC consists of administration of up to 4 monthly doses of a full treatment course of co-packaged Amodiaquine, Sulfadoxine + Pyrimethamine to all eligible children aged 3-59 months during the high malaria transmission season. The objective is to maintain therapeutic antimalarial medicine concentrations in the blood during the period of greatest risk. There were 58,246 children treated in the Boromo Health District and 50,007 at Dano during the 2017 campaign, representing 97.3% of the target population. IMC for its part in the campaign covered 3.6% of all children in the country. At Boromo, between 2016 and 2017, there was a decrease in cases of severe malaria by 16.6% (3900 to 3252) in the general population and by 25.4% (2057 to 1535) in children under 5. The number of malaria deaths in children under 5 also declined by 64.4% (45 to 16) between 2016 and 2017. In Dano, a reduction in severe malaria cases of 5.62% (4392 to 4145) and 26.4% reduction in deaths (53 to 39) was observed among children under 5 during the same period. Key challenges were: i) inability to conduct rapid assessment to measure coverage, ii) insufficient number of health staff for community distributors' supervision area, iii) access to some villages due to the rainy season iv) unknown adherence to drug administration during 2nd and 3rd doses. SMC appears to be an effective strategy in these districts for the reduction of cases of severe malaria-related illness and deaths in children under the age of 5. To better organize future SMC campaigns, the challenges identified during this campaign should be addressed.

2107

NEAR REAL-TIME REPORTING THROUGH DHIS2 TO DRIVE EFFICIENCY DURING LLIN MASS DISTRIBUTION CAMPAIGNS: LESSONS FROM KINSHASA, DRC, AND MALI

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Mass distribution campaigns of Long Lasting Insecticidal Nets (LLINs) are an intense effort and pose operational challenges, such as household (HH) registration for correct LLIN allocation and management of stock balances between LLIN needs and quantities distributed. In order to overcome these challenges, Population Services International (PSI) developed and used an innovative monitoring tool in DHIS2 to collect data on two critical phases of the LLIN campaign: allocation of nets during registration and their distribution. PSI piloted the use of the DHIS2 LLIN Distribution monitoring tool during the LLIN mass campaign in Kinshasa, DRC, in October 2016

and in Kayes region, Mali, in December 2017. A total of 4,964,938 and 1,437,111 nets were distributed and tracked in DHIS2 in almost real time in Kinshasa province and Kayes region, respectively. Key performance indicators monitored in DHIS2 include: number of HHs by distribution site and size, number of LLINs allocated by distribution site, number of LLINs received by distribution site, number of LLINs distributed by distribution site, and percentage of LLINs distributed out of LLINs received by distribution site. This innovation in the collection and management of LLIN distribution data reduces the long duration of data compilation and validation and improves the efficiency and effectiveness in activity monitoring, as well as stock management. Rapid access to information by all health authorities involved in the campaigns facilitated quick detection of inconsistencies and timely decision-making. In terms of challenges, the issue of poor or unstable telephone network coverage and lack of power supply in very remote areas have sometimes affected data entry and data syncing with DHIS2. The benefits of this innovative approach overcame these challenges and, as advocated by Dr. Joris Likwela Losimba, NMCP Director in DRC, the use of the DHIS2 LLIN Distribution tool is an excellent way of managing future mass campaigns. When used within the national management information system, it also contributes to strengthening the health system and can be used for other related areas.

2108

THE PREDICTORS OF HEALTH WORKER PRACTICES IN LOW- AND MIDDLE-INCOME COUNTRIES: A SYSTEMATIC REVIEW

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Health worker (HW) performance in low- and middle-income countries (LMICs) is often inadequate. Understanding the factors that influence HW performance is useful for anticipating where there might be quality problems and designing studies to test strategies to improve performance. To identify contextual and methodological predictors of HW practice outcomes expressed as a percentage (eg, % of patients treated correctly), we used random-effects linear regression modeling to analyze pre-intervention results from a systematic review on improving HW performance in LMICs. We searched 47 databases for published studies and 52 document inventories for unpublished studies up to early-2016. We screened 209,887 citations and identified 322 eligible studies for this analysis. These studies included 1,938 measures of HW practices. Mean performance was low, at 40.1% across all outcomes. The final model, which included all of the following factors, found that for every $1000 increment in gross national income, performance was 1.7 percentage-points (%-points) lower ($p = 0.02$). Performance in Europe and the Middle East was 10.3 %-points higher than in Asia ($p = 0.04$), with no differences for other continents. Performance in private health facilities was 8.3 %-points lower than in public facilities ($p = 0.03$). Compared to practice outcomes for multiple health conditions (eg, % of all patients receiving injections), performance was lower for specific acute and chronic diseases (–7.6 and –8.8 %-points, respectively, $p < 0.02$), but not for pregnancy. Compared to treatment outcomes, performance was 9–11 %-points lower for patient assessment, counseling, referral, and documentation ($p < 0.03$). Performance was 8.2 %-points lower in studies using patient interviews ($p = 0.005$), with no differences for other methods (eg, chart review). We found no differences between HW types and no evidence of a secular trend from 1990–2015. Selection bias was an important potential limitation. In conclusion, many factors were associated with HW practices. The results could help target quality improvement efforts and inform research on improving HW performance in LMICs.

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EQUITABLE BUT NOT PRO-POOR CHANGE IN MATERNAL AND NEWBORN HEALTH

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Health disparities persist in maternal and newborn health (MNH) with poorer families receiving less and lower quality care than wealthier families. To close this gap, many large-scale programmes have focused on pro-poor approaches to providing care. We present population level evidence on the equity of changes in MNH care from three high mortality settings where large-scale, community-based interventions were put in place: Gombe State in north-east Nigeria, Ethiopia, and the State of Uttar Pradesh in India. In each setting household surveys were carried out in 2012 amongst 7000, 3000 and 8500 women respectively, with follow-up data collection in 2015. The same survey methods were applied each time. Principle components analysis was used to determine socio-economic status (SES). Nine common indicators were identified as being targeted across the settings: four contacts (antenatal, delivery and postnatal care for mothers and newborns) and five interventions (handwashing and use of gloves by birth attendants, and clean cord care, early breastfeeding and thermal care of the newborn). At baseline, coverage was inequitable for all indicators with the poorest families consistently having lowest coverage. Different indicators showed improvement between settings but where changes did occur they were exclusively equitable in that coverage increased for all SES groups equally. However, because of the pre-existing inequity this meant that changes were not pro-poor and coverage remained unanimously inequitable. For example, handwashing with soap by delivery attendants increased between 2012-2015 from 55% to 81% in Gombe State (p-value <0.001). In 2012 the odds ratio of coverage between richest and poorest was 1.5 (95% confidence interval 1.2-1.9) and in 2015 it was again 1.5 (1.3-1.7). And the p-value for the likelihood ratio test for the interaction between time and SES was 0.84 indicating no evidence that indicator coverage between 2012 and 2015 differed by SES. Results across these settings are examined with reflection on findings from other disease control initiatives that have been designed for pro-poor change.

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THE RELATIVE IMPORTANCE OF FINANCIAL AND NON-FINANCIAL INCENTIVES FOR COMMUNITY HEALTH WORKERS: EVIDENCE FROM A DISCRETE CHOICE EXPERIMENT IN WESTERN KENYA

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Integrated community case management programs are increasingly being scaled up across sub-Saharan Africa to expand care for common illnesses in areas where access to formal health facilities is limited. These programs rely on trained community health workers (CHWs) to help diagnose and treat illnesses such as malaria, pneumonia and diarrhea. Most CHWs do not receive a salary, but are instead offered a mix of financial and non-financial incentives for participation in these programs. There is little evidence, however, on CHWs' preferred combination of incentives, which is key to ensuring high levels of CHW performance as well as improved retention in their role. We conducted a discrete choice experiment with 199 CHWs in Western Kenya to investigate their preferences for different types of incentives. In the experiment, respondents made a series of choices between two hypothetical CHW positions with varying levels of mobile phone airtime, training, transport reimbursement, and levels of

community and health facility staff appreciation for their work; CHWs' choices reflect how much they value these different job characteristics relative to each other. We found that CHWs most preferred job characteristic was high levels of community appreciation for their work (mean β=1.74, P<0.01). For CHWs, a high level of community appreciation was approximately equivalent to receiving a 2000 Kenya Shillings (~US $20) monthly transport stipend (mean β=1.67, P<0.01) and about twice as valuable as appreciation from the health facility staff (mean β=0.90, P<0.01). Furthermore, although CHWs generally preferred more mobile phone airtime and more frequent trainings, these were less preferred incentives compared to either high levels of community appreciation or a transport reimbursement. Our results demonstrate that CHWs value both financial and non-financial incentives. Moreover our findings suggest that investing in efforts to improve community members' knowledge and recognition of CHWs' contribution to community health may have a significant impact on CHWs' motivation and retention in their role.

2111

IMPACT OF ONE-ON-ONE DETAILING AND MASS MEDIA ON COMMUNITY USE OF ORS AND ZINC DURING A SCALE-UP PROGRAM IN GUJARAT AND UTTAR PRADESH

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The Clinton Health Access Initiative implemented a comprehensive, large-scale program to increase use of ORS and zinc as a treatment for diarrhea in India. The program included detailing private rural health care providers (RHCPs, a major source of care in India) to promote, share information about, and offer direct sales of ORS and zinc. A mass media campaign was also run concurrently to improve caregiver knowledge and demand for ORS and zinc. Using a difference-in-difference quasi-experimental approach we evaluate the potential impact of the detailing activities in Uttar Pradesh and Gujarat, as well as any additional effect of the mass media campaign on the likelihood of a child with diarrhea being treated with ORS or combined ORS and zinc. Twenty two of 31 districts in Gujarat and 39 of 75 districts in UP were purposely assigned to receive the detailing intervention, and the media campaign reached all districts. Cross-sectional surveys were conducted in detailed and non-detailed districts at two time points. Multilevel multiple logistic regression models were constructed and adjusted for relevant covariates and change over time. A total of 1,828 under-5 diarrhea cases in Gujarat and 4,624 in UP were captured and analyzed. Irrespective of media exposure, the odds of being treated with ORS were significantly higher for children living in detailed districts than those in non-detailed districts (Gujarat aOR 4.05, 95% CI 2.9, 5.5; UP aOR 1.61 95% CI 1.0, 2.6); in Gujarat, the odds of receiving both ORS and zinc were significantly greater for children in detailed districts when compared to those in non-detailed districts (aOR 4.80, 95% CI 1.1, 20.1). For children whose caregiver was exposed to the media campaign, the odds of receiving ORS were higher for children in detailed districts as compared to those in non-detailed districts (Gujarat aOR 5.75, 95% CI 3.3, 9.9; UP aOR 1.77, 95% CI 1.6, 2.0). This highlights the benefit of program design that addresses barriers on both the demand and supply side simultaneously, as the media campaign was found to have an amplifying effect on the positive impact of detailing activities when implemented together.

2112

HOW GROUP-BASED CARDIOVASCULAR HEALTH EDUCATION AFFECTS TREATMENT ADHERENCE AND BLOOD PRESSURE CONTROL AMONG INSURED HYPERTENSIVE NIGERIANS: A PRE-TEST, POST-TEST STUDY

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Cardiovascular diseases (CVD) are a major cause of death globally. Hypertension is a significant risk factor for CVD. To limit CVD access to affordable care is important. In sub Saharan Africa, access to affordable hypertension care is increasing through health insurance. Yet, hypertension treatment outcomes often remain poor due to poor adherence. Patient-centered educational tools can redress this. Using pre-test/post-test design, we investigated the effects of a structured cardiovascular health education program (CHEP) on treatment adherence, blood pressure (BP) control and body mass index (BMI) among Nigerian hypertensive patients who received guideline-based care in a rural primary care facility, in the context of community based health insurance. Participants were 149 insured patients with uncontrolled BP and/or poor self-reported medication adherence after 12 months of guideline-based care. All patients got 3 group-based educational sessions and usual primary care over 6 months. We evaluated changes in self-reported adherence to prescribed medications and behavioral advice (primary outcomes); systolic BP (SBP) and/or diastolic BP (DBP) and BMI (secondary outcomes); and beliefs about hypertension and medications (exploratory outcomes). Outcomes were analyzed with descriptive statistics and regression analysis. 140 patients completed the study (94%). At 6 months, more participants reported high adherence to medications and behavioral advice than at baseline: respectively, 101 (72%) versus 70 (50%), ($p < 0.001$) and 126 (90%) versus 106 (76%), ($p < 0.001$). Participants with controlled BP doubled from 34 (24%) to 65 (46%), ($p = 0.001$). The median SBP and DBP decreased from 129.0 to 122.0 mmHg, ($p = 0.002$) and from 80.0 to 73.5 mmHg, ($p < 0.001$), respectively. BMI did not change ($p = 0.444$). Improved medication adherence was associated with a decrease in medication concerns ($p = 0.045$) and improved medication self-efficacy ($p < 0.001$). By positively influencing patient perceptions of medications, CHEP strengthened medication adherence and, consequently, BP reduction among insured hypertensive Nigerians. This educational approach can support cardiovascular disease prevention programs for Africa's growing hypertensive population.

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EVALUATION OF ANTIMICROBIAL PRESCRIBING PATTERNS AT A REFERRAL HOSPITAL IN RURAL TANZANIA

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Bacterial resistance to antibiotics is a growing concern worldwide, with Sub-Saharan Africa being no exception. It is unclear what role inpatient antibiotic use plays in this growing problem, as antibiotic stewardship efforts are largely absent in this region and prescribing patterns are not well documented. We evaluated the current antibiotic prescribing patterns and patient access to antibiotic therapy at Mbeya Zonal Referral Hospital in rural Tanzania. We chronologically reviewed 155 unique inpatient adult medical records from admissions in January-February 2018, of which 100 utilized inpatient antibiotic therapy. Metrics for evaluation included documentation of infectious diagnoses, diagnostics obtained, choice and duration of antibiotic therapy administered, antibiotic course completion, patient health insurance status, and antibiotic cost data based on patients' method of payment. Data were summarized using descriptive statistics. Within the 100 patient records involving antibiotic therapy, 171 antibiotic courses were noted, of which 153 were evaluable. Patients received the entire prescribed course of antibiotic therapy 43.8% (67/153) of the time. Ceftriaxone and metronidazole, the most commonly used antibiotics, were administered in 40.4% (69/171) and 24.0% (41/171) of courses, respectively. Among patients who received antibiotic therapy, 38% (38/100) had health insurance. The most common documented infectious diagnosis was unspecified pneumonia. Of patients who received antibiotics, 27% (27/100) had no infectious diagnosis documented and 18% (18/100) had a culture of any type obtained. In summary, inpatient antibiotic utilization patterns at a teaching hospital in rural Tanzania are frequently suboptimal. Therapy is often abbreviated, documentation is sometimes incomplete, and diagnostic confirmation is frequently not performed, all of which may contribute to increasing antibiotic resistance. Lack of insurance coverage and thus cost of therapy is likely a significant contributor to this problem. These data may be used to enhance stewardship efforts and guide hospital policy in the future.

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ACCESS TO QUALITY CARE FOR PNEUMONIA, DIARRHEA, AND MALARIA AMONG CHILDREN UNDER FIVE IN RURAL MALI

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Rural Mali has one of the highest rates of child mortality globally; however, provider quality and access to care for common childhood illnesses in this setting have not been well studied. Using baseline data from a cluster-randomized trial, we conducted a comprehensive analysis of care seeking and treatment patterns for pneumonia, diarrhea, and malaria. From December 2016 to February 2017, mothers of 19,556 children under age five completed a household survey. Respondents provided information about whether children experienced diarrhea, fever, and/or cough in the preceding two weeks, care sought, and treatment received. We used mixed-effects regression models to assess odds of illness, likelihood of attending any care, care with a trained provider, and treatment according to protocol [oral rehydration therapy (ORT) and zinc for diarrhea without bloody stools, antimalarials for presumptive malaria (artemisinin combination therapy, quinine, artusenate, arthemeter), amoxicillin for uncomplicated pneumonia] adjusted for distance to nearest primary health center, child age/sex, household wealth, and maternal education. Among all children, 13.7% reported diarrhea without bloody stools, 3.4% reported fever and received a malaria rapid diagnostic test, and 4.9% reported cough with fast breathing. Of these 3,132 children, 60.3% accessed any provider; 13.7% attended a trained provider. Overall, 12.9% of children received correct treatment. Correct treatment rates were lowest for diarrhea without bloody stools (1.3%) and highest for presumptive malaria (49.0%). In adjusted models, attending a trained provider significantly predicted receipt of ORT and zinc (AOR=15.95, 95% CI 6.40 - 39.75) and amoxicillin (AOR=2.71, 95% CI 1.48 – 4.93), but not correct treatment for presumptive malaria. However, distance to nearest primary health facility was associated with receipt of antimalarials (AOR=1.58, 95% CI 1.19 – 2.09). Correct treatment of diarrhea, malaria, and pneumonia is low even among trained providers in rural Mali. Interventions should seek to improve quality among all providers while increasing access to trained providers.

2115

NOTHING IN BIOLOGY MAKES SENSE EXCEPT IN THE LIGHT OF EVOLUTION: AN ALVEOLATE-CONSERVED MECHANISM IS IMPLICATED IN RHOPTRY SECRETION IN APICOMPLEXA

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Host-cell invasion by apicomplexan parasites is governed by coordinated and sequential exocytosis of specialized apical organelles: micronemes and rhoptries. Despite decades of research on parasite invasion our knowledge on membrane fusion events and exocytosis mechanisms of these organelles remains largely unknown. Together with ciliates and dinoflagellates, apicomplexan parasites belong to the *Alveolata* superphylum. Although morphologically different, *alveolates* share several features, including the presence of secretory organelles. Previous studies in *Paramecium tetraurelia* showed that their defensive extrusive organelles, termed trichocysts, have a characteristic arrangement of particles on the membrane over the docking sites: a ring of intramembranous particles with a "rosette" of 8-10 particles in the center. Rosette assembly is required for membranes fusion and, was shown to be essential for trichocyst secretion upon stimulation. Remarkably, a similar structure was observed at the apex of several apicomplexan parasites, which has remained uncharacterized to date. The dissection of mutants lacking the rosette and defective for trichocyst exocytosis (called ND for "non-discharge") prompted the identification of essential components of the trichocyst-membrane fusion machinery in *Paramecium*. We have identified, localized and characterized the orthologs of nd6 and nd9 in the apicomplexan model, *Toxoplasma gondii*. While TgND6 localizes to the apical pole of the parasites, TgND9 is located in the cytoplasm. Conditional depletion of TgND6 and TgND9 didn't affect parasites intracellular replication, egress or motility. Importantly, micronemes secretion also remains unaffected, but depletion of TgND6 or TgND9 abolishes rhoptry secretion and blocks the host-cell invasion process. This study identifies novel proteins essential for rhoptries secretion, supports our hypothesis of an Alveolate conserved mechanism for organelles-dependent membrane fusion events, and finally, offers a fresh perspective to explore such a fundamental and yet unclear basic process.

2116

THE GREAT ESCAPE: INVESTIGATING THE ROLE OF SERA6 IN MALARIA PARASITE EGRESS FROM THE RED BLOOD CELL

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Malaria is a devastating parasitic disease. Resistance of the most dangerous causative agent, *Plasmodium falciparum*, to frontline antimalarial drugs necessitates an improved understanding of parasite biology to facilitate new approaches to disease control or eradication. Malaria pathogenesis arises from replication of asexual blood stages in the human host. Merozoites invade erythrocytes, replicate within a parasitophorous vacuole (PV) then subsequently burst out of and destroy the erythrocyte in a process called egress. The released merozoites immediately invade fresh erythrocytes to repeat the cycle.

Malaria parasite egress is a key biological pathway with much potential for identification of novel drug targets. Work over several years has shown that egress is tightly controlled by a parasite enzyme cascade in which activation of the single cGMP-dependent protein kinase PKG triggers the discharge of a parasite serine protease called SUB1 into the lumen of the PV. SUB1 in turn proteolytically processes a PV-located cysteine protease called SERA6 in *P. falciparum*. Whilst SUB1 is required for all the morphological changes that lead up to egress, including PV membrane rupture, SERA6 is essential only for erythrocyte membrane rupture, the final step in egress. However, the exact molecular mechanisms underlying the role of SERA6 in egress remain a mystery. Here we present recent insights into the regulation of SERA6 activity and how the enzymatic function of SERA6 leads to cleavage of erythrocyte cytoskeletal components, resulting in its collapse and eventual lysis of the infected erythrocyte.

2117

STUCK ON YOU: THE PLASMODIUM BASAL COMPLEX IS REQUIRED FOR PROPER DAUGHTER CELL SEGMENTATION

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During the blood stage of malaria infection, *Plasmodium* parasites must asexually divide via schizogony during each 48-hour life cycle. Over the course of schizogony, a single parasite produces 20 to 30 daughter cells within a common cytoplasm that form into invasive merozoites by segmentation. The basal complex is hypothesized to be important for segmentation by 1) forming a contractile ring around nascent daughter cells, 2) connecting the inner membrane complex to the parasite plasma membrane, and 3) mediating membrane fusion to "clip" daughter cells from the residual body, completing cytokinesis. We identified a *Plasmodium* protein with no previously known function that is a novel basal complex protein, PF3D7_0407800, which we named coordinator of nascen[[Unsupported Character - Codename]]t cell detachment, or PfCINCH. Using CRISPR-Cas9 we generated a parasite line where PfCINCH can be inducibly knocked down with the TetR-DOZI system and determined that PfCINCH is required for asexual replication. By time-lapse microscopy, super-resolution fluorescence microscopy, and transmission electron microscopy we observed that parasites deficient in PfCINCH mature normally throughout the majority of the asexual life cycle and even egress at 48 hours post invasion; however, daughter cells contain multiple nuclei and sets of organelles in a single parasite plasma membrane. Additionally, by focused ion beam - scanning electron microscopy (FIB-SEM) we elucidated the 3D structure of these mutants. Taken together, this suggests that the *Plasmodium* basal complex is required for defining the boundaries of nascent daughter cells in schizogony. Finally, by immunoprecipitating PfCINCH complexes we have identified several novel members of the *Plasmodium* basal complex. Therefore, in this work we have applied several state-of-the-art techniques to describe the function of a previously uncharacterized protein and shed light on the protein components of *Plasmodium* segmentation machinery.

2118

PATCH-CLAMP OF THE *P. FALCIPARUM* DIGESTIVE VACUOLE IDENTIFIES A NOVEL CHANNEL AND ANTIMALARIAL TARGET

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The malaria parasite digestive vacuole (DV) is required for hemoglobin digestion and is the target of several antimalarial drugs including chloroquine. The DV is presumed to have multiple transmembrane channels and pumps to maintain an acidic internal pH, export amino acids, and mediate flux of antimalarial drugs. Study of these transporters has been limited to indirect transport measurements and has been complicated by imperfect DV enrichment from parasite lysates. We have now addressed these limitations with cell-attached patch-clamp of individual, intact DV membranes from *Plasmodium falciparum*. High resistance seals (> 10 Gohms) were obtained after optimizing pipette glass composition and geometry. A large-conductance channel (450 pS in 320 mosm salt solutions) was the primary channel seen, but additional smaller conductance channels have also been detected. The primary channel exhibited weak voltage-dependence at large imposed membrane potentials as well as unusual gating that suggests a two-pore configuration on the DV membrane. Progress on identification of this channel's molecular basis and the effects of antimalarial drugs and nutrients on channel activity will also be described. These findings indicate that the DV membrane has robust transport activity with features distinct from those of the mammalian lysosome. Identification and molecular characterization of transporters on the DV membrane will provide foundational insights into vacuolar biology, clarify the resistance mechanisms for several antimalarial drugs, and guide development of new therapies for malaria.

2119

ANTIBODY RESPONSES AGAINST THE CANDIDATE VACCINE ANTIGENS *OV*-103 AND *OV*-RAL-2 ARE ASSOCIATED WITH PROTECTIVE IMMUNITY IN BOTH MICE AND HUMANS TO *ONCHOCERCA VOLVULUS* INFECTIVE LARVAE

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Onchocerca volvulus, a filarial nematode is an etiologic agent of river blindness that infects approximately 17 million people, mostly in Africa. The current strategy for elimination of *O. volvulus* focuses on controlling transmission through ivermectin-based mass drug administration (MDA) programs. Due to several limiting factors such as potential ivermectin resistance, the lack of macrofilaricidal activity by ivermectin and the prolonged time line (>20 years) needed for transmission interruption, there is a growing concern that elimination of onchocerciasis cannot be achieved solely through the current strategy. Additional tools are critically needed including the use of a prophylactic vaccine. Presently *Ov*-103 and *Ov*-RAL-2 are the most promising vaccine candidates against *O. volvulus* infection. Protection induced by immunization of mice with the alum adjuvanted *Ov*-103 *or Ov*-RAL-2 vaccines appeared to be

antibody dependent as AID$^{-/-}$ mice that could not mount antigen-specific IgG1 antibody response were not protected. Moreover, when cells were blocked from entering the parasite microenvironment in vaccinated mice, killing of larvae did not occur; indicating that vaccine induced protective immunity was dependent on IgG and cell contact. To assess the association between antigen-specific antibody responses and anti-larvae immunity in humans, we analyzed the presence of anti-*Ov*-103 and *Ov*-RAL-2 cytophilic antibody responses (IgG1 and IgG3) in individuals classified as putatively immune and in infected individuals who developed concomitant immunity with age. In both groups approximately 90% of the individuals had elevated IgG1 and IgG3 responses to *Ov*-103 and *Ov*-RAL-2. Moreover, human monospecific anti-*Ov*-103 antibodies but not anti-*Ov*-RAL-2 inhibited significantly the molting of L3 larvae *in vitro* by 46% in the presence of naïve human neutrophils, while both anti-*Ov*-103 and anti-*Ov*-RAL-2 antibodies significantly inhibited the molting by 70-80% when cultured in the presence of naive human monocytes. We have also found that sera from both putatively immune and infected individuals had significantly high levels of chemokines such as KC, IP-10, MCP-1 and MIP-1β that are known to be associated with neutrophil and monocyte recruitment. The vaccines characterized in this study have the potential of reducing infection and thus morbidity associated with onchocerciasis in humans. If further developed for human use the stimulation of cytophilic antibodies that function in antibody-dependent cellular cytotoxicity would be of importance in such a vaccine.

2120

THE NOTCH SIGNALING PATHWAY CONTROLS BASOPHIL RESPONSES DURING HELMINTH-INDUCED TYPE 2 INFLAMMATION

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Type 2 inflammation is characterized by production of the cytokines IL-4, IL-5 and IL-13 and promotes clearance of gastrointestinal helminths, which infect over 2 billion people worldwide. Basophils are innate immune cells that accumulate in the intestine during infection with the helminth *Trichuris muris*, and are known to be potent producers of inflammatory mediators. However, the molecular mechanisms that control basophil function and gene expression during helminth-induced type 2 inflammation remain unclear. We show that during *T. muris* infection, basophils upregulated components of the Notch signaling pathway, which regulates gene expression programs during development and inflammation. *In vitro*, Notch inhibition abrogated basophil cytokine production by directly targeting *Il4* and *Il6* transcripts. Transcriptional profiling of Notch-deficient basophils revealed that Notch directs basophil responsiveness to inflammatory cues and effector gene expression. *In vivo*, Notch-deficient basophils did not localize effectively within the intestinal lamina propria during infection, and displayed decreased interaction with CD4$^+$ cells. Consequently, mice lacking basophil-intrinsic Notch signaling had impaired worm clearance and decreased intestinal type 2 inflammation following *T. muris* infection. These findings demonstrate that Notch regulates basophil gene expression and effector function during helminth-induced type 2 inflammation, with repercussions for our understanding of type 2 immunity, for development of effective therapeutics aimed at this arm of host defense.

2121

ALLERGIC INFLAMMATION INHIBITS HELMINTH LARVAL DEVELOPMENT AND CONTROLS PARASITE BURDEN

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Although chronic helminth infection has been associated with diminished allergic reactivity (hygiene hypothesis), the impact of pre-existing allergic sensitization on altering the outcome of helminth infection is less well-studied. Having previously demonstrated an augmented Th2-dominated parasite specific CD4+ response in the context of human helminth infection with coincident house dust mite (HDM) allergy, we sought to identify the mechanism driving this response and to understand how allergic sensitization may influence parasite burden at the site of inflammation. Using a murine model of HDM-induced allergic asthma and concomitant *Ascaris* spp. infection, our data show that HDM sensitization prior to infection with *Ascaris* induces a robust Th2 inflammation in the lung characterized by an increase in both IL-5/IL-13 producing type 2 innate lymphoid cells ($17.9x10^3$ cells vs $2.4x10^3$ cells, $p<0.01$) and eosinophils ($2.92x10^5$ cells vs $0.02x10^5$ cells, $p<0.001$) when compared to non-allergic infected animals. This allergen-driven inflammation in the lungs leads to an IL-4-rich environment (8.39 pg/mL vs 3.33 pg/mL, p=0.006) that drives the differentiation of lung macrophages towards the M2 phenotype expressing Arginase-1 (17.6-fold change, p=0.0142), but not iNOS. When the *Ascaris* larvae migrate from the circulation to the lung tissue in their quest to reach the airways in these HDM-sensitized mice, the Th2 inflammation leads to a 72% marked reduction in the number (20±9 larvae vs. 63±50 larvae; p=0.0443) and in the development of lung-stage *Ascaris* larvae (size 24816 μM^2 vs 58170 μM^2, $p<0.001$) when compared with non-allergic infected mice. When eosinophil deficient (ΔdblGATA) mice are infected with *Ascaris* following HDM-induced asthma, the numbers of larvae are no longer reduced (p=0.1800), nor is there any alternation in larval development when compared to ΔdblGATA non-allergic infected mice (p=0.08). Taken together, our data suggest that allergic sensitization coincident with helminth infection drives an eosinophil-rich pulmonary Th2 response that limit helminth parasite numbers and also directly hinder their development.

2122

B CELLS ARE IMPORTANT DURING EARLY INFECTION IN SUPPORTING TH2 TYPE IMMUNE RESPONSES TO *TRICHURIS MURIS* INFECTION

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How B cells contribute to protective immunity against parasitic nematodes remains unclear, with their importance as regulatory cells under-explored. This study therefore investigates the role of B cells and antibodies in immunity to *Trichuris muris* (*T. muris*) infection using anti-CD20 mAb (Genentech) to deplete B cells prior to and post *T. muris* infection in both C57BL/6 and BALB/c strains of mouse. C57BL/6 mice receiving anti-CD20 treatment prior to infection failed to expel *T. muris* by d35 post infection (p.i.) and Th2 type cytokines produced by mesenteric lymph nodes cells were significantly lower than produced by cells from isotype control treated mice; in contrast, BALB/c mice were still able to expel the worms in the absence of B cells. To explore whether the susceptibility of C57BL/6 mice after anti-CD20 mAb treatment is due to the lack of B cells as antibody producers or as regulatory cells, we blocked IFN-γ using anti-IFN-γ mAb post B cell depletion. Interestingly, our data show that

worm expulsion now occurred by day 21 p.i. and the mice produced more *T. muris* specific IL-13, in the absence of *T. muris* specific antibodies. To investigate whether B cells are important in initiating or in maintaining Th2 type immune responses, we depleted B cells from day 14 p.i., culling mice at day 35 p.i. Surprisingly, removing B cells after the first two weeks post infection failed to prevent worm expulsion, with the balance of Th1/Th2 cytokines remaining the same to as seen in isotype control treated mice. Collectively, this study suggests that (a) the importance of B cells in mediating worm expulsion during *T. muris* primary infection varies according to genetic background and (b) the important role played by the B cell in resistance to infection is as a regulatory cell, acting to support Th2 type immune responses during the first two weeks post infection.

2123

MALARIA PARASITE TRANSLOCON STRUCTURE AND MECHANISM OF EFFECTOR EXPORT

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The putative Plasmodium Translocon of Exported Proteins (PTEX) is essential for transport of malarial effector proteins across a parasite-encasing vacuolar membrane into host erythrocytes, but the mechanism of this process remains unknown. Here we show PTEX is a bona fide translocon by determining near-atomic resolution cryoEM structures of the endogenous PTEX core complex of EXP2, PTEX150 and HSP101, isolated from Plasmodium falciparum in the engaged and resetting states of endogenous cargo translocation with CRISPR/Cas9-engineered epitope tags. Our work reveals the mechanism of P. falciparum effector export, enabling structure-based design of drugs targeting this unique translocon.

2124

PLASMODIUM AND HOST MICROBIOTA: FRIEND OR FOE?

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In malaria endemic areas, *Plasmodium*, the causative agent of malaria, and bacterial co-infections are highly concurrent, often with fatal outcomes like cerebral malaria(CM), acute lung injury (ALI) and severe anemia. Recently, it has been shown in patients from Sub-Saharan Africa that *Plasmodium* infection predisposes to high bacteremia. However, the causal link between *Plasmodium* infection and increased bacteremia and the putative mechanisms involved behind severe malaria associated pathology is currently unknown. In the present study, using different models of rodent malaria we show that infection with *Plasmodium* parasites causes an increase in the bacterial diversity in the lung specifically of mice that die of ALI. Moreover, we show by genetic knockout and complementation studies that this increase in the tissue specific bacterial load was dependent on parasite sequestration in the lung. Metagenomics analysis of the bacterial community showed marked alterations in the lung microbiome of mice that die of ALI. We report here that this increase in the bacterial burden is not associated with increased intestinal permeability or with histological alterations in the intestinal barrier. These alterations in the lung microbiome was significantly correlated with high levels of alveolar inflammation (IL-6, IL-10, IFN-γ and TNF-α). To definitively assess the impact of microbiota changes during *Plasmodium* infections on the clinical outcome of infection, germ free mice infected with *Plasmodium* strains causing lung injury had a significant delay in death (with majority dying with hyperparasitemia) compared to specific pathogen free mice. Interestingly, targeting specific microbiota of the lung with antibiotics prevented malaria associated ALI in mice and prolonged survival. All these results suggest that *Plasmodium* blood stage-mediated immunomodulation may result in aberrant expansion of bacteria in the lungs during malaria infection and also pave the way for interventional studies for malaria associated ALI.

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