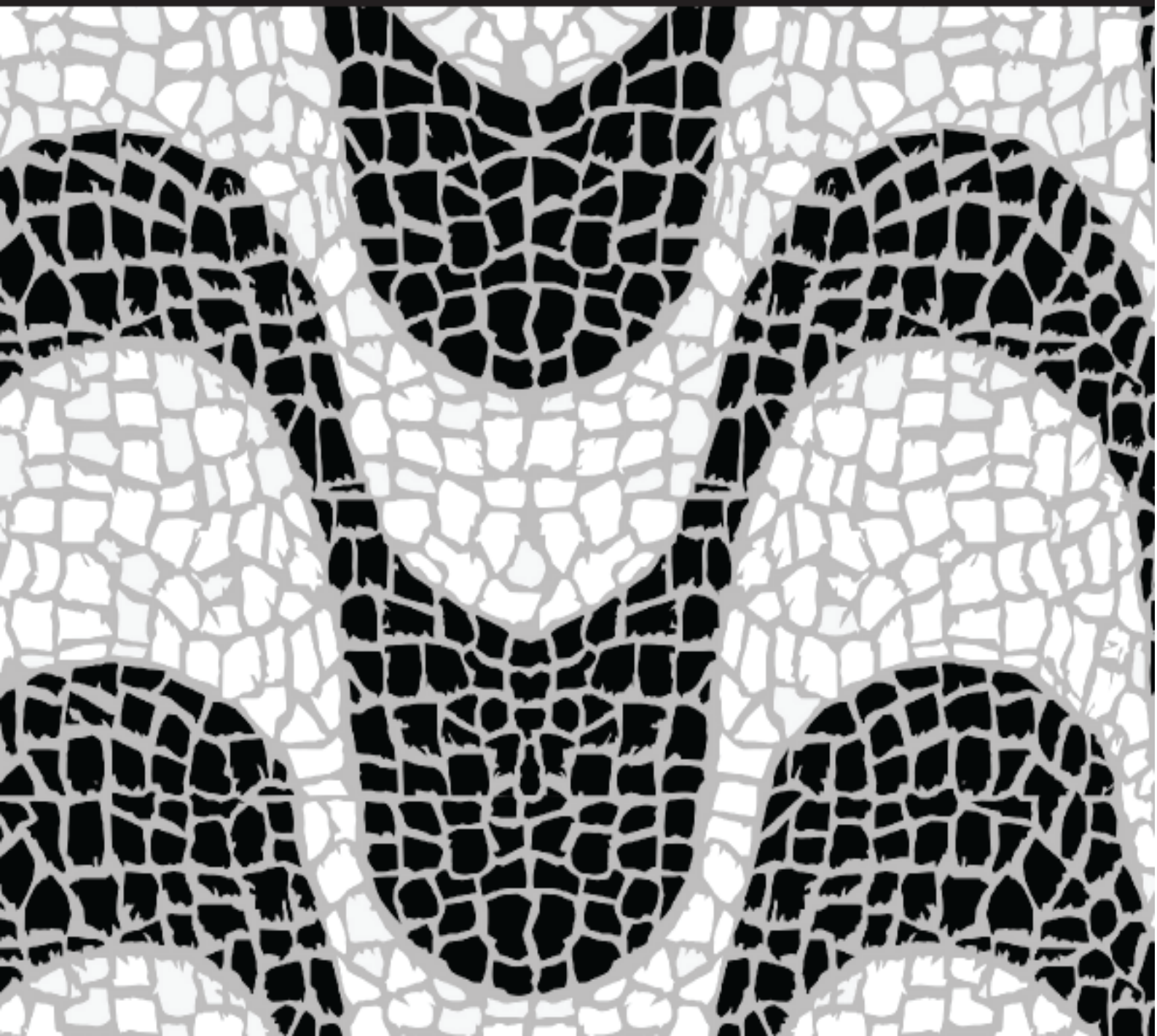
15 INTERNATIONAL SYMPOSIUM on SCHISTOSOMIASIS

01 a 03 de August de 2018

Rio Othon Palace - Copacabana

Rio de Janeiro - Brazil



CONFERENCES

Conference 1

Ministry of Health Plan for Schistosomiasis Elimination in Brazil

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Introduction: Schistosomiasis is a prevalent disease in Brazil and an estimated number of 1.5 million people would be infected in the country. The areas where the transmission is more frequent is among the north-eastern region and in the states of Minas Gerais and Espírito Santo in the south east region. Ministry of Health undertook the task of eliminating the schistosomiasis as a public health problem according to Plan of Action for the elimination of neglected infectious diseases and post-elimination actions 2016/2022 (55th CD 68th Session of the Regional Committee of WHO for the Americas) and will promote in partnership with states and municipalities an action plan for confronting the disease during the period 2019-2022. The aim of this plan is to intensify the actions towards the elimination of the schistosomiasis in the country and controlling the helminthiasis soil-transmitted in these areas as well. **Methods:** This plan is being elaborated by the three levels of management of SUS, Ministry of Health, State and Municipality Secretaries, intersectorial focusing. The selection of the municipalities to be contemplated by the plan has its base on the epidemiological data available in the official information systems, the analysis of the muncipalities' sanitation conditions and the results of the National Survey on Prevalence of Schistosomiasis mansoni and Geo-helminthes 2011-2015. The actions proposed will be submitted to validity in the SUS instances of agreement and the indicators and the aims will be defined as well as the ways of monitoring and evaluation of the results. **Results:** The general strategy lines of the action plan were defined at first. They are: 1) epidemiological surveillance strengthening; 2) enlargement of the access of the basic attention for the initial diagnosis and management of the clinic cases; 3) implementation of the sustainable measures for elimination and education in health and sanitation; 4) maping the areas of risk and surveillance of intermediate hosts; 5) foment to operational researches in areas of interest. Based on defined general lines, states and municipalities will be able to adapt the plan to the local reality. Approximately 250 priority municipalities were selected for the plan. **Conclusions:** It is expectable that this plan can focus this disease and it is prioritized among the SUS managers as well as intensify and strengthen the actions to face it and contribute to increase the access to the *Schistosoma mansoni* health's sufferers in the country.

Supported by Ministry of Health/Brazil, State and Municipal Health Secretariats

Conference 3

WHO recommendations for achieving elimination of schistosomiasis

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Schistosomiasis is endemic in 78 countries globally. Among them, 52 require preventive chemotherapy for an estimated number of 206.4 million people in 2016.

The regular treatment of affected population aims to reduce disease morbidity and transmission. Periodic treatment of at-risk populations procure immediate relief, cure symptoms and prevent infected people from developing severe, late-stage chronic disease.

Since the launch of the preventive chemotherapy strategy in 2006, millions of people have been treated in endemic areas resulting in a significant reduction of the prevalence of infection and of the burden of the disease. The number of people requiring PC decreased from 258.8 million in 2014 to 218.8 million in 2015 and 206.4 million in 2016.

However, mass treatment alone will not interrupt the transmission of the disease. Implementation of others strategies aiming to prevent reinfection and interrupt on longer term the transmission of the disease are required. This is the reason why WHO recommends comprehensive integrated strategies to control and eliminate schistosomiasis.

Integrated strategy mean combining preventive chemotherapy of at risk groups, access to safe water, improved sanitation, hygiene education, veterinary public health and snail control. Implementation of the others strategies will sustain the gains so far achieved and accelerate the progress toward the elimination of the disease.

Targeted mass treatment based on refinement of the distribution of the disease, and in particular in the transmission hots spots is essential and shall continue. Negotiations and creation of partnerships with other sectors need to be established for the improvement of WASH indicators and snail control through utilization of molluscicides or modification of the environment.

Improved, easy to use and more sensitive diagnostic tools are required for the monitoring and the surveillance of the disease at last stages.

Conference 4

Delivering integrated health solutions to fight schistosomiasis

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Introduction: Merck has a long-standing commitment towards improving the health of underserved populations: corporate responsibility has been an integral part of the Company's identity for 350 years. As a leading science and technology company, Merck continues playing a key role in tackling global health challenges through innovative top-quality health solutions. **Methods:** In 2017, Merck established the Global Health Institute highlighting the Company's continued emphasis on investment into solution developments for infectious diseases. The Institute aims to fulfill the Merck's mission to develop and provide access to transformative and integrated innovations (treatments, diagnostics, preventive measures, health system strengthening approaches) for the most vulnerable, children and women, suffering from schistosomiasis, malaria and microbial infections. The Institute operates as a social business enterprise and applies a model that synergizes internal expertise; it is based on public-private partnerships and innovative financial mechanisms to develop a sustainable portfolio of affordable products and services. **Results:** Beyond the Merck Praziquantel Donation Program, the 'One Merck for Schistosomiasis' program is implementing an integrated strategy that goes from R&D to Access. Amongst other projects, the program includes the development of a pediatric formulation of praziquantel via a consortium of partners, including Farmanguinhos in Brazil, to treat children (<6 years of age) – the program is currently preparing Phase III; the screening of Merck's compound library through a dedicated schistosomiasis drug discovery platform; and the development of highly sensitive schistosomiasis diagnostics in association with current efforts of the Bill and Melinda Gates Foundation. **Conclusions:** With these efforts and through the Institute, Merck wants to generate significant impact to strengthen control of infectious diseases as well as to contribute to elimination agendas.

Financially supported by Merck and selectively by grants from external funders.

Conference 5

Water and basic sanitation for health promotion and prevention of neglected diseases

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Concerns related to access to water and sanitation services have gained new momentum since the access to these services was designated as a dedicated goal in the sustainable development agenda. The scientific understanding of the effects of water and sanitation interventions on human health has evolved consistently over the preceding decades. There are currently a large number of published epidemiological studies on these relationships, which have been carried out with increasingly rigorous methodological approaches and data collection. Such studies cover different types of interventions (e.g. improved water quality, increased water quantity, improved sanitation, elimination of open defecation, improved hygiene through availability of water and soap), of health outcomes (e.g., diarrhoea, child and infant mortality, specific diseases) and of methodological approaches. Given the number of such studies, several systematic reviews and meta-analyses have emerged, summarizing the relationships between different environmental interventions and health outcomes. A recent study (Wolf et al., 2018) found that several types of interventions are associated with lower risk of diarrhoeal morbidity: household water treatment reduces diarrhoea risk by 61%; water of higher quality piped to premises by 75%; sanitation interventions by 25% and handwashing with soap by 30%. These studies have influenced policies at the global and national levels, such as the decision to promote household water treatment in the 2000's. As for parasitic diseases, there is also growing evidence of the role of water and sanitation interventions. For instance, Grimes et al. (2014) suggest through a meta-analysis that safe water supply is associated with lower odds of schistosomiasis (OR = 0.53), and that adequate sanitation is associated with lower odds of *Schistosoma mansoni* (OR = 0.59) and *Schistosoma haematobium* (OR = 0.69). Additional research is still needed to help understand the effects of improving services on more specific situations, such as comparing different levels of services and their impact on health outcomes; namely the water and sanitation ladders developed to monitor the SDGs. A better understanding is also required of the impacts of access to these services by specific groups and in specific settings, such as for homeless people, refugee camps, indigenous groups, women and girls, disabled persons and the elderly. Additionally, more evaluative research, assessing the effects of policies and programs, would benefit decision-making in the water and sanitation sector. Regarding the methods applied to understand the nexus between access to water and sanitation and health conditions, qualitative studies appear to have great potential to uncover key elements related to the acceptability and accessibility of services, which are pre-requisites to obtain the intended effects of interventions.

Finally, new approaches, such as the framework of the human rights to water and sanitation, once fully incorporated into the conception of the water and sanitation sector, have the potential to maximize the health benefits of related interventions.

Conference 6

Whole genome analysis of a schistosomiasis-transmitting freshwater snail

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Introduction: At the turn of the millennium, investigations of *Biomphalaria glabrata* as snail intermediate host responsible for transmission of *Schistosoma mansoni* had embraced molecular biology for gene discovery and characterization. Increasingly complex research questions provided strong motivation toward genome characterization to gain novel and more comprehensive perspectives on the biology of *B. glabrata*, also at a time of an ongoing *S. mansoni* genome project. The international research community organized the *Biomphalaria glabrata* Genome Initiative that mounted collaborative efforts to develop and successfully secure support from NHGRI (NIH-USA) for genome characterization of *B. glabrata*. **Methods:** A 2002 field isolate (Fiocruz-CMM, Belho Horizonte, MG, Brazil) susceptible to *S. mansoni,* was used to generate the genome strain BB02 *Biomphalaria glabrata*. The genome project initially yielded a bacterial artificial chromosome (BAC) library (Arizona Genomics Institute, USA). Subsequently, a succession of Sanger–, 454-, and Illumina sequencing led to the assembly of the *B. glabrata* genome (McDonnell Genome Institute, Washington University, MO, USA). **Results:** The BglaB1 assembly (916 Mbp, estimated 31,985 genes) was deposited in GenBank (ASM45736v1) and is also housed and curated by VectorBase. Genome analysis by a team of international investigators from ~50 institutes revealed *B. glabrata* as a complex biological entity and indicated biological properties that may afford persistence of *B. glabrata* in the field, a prerequisite for schistosome transmission, as well as likely determinants of the outcome of *B. glabrata-S. mansoni* interactions, including aspects of immunity and gene regulation. Already, the extensive information extracted from the *B. glabrata* genome may foster new strategies to interrupt snail-mediated parasite transmission, including modification of snail-snail communication, alteration of snail reproductive output, and development of novel, specific molluscicides. **Conclusions:** The initial genome characterization is driving additional and novel research by aiding characterization of full length gene sequences and interpretation of MS-proteomics, screening and testing of novel candidate snail genes for parasite susceptibility, and comparative study of other (*Biomphalaria*) snail vectors of schistosomiasis. Integration of outcomes of such efforts into current control strategies can support the WHO-stated future goal of global elimination of schistosomiasis as a public health threat.

Conference 7

Goals, Targets, Guidelines and Adaptive Strategies Based on Old and New Assays

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Introduction: The World Health Organization (WHO) is currently revising its guidelines for control of morbidity due to schistosomiasis (Control), for its elimination as a public health problem (EPHP) and for elimination of its transmission (Elimination). The SCORE program is providing data that will be useful for guidelines revision. For example, SCORE findings have increased our understanding of the variability of village responses to mass drug administration (MDA) and the use of the Point-of-Care Circulating Cathodic Antigen (POC-CCA) assay in varied prevalence settings. **Methods:** The critical starting points for any set of guidelines are clearly articulated goals and targets by which programs can assess when their goals have been achieved. Based on SCORE data and those of others, we offer possible targets for each of the stated goals. We propose these targets here to encourage discussion by the schistosomiasis community. SCORE data has documented the problem of persistent hotspots—around 30% of villages that do not demonstrate expected declines in prevalence despite adequate, multiple MDAs. These results suggest the need for adaptive strategies beyond general guidelines to help program managers achieve their goals. Such strategies could include periodic re-mapping, with subsequent program decision-making informed by the goals of the program (Control, EPHP, or Elimination) and whether the village or area is responding or not to MDA. We believe that clearly articulated adaptive intervention strategies would be helpful to programs if they choose to go beyond the WHO guidelines. **Results:** The following draft targets for *S. mansoni* are a starting place for constructive discussions that could contribute to the revised WHO guidelines and agreed-to adaptive strategies. For purposes of this discussion, we derived the relationship between prevalence by Kato-Katz (KK) and POC-CCA (trace as positive) from published reports. The proposed target for the Control goal is sustained prevalence in a defined region at ≤15% by KK or ≤35% by POC-CCA. The EPHP goal target is to achieve and maintain prevalence at ≤5% KK or ≤15% POC-CCA. The prevalence target for Elimination is: 0% KK or <6% POC-CCA, with confirmation of no positive cases using more sensitive and specific assays and comprehensive sampling strategies, yet to be determined. Similar goals and targets are needed for other human schistosome species. The question of how often to evaluate progress and on what geographic scale will also be discussed, since that is an integral issue for determining achievement of goals. Adaptive strategies for given situations, such as persistent hotspots or areas that achieve the target ahead of other areas in the intervention area, will be essential in many cases if program goals are to be achieved. **Conclusions:** Possible algorithms for achieving each of the proposed targets will be presented in hopes of generating robust and helpful discussion and debate that will lead to helpful input into guidelines development and development of adaptive intervention strategies.

Supported by the University of Georgia Research Foundation, Inc., which is funded by the Bill and Melinda Gates Foundation for the SCORE Project.

Conference 8

Moving beyond morbidity control toward elimination of transmission

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Introduction: World Health Organization guidelines for schistosomiasis control will be revised in 2018-2019. Recent evidence from a number of large-scale drug treatment-based *Schistosoma* control programs in Africa, South America, the Philippines, and Asia suggests that there are clear limitations to the MDA- or PCT-based approach to control. Those results have now prompted a re-examination of the objectives and proposed endpoints for disease control and prevention programs. Our understanding of what constitutes schistosomiasis-associated morbidity has expanded since 2002, when the current guidelines were developed. Managers have also indicated their need for greater clarity regarding the policies that are recommended for 'schistosomiasis control' at every stage of implementation. **Methods:** We have used systematic reviews and meta-analyses to quantify the impact of drug treatment in terms of reducing existing morbidity prevalence. The relative benefits of more intensive drug treatments have been assessed, and the links between schistosome infection and its more subtle disabling morbidities have been established. We have also examined the ability of environmental snail control to limit or prevent new schistosome infections in human populations. Based on the concept that prevention of infection is the most effective means for complete prevention of disease, we then assessed the relative costs and benefits of transmission interruption as compared to repeated MDA. To do this, we have used SCORE program results to develop calibrated model-based projections of infection and associated disease prevalence based on advanced dynamic models developed with colleagues within the NTD Modelling Consortium**. Results:** Although substantial treatment-associated reductions in egg output result in significant improvements in the prevalence of morbidity, 'bounceback' reinfections are common, which can lead to re-emergence of significant morbidity. Drug treatment is not completely curative (<70%), and the efficacy of MDA-based control is fragile; it is threatened by systematic non-compliance, human migration, and very local factors that perpetuate and greatly amplify transmission. Although some models have predicted a natural 'breakpoint' in transmission if prevalence is brought to low levels, this seems unlikely to happen according to our new model projections. In cost-effectiveness analysis, combined MDA with snail control showed promise in low to moderate transmission areas. However, high transmission areas (persistent hot spots) will require more complex environmental controls (sanitation, alternative water use) to approach elimination. **Conclusions:** Programs must expect that current MDA-based approaches will provide only partial success in control of schistosomiasis. Such control is beneficial, but will probably have to be continued for an indefinite period of years or even decades. It is important now to establish the 'next-step' objectives and tools for transmission interruption. Enumeration of promising evidence-based practices, and linkage with national development goals, will provide the needed guidance for full disease elimination.

ROUNDTABLES

Roundtable 1

Global and multi-country initiatives for schistosomiasis elimination

Developing an Action Plan for Schistosomiasis Control and Elimination

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Global Schistosomiasis Alliance

The Global Schistosomiasis Alliance (GSA) has as its vision the elimination of schistosomiasis as a public health problem. The Alliance aims to provide a convening platform for schistosomiasis control and elimination expertise, tools and practices. Considerable efforts are being made to control and eliminate schistosomiasis across the endemic regions of the world. In Africa the need is greatest with over 90% of all known cases and with many areas where prevalence and intensity of infection is still high leading to unacceptable morbidity. GSA is working with partners and stakeholders to establish an Action Plan to highlight and coordinate activities that are needed to accelerate progress towards elimination of the disease. A number of important issues need to be addressed: improving praziquantel supply and delivery, reaching complete coverage of at-risk populations, determining the key criteria for transitioning from morbidity control to elimination goals, optimizing existing tools and developing new ones for diagnosis, developing additional control interventions including snail control and behavior changes. The Action Plan will be discussed with special mention as to how improved surveillance and response systems are required especially in areas that may be close to interrupting transmission. The GSA uses working groups to bring members with relevant expertise together to produce tangible outputs targeted at specific issues or challenges for schistosomiasis control & elimination and the Research Working Group especially would encourage further input to help define operational research needs and refine how GSA can be of service to the schistosomiasis research community.

Roundtable 1

Global and multi-country initiatives for schistosomiasis elimination

Targets for control of NTDs with specific reference to African schistosomiasis

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Sustainable control of neglected tropical diseases (NTDs) is an important endeavour, especially in Africa where helminthiases are common and several national control programme are ongoing. The backbone of control is preventive chemotherapy as well as integration of complementary interventions that may require vector control, WASH improvements or behavioural change. To make this happen multi-disciplinary approaches are needed and I discuss the formation of COUNTDOWN, a DFID_UK funded implementation research consortium, which aims to fosters the scale-up and more balanced equity of current and future interventions. During the talk I will pay particular attention to schistosomiasis and discuss pertinent issues such as precision mapping, expanded access of praziquantel treatment and the importance of female genital schistosomiasis.

Roundtable 1

Global and multi-country initiatives for schistosomiasis elimination

What is the score after 9 years of SCORE?

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Introduction: The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) began in December 2008 to address implementation issues facing Neglected Tropical Disease (NTD) program managers trying to control morbidity due to *Schistosoma mansoni* and *S. haematobium*. During 2009, SCORE held 8 planning meetings to define the key questions and best approaches to answering them within the available resources. **Methods:** The major studies were designed to evaluate approaches to gaining schistosomiasis control (villages with prevalence ≥ 25%) or sustaining control (villages with 10%-24% prevalence) through mass drug administration (MDA) with praziquantel (PZQ). Gaining control studies, conducted in Kenya, Tanzania and Mozambique, included 6 arms of randomized villages that received MDA either annually for 4 years or twice in 4 years, either through school-based treatment (SBT) or community-wide treatment (CWT). Sustaining control studies (conducted in Cote d'Ivoire and Kenya) had only three SBT arms (annual MDA or MDA twice over 4 years). Another major study involved comparing approaches to schistosomiasis elimination in Zanzibar. Studies were also designed to evaluate the Point of Care Circulating Cathodic Antigen (POC-CCA) assay. Additional studies leading to elimination allowed SCORE to evaluate mapping with the POC-CCA assay in several low-prevalence countries. Several important findings have emerged, and data analyses are continuing. **Results:** The major sustaining, gaining, and elimination studies demonstrate that MDA – whether SBT or CWT, annual or biennial – lowers prevalence and intensity in large-scale operational studies. However, the responses of individual villages to MDA varied a great deal, regardless of the study or study arms. Prevalence in some villages continued to go down with 2 or 4 treatments, but other villages treated in the same way with equal coverage maintained high prevalence. Intensities of infection decreased more consistently, but not in all villages. This variability was true whether villages started at high or low prevalence and in each study, about 30% of villages were persistent hotspots in the face of adequate MDA. Another important finding is that 4 years of annual MDA by SBT also produced decreases in prevalence and intensity in untreated first-year students and adults. The use of the POC-CCA in a variety of settings indicated that when the Kato-Katz assay indicates >50% prevalence, the two assays perform equivalently. However in low to moderate prevalence areas (10-50% by Kato-Katz), the number of people testing positive with the POC-CCA assay is typically at least double the number of infected persons. Over half of these have been demonstrated to be true-positives based on additional studies, but some are false positives. **Conclusions:** Key findings from the SCORE studies thus far are: 1) MDA with PZQ lowers prevalence and intensity of infection; 2) Four annual treatments over 4 years are better than 2; 3) SBT lowers prevalence and intensity in "non-target" populations; 4) Villages vary a great deal in how well they respond to MDA, with ~30% in these studies staying persistent hotspots; 5) There is more schistosomiasis than we thought. Specific examples supporting these conclusions will be presented.

Supported by the University of Georgia Research Foundation, Inc., which is funded by the Bill & Melinda Gates Foundation for the SCORE Project.

Roundtable 2

Vaccines and therapeutic targets for schistosomiasis control

Identification of new targets and anti-schistosomiasis vaccine formulations

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A vaccine against schistosomiasis would overcome the limitations presented by a control strategy based on chemotherapy and would have a great impact in disease transmission leading to elimination. Recent mathematical models indicate that a vaccine capable of inducing partial protection against schistosomiasis would result in the elimination of the disease over a period of 18 to 22 years. The impact would be even greater if vaccination is associated with chemotherapy in residents of endemic areas. Currently, three Schistosoma vaccine candidates are under clinical trials (Sh28GST, Sm14 and SmTSP2), but the identification of new candidates is still needed in order to improve vaccine formulations.

In these regards, our group has been working in the identification of potential antigens in the tegument of the *Schistosoma mansoni* parasite. The *S. mansoni* schistosomula's tegument is an interesting source of vaccine candidates, since schistosomula have been described as the parasite life stage most susceptible to host immune response attack. Additionally, the tegument represents the interface between parasite and host and is involved in essential functions to parasite survival, as nutrition, immune evasion, osmoregulation, excretion, sensory reception and signal transduction.

Here we are going to present some results obtained by our group in the screening of promising candidates to compose an anti-schistosomiasis vaccine. From the identification of targets to the its characterization and evaluation as a protective antigens in different vaccine formulations and immunization strategies in pre-clinical trials.

Roundtable 2

Vaccines and therapeutic targets for schistosomiasis control

Drug discovery for schistosomiasis at UC San Diego: new tools, old drugs and the Brazilian connection

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With the ever-increasing reliance on just one drug, praziquantel (PZQ), to treat and control schistosomiasis, alternative drugs should be identified. As part of the CDIPD's contribution to developing new drugs for parasitic diseases of poverty, I will first outline the development of an automated high-throughput / high-content drug screening (HTS/HCS) platform to quantify the chemically induced responses of *Schistosoma mansoni*. I will describe its development; including its training with anti-schistosomal drugs and implementation via a customized graphical user interface to measure a range of static and dynamic parasite responses. Data from screening campaigns of high-value small molecule collections will be presented. Secondly, I will re-examine the case for a group of compounds known as alkyl amino alkanehio sulfates that were originally developed as radiation-protection agents and subsequently shown to be potently parasiticidal in a mouse model of *S. mansoni* infection by the groups of José Pellegrino and David L. Nelson at the Universidade Federal de Minas Gerais. In our mouse model of infection, one compound in particular was competitive with PZQ in terms of its single-dose efficacy against mature and PZQ-refractory juveniles. *In vitro* metabolic profiling of this lead and its disposition in mice identified a putative metabolic pathway. Chemical synthesis and *in vitro* and/or *in vivo* testing of two key metabolites confirmed schistosomicidal activity for one of them, including against *Schistosoma haematobium*. The identification of the lead and at least one cidal principle reinvigorates the pursuit of a chemical series which may yet yield a drug that either complements or provides an alternative to PZQ.

Supported by a UCSF Clinical and Translational Science Institute T1 Catalyst Award, and NIH-NIAID R01AI089896 and R21AI107390 awards.

Roundtable 2

Vaccines and therapeutic targets for schistosomiasis control

Searching for new antischistosomal therapeutic options by combining phenotypic and target-based approaches

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Introduction: By all practical means, the only available therapeutic option to treat human infections by all major species of *Schistosoma sp* is praziquantel (PZQ), an already ancient drug with more than four decades of continuous use. Although cheap and relatively safe, PZQ has known caveats such as low efficacy against juvenile worms and difficulties in oral administration to school-aged children. Furthermore, induction of resistance in laboratory strains has been demonstrated and increased exposure in the field due to mass drug administration programs in Africa has made the threat of resistance rising a major concern. With the goal to find new therapeutic options to schistosomiasis our group has engaged on an interdisciplinary program for antischistosomal drug discovery. **Methods:** Phenotypic screening keeps as the mainstay of antihelminthic drug discovery but traditionally suffers from lack of unbiased methods for evaluating drug effects on cultured worms. We have developed and employed automated imaged-based assays for the high-content analysis of phenotypic responses by adult worms incubated with drug candidates. The later were selected from in silico analysis of virtual chemical libraries in the search for repurposed drugs or inhibitors of known drug targets, such as the Thioredoxin Glutathione Reductase (TGR) and Cathepsin D-like enzymes. Alternatively, we have adapted a metabolic viability assay based on the reduction of the soluble XTT dye for screening the 400 compounds in the MMV's Pathogen Box (PB) against juvenile worms. As a third approach, we are exploring fragment-based drug discovery (FBDD) by X-ray crystallography for the structure-based development of new inhibitors of *S. mansoni* TGR (SmTGR). **Results:** In this presentation, we will summarize our successful application of combined phenotypic and target-based approaches for antischistosomal drug discovery. We will also show the results of the PB screening on juvenile worms where eight new antischistosomal hits were found. Interestingly, cheminformatics analysis has indicated that one of the PB hits has SmTGR as its putative molecular target. Finally, our latest results on the application of FBDD to SmTGR inhibitor discovery will also be briefly presented. **Conclusions:** There is an urgent necessity for developing new antischistosomal agents for use in combination with or in substitution to PZQ. Several difficulties for achieving this goal are posed but we show that the combination of computational tools with modern phenotypic assay methods and the latest advances in structure-based drug discovery approaches has the potential to overcome the usual bottlenecks and help accelerate antischistosomal drug discovery.

Supported by CAPES/CNPq/FAPERJ/Fiocruz/Newton Fund

Roundtable 3

Parasite-Human-host interaction

Schistosoma mansoni S*m*KI-1 serine protease inhibitor binds to elastase and impairs neutrophil function and inflammation

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Protease inhibitors have important function during homeostasis, inflammation and tissue injury. In this study, we described the role of *Schistosoma mansoni*S*m*KI-1 serine protease inhibitor in parasite development and as a molecule capable of regulating different models of inflammatory diseases. First, we determine that recombinant (r) S*m*KI-1 and its Kunitz domain but not the C-terminal region possess inhibitory activity against trypsin and neutrophil elastase (NE). To better understand the molecular basis of NE inhibition by S*m*KI-1, molecular docking studies were also conducted. Docking results suggest a complete blockage of NE active site by S*m*KI-1 Kunitz domain. Additionally, rS*m*KI-1 markedly inhibited the capacity of NE to kill schistosomes. In order to further investigate the role of S*m*KI-1 in the parasite, we designed specific siRNA to knockdown S*m*KI-1 in *S. mansoni.* S*m*KI-1 gene suppression in larval stage of *S. mansoni* robustly impact in parasite development in vitro and in vivo. To determine the ability of S*m*KI-1 to interfere with neutrophil migration and function, we tested S*m*KI-1 anti-inflammatory potential in different murine models of inflammatory diseases. Treatment with S*m*KI-1 rescued acetaminophen (APAP)-mediated liver damage, with a significant reduction in both neutrophil recruitment and elastase activity. In the model of gout arthritis, this protein reduced neutrophil accumulation, IL-1β secretion, hypernociception, and overall pathological score. Finally, we demonstrated the ability of S*m*KI-1 to inhibit early events that trigger neutrophil recruitment in pleural cavities of mice in response to carrageenan. In conclusion, S*m*KI-1 is a key protein in *S. mansoni* survival and it has the ability to inhibit neutrophil function as a promising therapeutic molecule against inflammatory diseases.

Supported by CNPq, FAPEMIG and CAPES

Roundtable 3

Parasite-Human-host interaction

The use of RNA interference technology to understand the role of parasite proteins in the host-parasite interaction

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The *Schistosoma mansoni* genome project identified 10,852 protein-coding genes of which almost half are annotated with “unknown function”. Those genes could be parasite-specific and represent genes whose biological functions are of interest for basic and applied science. Despite of great advances in the genomic field, application of technologies for schistosomiasis control have not kept pace; treatment of this disease still relies on a single drug and no vaccines are yet available. Therefore extracting meaningful functional information from the accumulated genomic data is critical to discovering new chemotherapeutics and other novel approaches to disrupting development within the snail and human hosts. Currently, RNA interference (RNAi) is the most effective reverse genetic tools for manipulating gene expression and determining gene function in schistosome parasites, both *in vitro* and *in vivo* in the association with their hosts. Our group has dedicated efforts to understand the role of schistosome genes and their encoded protein products in the host-parasite interaction, with the goal of identifying and validating promising “druggable” targets. This talk aims at presenting our work applying the RNAi approach in the different parasite life cycle stages to assess the function of diverse genes such as antioxidants, kinases and histone modifying enzymes, to rationally identify efficient therapeutic targets for schistosomiasis control, as well as understanding the mechanisms by which *S. mansoni* survives within its hosts.

Apoio Financeiro: FAPEMIG; European Commission, 7th Framework Programme under Grant Agreement 602080 (A-PARADDISE); CAPES/University of Nottingham-Drug Discovery; FIOCRUZ; CNPq

Roundtable 3

Parasite-Human-host interaction

Understanding the roles of Immunoglobulin E (IgE) in human resistance to infection with *Schistosoma* sp.

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Helminth infection is well known to induce a strong Immunoglobulin E (IgE) response in infected hosts. This IgE response is in part directed against parasitic antigens, but helminth infection can also lead to a strong IgE response to non-parasite–related bystander proteins (Orr and Blair 1969). It is widely accepted that IgE has evolved to protect mammals against parasitic infection. For schistosomes, the first demonstration of an association between parasite-specific IgE and protection against reinfection goes back to the work of Hagan *et al.* (Nature 1991). While it is plausible to assume that such a potent, potentially life-threatening immune response has not evolved to protect against innocuous environmental allergens such as pollen, the IgE/parasite connection has been more recently questioned by other authors (e.g. Medzithov and Galli) who offer an alternative evolutionary explanation, the IgE/toxin hypothesis. This states that IgE-dependent responses, in combination with effector cells (mast cells and basophils), protect against noxious substances such as apitoxins or snake venom. In contrast, the IgE/parasite hypothesis postulates that IgE responses, including those to innocuous allergens, are misdirected responses to antigenic epitopes cross-reacting with 'true' parasitic allergens. Irrespective of one's position in the parasite vs toxin dispute, the issue has clear practical repercussions for anti-helminth vaccine development. If parasite-specific IgE is indeed the evolutionarily favoured, host-protective immune response, then a good protective vaccine should elicit such a response in vaccinees. Vaccinating with allergens however bears an inherent risk of inducing systemic anaphylaxis, in particular when vaccinating in endemic areas. On the other hand, if parasite-specific IgE is not needed for host-protective responses, then we need to revisit our understanding of IgE in parasitic infection.In both cases however, it is of paramount importance to develop techniques which allow assessment of potential allergenicity of vaccine candidates, by combining bioinformatic predictions with experimental validation. Such techniques are complicated by difficulties such as predicting allergenicity of proteins without homology to known allergens, or dealing with conformational (as opposed to linear), reduction-sensitive epitopes. Here, we present how parasitologists' understanding of IgE, of the nature and structure of allergens and the underlying mechanisms of protection, lags decades behind the large body of evidence accumulated by allergists and allergologists. If 'true', 'primordial' allergens are indeed primarily parasite-derived, it is an irony that the vast majority of the thousands of allergens deposited in databases such as the Allergome are derived from plants and fungi, and only a few dozen from parasites! We also present an experimental workflow allowing fast assessment of allergenicity in vitro using novel IgE reporter systems, together with our attempts to handle conformational, reduction-sensitive epitopes in this system.

Roundtable 3
Parasite-Human-host interaction

Interleukin-4-inducing principle from schistosome eggs (IPSE): immunomodulatory effects

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Introduction: *Schistosoma haematobium* infection, also known as urogenital schistosomiasis, affects >112 million people globally. *S. haematobium* worms live in host pelvic veins and deposit eggs in the bladder. The eggs secrete antigens that induce granuloma formation, in turn provoking urothelial hyperplasia and carcinogenesis. The IL-4-inducing principle of *Schistosoma mansoni* eggs (M-IPSE) is an abundant antigen released by schistosome eggs. M-IPSE binds immunoglobulins and chemokines, translocates into host cell nuclei and modulates gene transcription, and induces basophils and mast cells to release IL-4, thereby orchestrating a type 2 immune response. Herein we describe *S. haematobium* orthologs of IPSE (H-IPSE) and their immunomodulatory capacities. **Methods:** *S. haematobium* orthologs of IPSE (H-IPSE) were cloned and recombinantly expressed in mammalian cells. ELISA assays were performed to measure IPSE affinity for IgE and IgG. A multiplexed protein array was used to profile H-IPSE's chemokine, cytokine, and nuclear protein binding partners. A rat basophil cell line was used to define H-IPSE's ability to trigger NF-AT activation in IgE-bearing basophils. Histamine assays were performed on peripheral blood of mice injected with H-IPSE. Wild type and IL-4 reporter mice were injected with H-IPSE, with or without ifosfamide to induce bladder inflammation, and peripheral and bladder IL-4 expression and inflammation were assessed by flow cytometry, histology, RNA-Seq, and qPCR. **Results:** H-IPSE orthologs clustered into two clades exemplified by H03-H-IPSE and H06-H-IPSE. H03, H06, and M-IPSE could bind IgE and IgG, albeit at differing affinities. H06 and M-IPSE featured overlapping affinities for numerous chemokines, cytokines, and nuclear protein binding partners. Like M-IPSE, H06 was able to trigger NF-AT activation in IgE-bearing basophils. Surprisingly, H06 injected intravenously into mice did not elevate circulating histamine levels. H06 injected in this fashion triggered IL-4 production by peripheral basophils. A single injection of H06 was profoundly anti-inflammatory and promoted tissue repair (as assessed by histology, bladder hemoglobin levels, pain and voiding-related behaviors, and transcriptomics) in the ifosfamide-induced hemorrhagic cystitis mouse model. **Conclusions:** H-IPSE orthologs are highly immunomodulatory, suggesting that akin to M-IPSE, these egg stage-specific proteins play a major role as inflammatory rheostats in schistosomiasis. The anti-inflammatory properties of H-IPSE suggest that these proteins could be therapeutically exploited in ifosfamide-induced hemorrhagic cystitis and other inflammatory diseases unrelated to schistosomiasis.

This work was supported by the U.S. National Institutes of Health (R01 DK113504)

Roundtable 4

WASH and IEC strategies for schistosomiasis control/elimination

Distance Learning Course: Schistosomiasis - clinical and epidemiological management in Primary Health Care

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Introduction: Schistosomiasis mansoni represents an important public health problem in Brazil, affecting around 1.5 million people in the country. Given this scenario, and considering that Basic Health Care (ABS) is the main access for the population to the Unified Health System (SUS), the Ministry of Health of Brazil (MS) decides to invest in the qualification of health professionals that work at the Family Health Units (USF). By doing that the MS expect that patient management following an adequate flow, in which the sick individuals are picked up at a USF, diagnosed, treated and forwarded to a referral service when necessary, until the reestablishment of its health. **Methods:** To achieve this goal the MS will offer a Distance Learning Course (EAD) called: *Schistosomiasis - clinical and epidemiological management in Primary Health Care*. This course will be offered by the Open University of SUS (UNA-SUS / MS) to medicals and nurses that work in the ABS, especially those linked to the Program More Doctors and to the Program of Valorization of the Professional of Basic Attention Health. The course has a workload of 45 hours and is composed of an introductory unit, containing information about the course and the use of the Virtual Learning Environment. And five thematic units which will address the epidemiological aspects of the disease in the country, passing through the life cycle of the parasite, clinical manifestations, diagnosis, treatment and prevention. Each unit will present a problem situation, based on real facts, that will contextualize the content that will be addressed. The student should study all didactic material available in each unit and to advance to the next unit it will need to respond to an evaluative activity and have at least 70% correct response. By submitting the evaluative activity, the student will receive feedback on all the right and wrong answers and will be able to retake the evaluation until achieve the minimum performance. **Results:** With this course, it is expected that professionals working in ABS may be better prepared to properly screen, identify and treat patients with schistosomiasis, in order to ensure health recovery and minimize complications related to this morbidity. **Conclusions:** The Ministry of Health, through UNA-SUS, has been investing in the EaD understanding that this is a viable solution to propagate knowledge in a universal and simultaneous way in Brazil. It is a strategy to guarantee the continuous training of health professionals that work in Basic Health Care units, enabling MS standards, procedures and guidelines to be followed more quickly and homogeneously throughout the country. The final goal of this strategy is to reduce health indicators related to schistosomiasis in national level.

Supported by UNA-SUS/ Ministry of Health/ Brazil

Roundtable 4

WASH and IEC strategies for schistosomiasis control/elimination

Lic Health: Education Through Interschool Comunication and Technological Convergence

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This study involves an analysis of a health education intervention carried out both online, through a social network (Facebook), and in face to face encounters with teenage students living in rural areas in Vale do Jequitinhonha, Minas Gerais, Brazil, where schistosomiasis is endemic. Health Education is regarded as an effective strategy to control schistosomiasis when it is conducted in conjunction with actions geared towards improving living conditions of the subject population. Interschool communication and technological convergence were adopted during the health education intervention with the goal of helping teenagers to problematize ways of life in their respective existential territories and, at the same time minimize time and space barriers amongst participants as well as between them and the researcher. This article intends to analyze how powerful such intervention was in terms of producing knowledge and new modes of perceiving and experiencing their own territories as well as health issues. The selected methodology was Intervention research conducted in three different localities on the municipality of Ponto dos Volantes with forty-nine (49) students. The reports derived from face to face meetings with audio recordings and the record of interactions carried out through Facebook constituted the database that formed the basis for the analysis of the content produced. During the research the students produced and shared videos, parodies, aesthetic works, games and projects for local action. The intervention, by promoting and making more evident the linkage between happenings in each place, helped to foster movements that shifted identities, strengthened processes of singularization and encouraged the participants to share their conflicts and to create new concepts. The exercise of videomaking acted as a disruptor of hegemonic imagery and representations in each territory, provoking a fissure on the stablished views and feelings of the teenagers about their reality. Parody creation fostered an exchange of automatic and unquestioned behaviors and of hegemonic patterns that impact the ways of life in each of the three places under study. Aesthetic works enabled the production of new concepts about health while the games helped them to problematize the relation between schistosomiasis and the teenagers' conditions of existence. The intervention was characterized by the constant tension between the silences of the online aspects of the intervention and the powerful desire of subjective production manifested in the face to face encounters. The silences that marked the online interactions also carried an undertone of denunciation in the sense that it showed that the participants were more interested in pursuing other interests during their brief time online instead of actively engaging in the intervention. This shows that the limited capillarity of networks that still prevents access to the internet in rural areas is a problem. At the same time, the importance of expanding studies that rely on virtual spaces for the collective production of meaning in endemic communities living in rural areas became clear.
Financial support: Nacional Institutes of Health – TMRC (AIO 98507); CAPES; CNPq; INCT – DT.

Roundtable 4

WASH and IEC strategies for schistosomiasis control/elimination

Promotion of sustainable and healthy territories: the experience of ecological sanitation in Bocaina, Brazil

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In BocainaMosaic - Brazil, an area of high socio-environmental vulnerability, based on the demands of the Traditional Communities Forum of Angra dos Reis, Paraty and Ubatuba (FCT) a partnership was established with the Oswaldo Cruz Foundation (FIOCRUZ), supported by the National Health Foundation (FUNASA), for the creation of the Observatory for Sustainable and Healthy Territories of Bocaina (OTSS), a territorial and coordinated technopolitical space that generates social technologies in order to promote health and sustainable. As a demand from the communities, the ecological sanitation in Caiçara Community of Praia do Sono, in Paraty, Rio de Janeiro, Brazil, was chosen as a starting point for OTSS. As pointed in Agenda 2030, sanitation is a critical dimension for health promotion, especially ODS 6, highlighting the relationship between health and sanitation. Therefore, territorialized actions for sanitation in Traditional Communities, which contemplate the various actors involved with the aim of promoting sustainability, equity and autonomy, are highly important. The use of action research was defined to build solutions based on consensus, combining the work of academic and community researchers, with data collection through participant observation. The whole process of choosing the territory, building the project, agreeing on the technology to be used, the changes throughout the process, the implementation, the environmental education and the construction of sanitation modules was carried out in constant dialogue, through discussion meetings with the involvement and active participation of the various local actors and the community. To conclude, the implementation of ecological sanitation aims to create proper conditions for strengthening Traditional Communities and to promote a transformation pulse that fosters territorialized and inclusive public policies in other territories.

Roundtable 5

Schistosomiasis in pre-school age children

Schistosomiasis in PSAC, Epidemiology, available clinical/safety data on treatment with Praziquantel, and recent WHO recommendation

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Introduction: Preschool-aged children (PSAC) were thought to be at low risk of schistosome infection and received little research attention. Our studies have been to characterize and quantify the overall health effects of paediatric schistosomiasis, assessed variability in the schistosome infection in early childhood and determined how prior schistosome infection affects the clinical presentation and progression of the infection. This has been timely as development and clinical testing of a paediatric formulation of praziquantel for use in children aged 6 years and below is currently underway. Despite increased international efforts to control schistosomiasis using preventive chemotherapy, several challenges still exist in reaching the target populations. Until recently, pre-school children had been excluded from the recommended target population for Mass Drug Administration. **Methods:** The study involved community assessment of 1502 Zimbabwean children aged 1-5 year old in a high schistosome infection area recruited at health delivery centres. Demographics, anthropometric and parasitology data were collected from the children. Urinary morbidity was assessed by macro and microhaematuria. Growth-related morbidity was assessed using standard WHO anthropometric indices. Children negative for *S. haematobium* infection were followed-up quarterly to determine infection and morbidity incidence over one year. **Results:** At baseline, the prevalence of *S. haematobium* infection and microhaematuria was 8.5% and 8.6%, respectively. Based on different anthropometric indices, 2.2 - 8.2% of children were malnourished, 10.1% underweight and 18.0% stunted. The fraction of morbidity attributable to schistosome infection was 92% for microhaematuria, stunting at 38% and malnutrition at 9-34%. Children with *S. haematobium* infection were at greater odds of presenting with microhaematuria (AOR=25.6) and stunting (AOR=1.7). Annual incidence of *S. haematobium* infection and microhaematuria were 17.4% and 20.4%, respectively. Microhaematuria occurred within 3 months of first infection and resolved after praziquantel treatment, with significant reductions observed (42.3% vs. 10.3%; P<0.0001). The work describes for the first time, the incidence of schistosome infection and morbidity in PSAC, showing that morbidity assessed by microhaematuria occurs rapidly, within 3 months of first infection and resolves after praziquantel treatment. We also showed that a proportion of stunting and malnutrition are attributable to *S. haematobium* infection. **Conclusion:** Our study and those of others provided the evidence base for the need to treat pre-school children that led to recommendations by the World Health Organization for including pre-school children in treatment programs. The major challenge now lies in the unavailability of a child size formulation of the appropriate praziquantel. The target product profile for paediatric praziquantel as well as knowledge gaps pertinent to the successful control of schistosome infection and disease in pre-school children is now well established with praziquantel clinical trials entering Phase III. We describe the incidence and dynamics of first schistosome infections and morbidity in PSAC, and provide evidence base supporting the need to treat millions of PSAC in sub-Saharan Africa.
Financial support: Thrasher Research Fund, British Academy and The Wellcome Trust.

Roundtable 5

Schistosomiasis in pre-school age children

Process development and manufacturing of pediatric praziquantel orodispersible tablets

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Introduction: The gold standard treatment for schistosomiasis employs a single oral dose of praziquantel (PZQ) tablets, indicated to adults and school-age children (> 4 years). The PZQ, a 1:1 mixture (racemate) of the two enantiomers, levo-praziquantel (R-(-)-Praziquantel or L-PZQ) and dextro-praziquantel (S-(+)-Praziquantel or D-PZQ), has a bitter taste, which can lead to gagging or vomiting when chewing the tablets, and younger children have difficulties in swallowing the current tablets due to their large size. This leads to the issues of non-compliance and of under-dosing. The non-profit Pediatric Praziquantel Consortium was set-up to develop a suitable orally disintegrating tablet (ODT) formulation of PZQ for children aged 3 months to 6 years. As part of its overall development risk-mitigating strategy, the Consortium has decided to develop two ODT pediatric formulations in parallel until the end of the phase II clinical trial: one containing the L-PZQ enantiopure active pharmaceutical ingredient (API) and the other containing the racemic PZQ API. **Methods:** Development one synthetic route for R-(-)-PZQ API and two respective ODT formulations (L-PZQ ODT and Rac-PZQ ODT), to supply the Investigational Medicinal Product (IMP) for the clinical trials and meeting the requirements for future file registration. **Results:** An economic and robust chemical synthesis for the R-(-)-PZQ API was developed by Merck KGaA. API process and technology transfer including scale-up to commercial batch size is ongoing including continues process optimization. The initial ODT formulations were developed at lab scale by Astellas Pharma Inc., through conventional manufacturing technology according to the key criteria: small tablet size; rapid disintegration time; improved palatability and acceptable stability profiles. The formulation process was transferred and further optimized by Merck KGaA and Farmanguinhos/FIOCRUZ according to GMP standards for phase I and II clinical trials supply. From results of phase II the L-PZQ ODT was selected and scale-up trials to reach industrial size is ongoing at Farmanguinhos/FIOCRUZ to manufacture the IMP for phase III clinical trials. Furthermore, both the API as well as the ODT manufacturing processes are optimized and validated to meet regulatory expectations and to ensure sustainable future supplies. Finally, in accordance with the Consortium's access plan, a 2nd drug product manufacturing site in Africa is currently being selected which, after successful process and technology transfer, will be able to meet projected demands in the future. **Conclusions:** Both PZQ ODT formulations were successfully developed by the Consortium up to and including phase II, with appropriate efficacy and safety data on the final selected L-ODT formulation now to be gathered during phase III. In parallel, extensive Chemistry, Manufacturing and Control (CMC) development work packages are already underway in compliance with regulatory requirements and launch demands. These activities are supported by Bill & Melinda Gates Foundation and Global Health Innovative Technology Fund, and are part of the EDCTP2 programme supported by the European Union.

Roundtable 5

Schistosomiasis in pre-school age children

Development of a new oral dispersible tablet (ODT) formulation of praziquantel to treat pre-school age children infected with Schistosomiasis

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Introduction: Preschool-aged children (PSAC) are a high-risk group for schistosome infections counting for 20-25 million of the global prevalence. The Pediatric Praziquantel (PZQ) Consortium is developing a 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 the other one containing the racemate PZQ (rac-PZQ) molecule. **Methods**: The clinical studies conducted until now include two bioavailability (BA) studies in healthy adults conducted in South Africa (studies ClinicalTrials.gov Identifier NCT 02271984 and NCT 02325713), a taste study in children aged 6 to 11 years conducted in Tanzania (study PACTR201412000959159) and a Phase II dose finding study in *Schistosoma mansoni* (intestinal schistosomiasis) infected children age 3 months-6 years in Côte d'Ivoire (ClinicalTrials.gov Identifier: NCT02806232). **Results:** In the BA studies in adult healthy volunteers, the L-PZQ and rac-PZQ ODTs were assessed in with the commercial rac-PZQ tablet (Cisticid® 500 mg) as a reference. The systemic exposure of L-PZQ when given as a single enantiomer (i.e., as L-PZQ ODT) was around 40% of the exposure of L-PZQ when administered as racemate (Cisticid®). The relative BA of L-PZQ when given in rac-PZQ ODT was comparable to the levels observed after administration of Cisticid®. The overall palatabilities of the new ODTs were better than Cisticid® in Tanzanian children age 6 to 11 years. In the phase II dose-finding study, a total of 420 children age 2 to 6 years old, 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 rac-PZQ ODT, or 30 mg/kg, 45 mg/kg or 60 mg/kg of L-PZQ ODT. Cure rates 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. Egg reduction rates were ≥90% for all cohorts. Both ODT formulations were well tolerated at all doses tested. **Conclusions:** Supported by the current clinical results, the program is preparing Phase III. The Phase III program includes a confirmatory clinical study in *Schistosoma*-infected PSAC. The Consortium aims to submit a Marketing Authorization Application in 2020, and to have the product available in 2021, for launch in the first endemic countries in Africa and later in Brazil. Financially supported by Merck, in-kind contributions by Consortium partners and by grants from external funders.

Roundtable 6

Epidemiology and control

Analysis of impact of mass drug administration for the control of schistosomiasis in hyperendemic areas of the State of Pernambuco, Brazil.

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INTRODUCTION: Among the Brazilian states, Pernambuco had a higher prevalence (7.9%), a higher number of deaths (average of 200) and a higher mortality rate (2.7 p / 100,000 inhabitants) due to schistosomiasis in 2010. After 2011 , the State Health Department created the SANAR program, which defined, among other objectives, the reduction of the positivity of schistosomiasis to less than 10% by 2014, in 118 hyperendemic areas (HA) using the strategy of mass drug administration (MDA) for controls of Schistosoma mansoni (Sm). In these areas, about 146 thousand people live. This work aims to describe and analyze the results of MDA (Sm) in (HA). **METHODOLOGY**: This is a descriptive cross-sectional study with information on the positivity of Schistosoma mansoni available in the Information System of the Schistosomiasis Control Program (SISPCE) during the years 2011 to 2014, in order to analyze the results of the actions carried out by the SANAR Program. We considered the information from the 118 (AH) where the average positivity prior to 2011 was = or> 10%. We analyzed the results of 03 rounds of massive drug administration (MDA) between the years 2012 to 2014. We used for Microsoft Excel 2010 software analyzes. **RESULTS:** The mean positivity before MDA was 18.6% in the 118 (HA), ranging from (10.0% to 70.6%). Twenty-five of the 118 HA (21%) completed three rounds of MDA by 2014; 75 (63%) completed two rounds and 118 (100%) performed at least one round of MDA. After the rounds of MDA, the mean in the positivity for (SM) in the 118 (HA) was of 2.9% (0% to 25%) in 2014 and therefore a reduction in 84%. The median MDA coverage for the eligible population was 75% (12% to 100%), with little variation when analyzing the three rounds of treatment. **CONCLUSION:** Although most of the HA did not perform the three rounds of MDA, there was a significant reduction in the positivity for Schistosoma mansoni. There was good adherence of the population observed through median MDA coverage. However, the guarantee of the low positivity for (Sm) in these areas requires the maintenance of the priority in the actions of control of schistosomiasis and mainly actions of improvements of the conditions of basic sanitation.

Supported by: IHMT-UNL

Roundtable 6

Epidemiology and control

Moving towards the elimination of schistosomiasis in Sub-Saharan Africa: the PHASE initiative

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In Sub-Saharan Africa, morbidity control is currently the objective of schistosomiasis control programmes in many countries. Interventions are limited to chemotherapy with praziquantel. However, it is known that treatment alone will not be sufficient to achieve the interruption of schistosomiasis transmission. As, the ultimate goal of schistosomiasis intervention efforts should be the elimination of this infection, endemic countries should adopt alternative strategies combining intensified preventive chemotherapy and the implementation of complementary public-health, environmental and educational interventions. These complementary interventions – recommended in areas approaching elimination as a public-health problem – include health education for behaviour change, provision of safe water and sanitation, environmental management and snail control. To foster endemic countries in this direction, WHO/AFRO highlighted it in its 2014-2020 Regional Strategic Plan for schistosomiasis. WHO/AFRO defines this approach as PHASE, standing for preventive chemotherapy, health education, access to clean water, sanitation improvement, and environmental snail control and focal mollusciciding. Increasing access to safe water is an intervention that will significantly reduce the risk of schistosomiasis transmission. Its achievement requires inter-sectoral collaboration and partnership. Poor sanitation is a major contributor to transmission of schistosomiasis and causes rapid re-infection among treated children and adults. Improvement in waste disposal and a reduction in open defaecation is essential for achieving interruption of transmission. Improvement in sanitation not only contributes to prevention of transmission, but also to the prevention of many diarrhoeal diseases. Environmental management for snail control has not been generally undertaken in the sub-Saharan African region due to cost limitations and lack of identification of the water bodies where this is feasible. As snail control is generally challenging especially in large water bodies, there is need to identify areas with high water contact and intensive schistosomiasis transmission so that targeted snail control can be limited to such locations. However, technical capacity and funding to implement reliable snail surveys is lacking in many countries. The presentation will highlight and discuss some of the key interventions of the PHASE approach, as well as the opportunities and challenges for their implementation.

Roundtable 6

Epidemiology and control

Schistosomiasis and soil-transmitted helminthiases in the Americas: analysis of the current situation

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Schistosoma mansoni, the only *Schistosoma* species present in the Americas, continues to be endemic in Brazil, Venezuela and the Caribbean. An estimated 25 million people are at risk of contracting schistosomiasis in the Americas, 90% of them in Brazil. It is estimated that 1.6 million school-age children need preventive pharmacological treatment (with praziquantel) primarily in Brazil and Venezuela. Suriname appears to have focalized transmission at very low levels. Antigua and Barbuda, Guadeloupe, Martinique, Montserrat, and Saint Lucia seem to be close to having interrupted transmission but surveys need to be done to verify this. Schistosomiasis has apparently been eliminated in Puerto Rico, but this needs to be documented.

Notably there has been recent progress in the countries still considered endemic. There has been a reduction in the overall prevalence of schistosomiasis in Brazil primarily due to investments in basic sanitation and safe water supply, improvement in the population's income levels and quality of life as well as the availability of praziquantel being produced nationally in Brazil. There are plans to carry out transmission surveys in Venezuela to reactivate MDA in areas where it might be necessary. In St. Lucia a national survey to determine the epidemiological status of the disease was carried out in 2017; approximately 1500 school-age children were examined by both antigen and antibody detection methods; none of them was positive. Studies in adults as well as malacological studies are needed to complement the evidence and an action plan is being updated to improve surveillance. In Suriname, interventions are being intensified to eliminate schistosomiasis in areas where cases have been detected among school-age children and plans to eliminate the disease are being developed and a survey in school-age children is being planned. The available evidence suggests that schistosomiasis transmission has been eliminated in Dominican Republic, Antigua and Barbuda and Puerto Rico, but this needs to be confirmed. Surveys in Antigua and Barbuda and Dominican Republic are also being planned.

PAHO/WHO estimates that 44 million children were at risk of soil-transmitted helminthiases in the Region, 54% of them in three of the 24 affected countries (Brazil, Colombia, and Mexico), and 35% in seven other countries (Bolivia, the Dominican Republic, Guatemala, Haiti, Honduras, Nicaragua, and Peru). Almost 8 million preschool aged children and 23 million school-aged children in need of PC were treated in 2016, reaching a regional coverage of 64%, and 74% respectively. The minimum 75% national coverage target was reached in 7 countries. About 26% of school-age children and 36% of preschool-age children (approximately 12.8 million children) living at risk of soil-transmitted helminth infection still need to be dewormed.

Roundtable 7

New trends on the intermediate host

Brazil's contribution to the characterization of the genome of *Biomphalaria glabrata* (Say,1818)

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The construction and analysis of *Biomphalaria glabrata* genome were carried out by the effort of 117 researchers from 10 countries, among them 16 Brazilians from Fiocruz-Minas and Federal University of Uberlândia/Campus Patos de Minas (UFU). In Brazil, the molluscs were collected, and the F1 generation was exposed to *Schistosoma mansoni* miracidia to define the rate of infectivity of the population. In parallel, the molluscs were taxonomically identified. *Biomphalaria glabrata* species with an infectivity rate higher than 90% was confirmed. Then, the molluscs were sent to the USA for sequencing. After genome sequencing, a Brazilian team analyzed the miRNA and piRNA pathway genes and also identified and characterized conserved miRNAs (mature and precursors) and their target genes in *B. glabrata* genome and transcriptome. It was identified nine putative proteins involved on biogenesis of the miRNA and piRNA (Bgl-Argonaute, Bgl-Dicer, Bgl-Drosha, Bgl-Fmr1, Bgl-Partner-Dicer, Bgl-TDRD1, Bgl-PIWI, Bgl-Tudor-SN and Bgl-SPN-E) with high similarity with their orthologs from *Drosophila melanogaster*, *Lottia gigantea* and *Aplysia californica*. Conserved miRNAs, including clustered miRNAs, duplicated miRNAs, intronic miRNAs and intergenic miRNAs were found in *B. glabrata* genome and were characterized thermodynamically, structurally and phylogenetically. Most *B. glabrata* miRNAs displayed conservation in primary and secondary structure highlighting the main part of the precursors, mature sequences and their seed region. Most of the *B. glabrata* precursor miRNAs demonstrated conserved phylogenetic distribution with their orthologs from animal species. Different conserved precursor miRNAs (36) were identified in 12 organ RNAseq libraries (ovotestis, digestive gland, stomach, albumen gland, heart/APO, terminal genitalia, mantle edge, kidney, headfoot, salivary glands, central nervous system and buccal mass). The discovery of these small RNAs and their processing pathway in *B. glabrata* opens a range of opportunities for the study and better understanding of the *B. glabrata/S. mansoni* interaction. In addition, it makes these potential molecules targets for the search for new therapies for the control of schistosomiasis.

Support: Fapemig, CNPq, Fiocruz and UFU

Roundtable 7

New trends on the intermediate host

New Approaches to the Study of Snails Involved in Schistosomiasis Transmission

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There is today a renewed emphasis on the importance of the snail host in transmission, control and elimination of schistosomiasis, and it is gratifying that our underlying understanding of the biology of schistosome-transmitting snails is rapidly increasing on many fronts. However, we must also acknowledge that there is a shortage of expertise within medical malacology, even for basic but critically important topics like proper identification of snail species, and in understanding the roles of specific snail species in transmission. For this presentation, first a summary will be provided of discoveries stemming from recent RNA seq studies of schistosomes in snails. Such studies provide both distinctive new overviews for how schistosomes affect snail transcriptional responses and also highlight a number of key genes worthy of further study for their central role in schistosome-snail interactions. Next to be discussed will be recent field-oriented studies on the impact of biodiversity on the transmission of *Schistosoma mansoni* in and around Lake Victoria, focusing on the role played by competing trematodes in modulating schistosome transmission. Then various approaches to snail control will be discussed. I will conclude by accentuating the fact that we need to know much more about the basic biology of schistosome-transmitting snails in the wild, including the impacts on them of climate change, invasive species and other impending environmental changes. There is a need for innovative new approaches for how to both diagnose and control schistosomiasis beneath the waves, approaches that are preferably both specific and environmentally acceptable so they can attain acceptance for use in the modern world.

Supported by NIH R37 AI101438.

Roundtable 7

New trends on the intermediate host

Strategies for snail control and surveillance: *the neglected essential component for effective schistosomiasis control*

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Because the life cycle of schistosomiasis has for a very long time been fully understood, the parasite's vulnerable points are known. Treatment of infected individuals (definitive host) cures both intestinal and urinary forms of the disease. The drug of choice, praziquantel, is safe and efficacious against both adult and egg stages. Indirectly killing the parasite through targeting intermediate host snails ensures that no infective cercariae are released into water to infect the definitive host. Snail control is an option for schistosomiasis control that has stood the test of time and its impact demonstrated in many endemic countries for the disease. Although snail control can be used to complement preventive chemotherapy programmes, this aspect has become very controversial from an environmental perspective because other non-target organisms may be affected. Provision of safe water supplies ensures limited contact of vulnerable individuals with water infested by cercariae and hence protects them against being infected. In-addition, provision of latrines limit contamination of natural water with urine or stool that may contain schistosome eggs and hence reduces the force of transmission by restricting the number of miracidia that penetrate snails and multiply. The strategy for controlling schistosomiasis recommended by WHO seriously underplays the need to control or eliminate (in certain habitats) snails and the importance of water and sanitation. The WHO Global vector control response 2017–2030 mentions the word snails only two times compared to the 30 times that it mentions the word mosquito while the overall strategy recommended for controlling schistosomiasis mentions, snail control, and provision of water and sanitation as value adding. In this paper, we review the efforts to control snails and the impact that has had on schistosomiasis transmission as well as in reducing the burden of the disease. We highlight the challenges of the approaches and direct the audience to the more innovative methods that may be used for snail control in an integrated schistosomiasis control programme. The discussion on snail control is categorized as follows; i) biological, ii) molluscicides (synthetic and plant based), iii) environmental and iv) engineering.

Roundtable 7

New trends on the intermediate host

Integrated transcriptomics, proteomics and ultrastructural analysis to evaluate the schistosomastatic effect of a natural molluscicide

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Introduction Schistosomiasis has been reported in 78 endemic countries and affects 240 million people worldwide. The digenetic parasite *Schistosoma mansoni* needs fresh water to compete its life cycle. There, it is susceptible to soluble compounds that can affect directly and/or indirectly the parasite's biology. The cercariae stage is one of the key points in which the parasite is vulnerable to different soluble compounds that can significantly alter the parasite's life cycle. Molluscicides are recommended by the World Health Organization for the control of schistosomiasis transmission and *Euphorbia milii* latex is effective against snails intermediate hosts. **Methodology:** We used parasitological tools and electron microscopy to verify the effects of cercariae exposure to natural molluscicide (*Euphorbia milii* latex) on morphology, physiology and fitness of adult parasite worms. In order to generate insights into key metabolic pathways that lead to the observed phenotypes we used comparative transcriptomics and proteomics. **Conclusions/Significance:** We describe here that the effect of latex on the adult is not due to direct toxicity but it triggers an early change in developmental trajectory and perturbs cell memory, mobility, energy metabolism and other key pathways. We conclude that latex has not only an effect on the vector but applies also long lasting schistosomastatic action. We believe that these results are of interest not only to parasitologists since it shows that natural compounds, presumably without side effects, can have an impact that occurred unexpectedly on developmental processes. Such collateral damage is in this case positive, since it impacts the true target of the treatment campaign. This type of treatment could also provide a rational for the control of other pests. Our results will contribute to enforce the use of *E*. *milii* latex in Brazil and other endemic countries as cheap alternative or complement to mass drug treatment with Praziquantel, the only available drug to cure the patients (without preventing re-infection).

Supported by: Wellcome Trust (Flatworm Functional Genomics Initiative (FUGI)-107475/Z/15/Z), CNPq fellowships (205872/2014-0 – SWE), CAPES Parasitologia Básica/2011.

Roundtable 8

Diagnostic tools in post-elimination settings

The use of Helmintex test for monitoring interruption of schistosomiasis transmission

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Morbidity of schistosomiasis has decreased in Brazil and prevalence is also reducing after many decades of successful control programs coordinated by the Ministry of Health. There are many low endemic areas across the country. But it is also eventually expanding, like the introduction of the parasite in its southernmost transmission focus in Brazil and South America, the locality of Esteio. Our research group has been involved since 1997 in the efforts to investigate the transmission in Esteio. The recently established focus presented with very low prevalence and intensities of infection, presenting the challenge of lack of sensitivity of classical egg-detecting methods. Molecular methods would be an alternative, but the lack of a gold standard for performance evaluation is also a problem. Schistosomiasis transmission in Esteio is now probably interrupted, but the challenge persists: sensitive diagnostic methods to certify elimination. We discovered the possibility of isolation *Schistosoma mansoni* eggs from 30g of feces, through interaction with paramagnetic particles in a magnetic field. Helmintex (HTX) is based on this mechanism and is a very sensitive egg-detection method: 100% with egg burdens higher than 1.3 eggs per gram of feces (epg). A field study in a highly endemic area has shown that HTX is 3 times more sensitive than Kato-Katz thick smear method. It also has demonstrated the limitations of rapid, point of care, antigen detection method (POC-CCA), especially in individuals with lower epg and low positive predictive value due to 38% false-positive POC-CCA results. There are several ongoing developments with HTX: (i) a fluidic tridimensional sieving process, (ii) a magnetic-gradient probe for isolation of eggs, (iii) radiant energy emission for detection of eggs. These improvements have the potential for greatly reducing time and effort to perform HTX. Time for detection of eggs by microscopy has already been reduced by introduction of detergents in the sediment suspension and staining of eggs with ninhydrin. The main role for HTX is as a reference method, with confirmatory result based on detection of eggs. Magnetic detection of eggs enables for the first time the evaluation of performance of molecular and immunological methods with an adequate gold standard. Modeling the comparative study of HTX with other methods, through Latent Class Analysis has confirmed the estimation produced by a consolidated standard reference. With a proper and extended evaluation of performance, several diagnostic methods may be combined for a cost-effective investigation of epidemiological status, elimination monitoring and certification of transmission interruption of schistosomiasis.

Support: CNPq and CAPES.

Roundtable 8

Diagnostic tools in post-elimination settings

CAA and CCA detection in schistosomiasis: ASSURED diagnostic tools to be employed when moving from control to elimination.

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The renewed interest in mapping, intensified control and elimination of schistosomiasis (World Health Assembly Resolution WHA 65.21) has put the need for highly accurate diagnostic assays high on the agenda. Based on the well-studied schistosome antigen detection (CCA and CAA) ELISA's, a visual, field-friendly point-of-care urine test for CCA and a quantitative, ultra-sensitive reader-assisted assay for CAA have been developed. The CCA test is commercially available and may replace the Kato-Katz for prevalence mapping of community-level *S. mansoni* infections using a single drop of urine and also allows quick evaluation within days of treatment efficacy. The recently developed test for CAA is applicable to serum or urine of all schistosome species at sub-picogram levels, which allows finding single worm infections. The assay has been transformed into a robust, dry-reagent test, used in several low-resource settings in Africa. Recent studies using the 2 ml urine format show that in near-elimination settings in China, South-East Asia, Africa and Brazil, prevalence of active schistosome infections by egg microscopy may be underestimated up to 10-fold. Alsodecrease of CAA serum and urine levels after treatment has been shown in all effectively treated cases, also indicating that complete cure rates are very limited. In combination with optimized sampling schedules involving adequate pooling strategies and (sub) regional identification of potential transmission sites, the CAA could rapidly identify foci of low prevalence/intensity of all human schistosome infections. The CAA strip assay therefore presents itself as a highly accurate diagnostic tool, with a clear value for application in control and elimination settings. Initiatives supported by FIND, Merck and the Gates Foundation will have to lead to commercial production and availability in the near future.

Roundtable 8

Diagnostic tools in the post-elimination settings

Challenges in diagnosis of schistosomiasis in patients with low parasite load

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Introduction: One of the greatest problems in epidemiology and control of schistosomiasis is the difficulties in detecting the infection in patients with low parasite load and additionally, to control the cure after therapy. In Brazil, for instance, the great majority of infected people present few eggs in feces and are very hard to be detected as positive using 1 or 2 slides of Kato-Katz method, as recommended by the Brazilian Health Ministry. **Methods:** Our group developed new methods for the improvement of the diagnosis of schistosomiasis, for use in endemics areas, with patients with low worn burden. We use the parasitological technique of Gradient saline and immunodiagnostic separation with magnetic microspheres and improvement in POC/CCA by urine concentration (liophylization and millipore filter). **Results:** The techniques used presented good performance in sensitivity and specificity . The concentration of urine clear up the concept of “trace” in the reading of POC results. In people with result “trace” and with eggs in feces, after urine concentration, 90% changed “trace” to positive. On the other hand, the “trace” in individuals with negative results in feces examination, maintained the results as “trace” after concentration. **Conclusion:** The obtained results showed promising perspectives for the use of this technique and for the improvement epidemiological studies.

Supported by: CNPq/Fiocruz/MS/DECIT

Roundtable 9

Persisting schistosomiasis transmission

High-risk tourism in vulnerable areas

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Introduction: The Traveler's Health is a government sector that provides information to protect travelers about the risks when visiting a country, in order to protect their health. The state of Pernambuco, Brazil, has a mild climate throughout the year and a wide network of natural waters formed by rivers and waterfalls that are attractive for aquatic recreation. The practice of dumping sewage into natural or artificial freshwater has established schistosomiasis disease in rural resorts and water parks where travelers are exposed to water contaminated by fecal material and can develop the acute form of the disease. In this state schistosomiasis is endemic in the rural zone with foci occurring in coastal areas, presenting high rates of human infection, severe clinical forms and expressive number of deaths. **Objectives:** Identify and spatialize the risk for schistosomiasis transmission in localities of Pernambuco with aquatic environments for recreational tourism. **Methodology:** The areas for this study were selected in the Pernambuco Touristic Rural Guide and by active search in lodging sites. In the selected localities, malacological surveys were carried out during 2016-2017 to identify and georeferencing breeding sites and collecting snails vectors of schistosomiasis. The snails were examined in search of the positivity for the *Schistosoma mansoni* by the technique of light exposure and by molecular diagnosis. The breeding sites identified were categorized as natural (lagoon, river, waterfall) and artificial (pools, fishing grounds, ditches). The collected data were analyzed and spatialized pointed out the areas of risk through regression statistical techniques with spatial analysis and Kernel estimation. **Results:** In 72 analyzed locations were found 33 breeding sites and 13 foci of schistosomiasis vectors. Thematic maps shows the geographical distribution of breeding sites, quantities and species of schistosomiasis molluscs, highlighting 02 breeding places in Itamaracá and Porto de Galinhas, that are important places of local leisure and tourist destination, where a large quantity of infected *B. glabrata* was found. The maps indicate the location of the breeding sites as well as the intensity of the risk of transmission of the disease in each outbreak, calculated on the basis of number and infectivity of the snails. Themathic maps shows the "hot spots" of each event, allowing future analyzes to predict the epidemiological and spatial dimension of the foci. The descriptive model located unhealthy tourist sites frequented by people seeking leisure and who become vulnerable to *S. mansoni* infection. It is necessary to expand this knowledge to evaluate the environmental characteristics responsible for maintaining the foci of disease vectors, producing more significant information that can be reverted in prevention strategies to minimize the impact of the disease transmission to travelers and local populations. **Conclusion:** The thematic maps points tourist recreational aquatic environments with risk of schistosomiasis transmission in Pernambuco. Traveler agencies and sanitary surveillance should be responsible for providing adequate information to travelers seeking water recreation in countries where contaminated environments are still a reality.

Roundtable 9

Persisting schistosomiasis transmission

Effect of recurrent treatment in areas of persisting transmission

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Introduction: Although transmission levels of *S. mansoni* are significantly lower than in the 1970's, Brazil remains a patchwork of high and low transmission areas. This is in contrast to some areas in Africa where the full force of transmission can be seen over wide areas. All problems with transmission would of course be solved by adequate sanitation, but until this becomes universal, Brazil may provide a preview of Africa's endgame in schistosomiasis elimination. In many areas, partial adequate sanitation has been achieved, yet schistosomiasis has not been eliminated. Studies in rural and urban areas have produced some telling indications of what the parasite population experiences under pressure from repeated treatments and reveal important aspects of the dynamics that help maintain the infection. **Methods**: In 2 small rural communities, there were repeated treatments of all identified infections every 2 years for 8 years. Before each treatment, we performed epidemiologic surveys of the whole population and genotyped eggs isolated from whole stool samples of all those infected using 15 microsatellite markers. All those infected were treated with praziquantel and if eggs persisted in stool 4-6 weeks later, they were re-treated. This same approach was taken in 3 urban areas. **Results**: In the rural communities, there was a constant decline in prevalence from 45% down to <6%. There was little evidence of human or parasite migration. Community-wide egg production decreased 20-50-fold and Ne fell from >30,000 to <1000. Each of the 3 urban foci of schistosomiasis surrounded a surface water collection. Two were associated with urban agriculture, and were found to have prevalences >20%. The other had a prevalence of only 2%. Genetic analysis indicated that in the high prevalence areas, transmission was primarily local. The genetic evidence in the rural area as well indicated mainly local transmission and little immigration. In none of these areas was there phenotypic or genotypic evidence of selection/resistance. **Conclusion**: The levels of partial sanitation in Brazil and community-wide treatment that includes adults may account for the good long-term responses to praziquantel. Also the starting parasite diversity in Brazil was lower than we observed in Kenya, which may be a contributing biologic factor. Treatment reduces parasite diversity, and Low Ne counterbalances adaptive selection. Schistosome infections in this part of Brazil exists on a fragmented landscape with weak connections between nodes of infection. As sanitation improves, the widespread and intense use of praziquantel is recommended.

Supported by: National Institutes of Health, USA, Fulbright Scholar Program, Conselho Nacional de Desenvolvimento Científico e Tecnológico.

Roundtable 9

Persisting schistosomiasis transmission

Schistosomiasis Outbreak in Lençóis, Bahia.

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Introduction: Schistosomiasis still persists as a serious public health problem in Brazil, especially in impoverished rural and urban areas. Control depends on the universalization of primary health care and the strengthening of other public policies capable of promoting better living conditions for the population. Lençóis is one of the 24 municipalities that make up the Chapada Diamantina in Bahia. It has an estimated population of 11,636 inhabitants (IBGE, 2017) and a population density of 8.12 hab / km². Known for its natural beauty in the year 2017, it emerged on the national scene with the news that tourists from the state of Minas Gerais - Brazil, were contaminated by schistosomiasis after a river bath. The present study aims to describe the epidemiological situation of schistosomiasis in the municipality of Lençóis in the year 2017 and the measures of prevention and control of schistosomiasis adopted according to the Technical Guidelines of the Control Program of Schistosomiasis (PCE). **Methods:** This is a descriptive study, with the municipality as unit of analysis. Indicators of positivity, treatment and *Biomphalaria* (*sp*) infected by *Schistosoma mansoni* were described. Data were obtained from the Schistosomiasis Control Program Information System (SISPCE), processed using the Datasus Tabwin data tab, version 4.14, and the Microsoft Office 2013 excel package. **Results:** The mean positivity found was 10.1% and 99.3% of the positives were treated. In the healing evaluation stage, the mean positivity was 3.1%. In the malacological survey, snails of the genus *Biomphalaria* (*sp*) were captured in water bodies of 15 of the 58 sites surveyed, and *S. mansoni* infected snails were found in 05 locations. The prevention and control measures were consolidated in an Action Plan initially developed by the municipal and state health team and updated by the members of the State Committee of Endemias da Chapada, instituted in January 2018, where they are monitored through monthly meetings. Prominent actions: two public hearings, monthly monitoring of water collections, establishment of the municipal mobilization committee, prevention and control of schistosomiasis, training of multipliers in health, education, tourism, social assistance and community representatives, insertion of signaling signs in an area with risk and without risk for schistosomiasis, elaboration of the Project of a pier in the locality with greater positivity for humans and snails; intensification of actions of environmental education in health in the community and prioritization of public investments for universal access to basic sanitation services, especially drinking water supply and sewage services. **Conclusions:** The positivity in the municipality of Lençóis in 2017 may be related to the discontinuity of the actions recommended by the PCE, as well as the absence or inadequacy of public water supply and sewage services in localities with *Biomphalaria* (*sp*). The experience of the State Committee of Endemias da Chapada has been encouraging the integration of several actors necessary for the control of schistosomiasis, subsidizing municipal management in the implementation of the guidelines recommended by the PCE.

Roundtable 10

Clinical and Pathological aspects

Line of care for chronic and severe schistosomiasis patients: the experience of the decentralized care and referenced network in the state of Pernambuco, Brasil.

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The prevalence of schistosomiasis has reduced in the last years and has been observed a significant reduction in *S. mansoni* severity indicators in Brazil. Although had still been noticed cases of Hepatosplenic forms in the state of Pernambuco which entails almost 200 cases of deaths/year.

Based in an evaluation made in the Emergency Hospital during a year in Recife, had been observed that seventy percent of the cases of bleeding for esophageal gastric varices admitted had been caused by schistosomiasis. Furthermore, the mortality rate of these cases has been related to rebleeding, to patients age and the hepatic disease severity.

Therefore, the aim of the analysis has been to demonstrate the corresponding data of the follow-up of schistossomotic patients in the Hospital das Clinicas da Universidade Federal de Pernambuco, during the last seven years (2010-2017).

During this period 1394 patients was attended, in which 57,32%, were female and median of age 60 (46-68) years. In addition, 25,7% of them, has already been splenectomized due to bleeding episodes. The patients, were recruited from Units of Health of Pernambuco state: 54% from Zona da Mata; 23% Região Metropolitana do Recife; 20% from Agreste and 1% Sertão, which represents the disease distribution in the state.

Moreover, the following using ultrasound between two to seven years after the first exam and the disease treatment of the disease was performed in 621 patients (44,55%). The ultrassound results has been shown that the periportal fibrosis became stable in 68,1%, improved in 22,9% and became worse in 14% of the cases.

In summary, has been hypothesized that the chronicity of the disease, advanced age and absence of contact with contaminated water for more than 10 years was related in the majority of the patients as cause of the disease stabilization in almost 70% of the cases.

Roundtable 10
Clinical and Pathological aspects

Validation of home-based sampling and vaginal self-sampling for the diagnosis of female genital schistosomiasis (FGS) in Zambian women with and without HIV seroconversion. The BILHIV study.

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Introduction: Female genital schistosomiasis (FGS) affects over 45 million women worldwide and in sub-Saharan Africa, it is possibly the most underestimated gynecological affliction caused by an infectious agent, the parasite *S. haematobium* (Sh). FGS is associated with genital symptoms and infertility. Diagnosis is challenging, as it relies on expensive equipment that is seldom available in resource limited areas. There is evidence of a fourfold increase in HIV prevalence in women with Sh. The BILHIV study is nested within the HIV prevention trial, HPTN 071 (PopART) in Livingstone, Zambia. The aim of the study is to validate home-based self-sampling strategies for the diagnosis of FGS against vaginal lavage as gold standard, in women with/without HIV seroconversion. **Methods:** Ongoing recruitment from January- September 2018, after PopART's follow up at 36 months. Women ages 18-31 are approached at home from two districts (Dambwa, Maramba) and are given self-collection swabs (vaginal and cervical) after a thorough explanation, they provide a single 10 ml urine sample and fill out questionnaires on gynaecological symptoms and acceptability. They then attend a cervical cancer-screenning clinic where a trained midwife performs a vaginal lavage and a colposcopic evaluation with a point-of-care device. Routine cervical cancer screen is offered. Samples are stored at -80 C for and will be processed in The Netherlands for circulating anodic antigen (CAA), Sh DNA and inflammatory biomarkers. All women testing positive for *S. haematobium* and/or suspicious genital lesions are offered praziquantel. Treatment for other infections/pathologies is facilitated as per national guidelines. **Results:** To date, 538 women have been recruited to the study with 83% clinic follow up (446/538). Median age was 24 (IQR 22-27); 439/538 (81%) have colposcopic images available. Urinary *S. haematobium* egg-detection was significantly different in both districts: 9.1 % (23/254) in Maramba and 2.5 % (7/280) in Dambwa. Self-swabs are well accepted by participants. Abdominal pain is the most commonly reported symptom followed by dyspareunia (pain with coitus), vaginal discharge, fear of pain/bleeding after coitus, dysuria, vaginal ulcers and bleeding. CAA, PCR and colposcopic image analysis are ongoing. HIV seroconversion data will be released to the BILHIV study by PopART by the end of 2019. **Conclusions:** This study is an innovative approach to FGS diagnostics at the community level. Results have the potential to be used at scale for increased FGS surveillance and strengthen the FGS-HIV association.
Supported by the Wellcome Trust, UK

Roundtable 10

Clinical and Pathological aspects

Update and proposed improvement of the WHO-Niamey-Belo Horizonte point-of-care-protocol for ultrasound abnormalities due to *Schistosoma mansoni*

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In 2000 a standardized protocol for field ultrasound examinations of hepatosplenic schistosomiasis was published by the WHO. During the following years, practical experience with this protocol was obtained by schistosomiasis working groups worldwide. The following improvements of the protocol are proposed based on a systematic analysis of the respective publications.

For standard investigations: 1. Omit all measurements except for the portal stem. 2. The ultrasonographist should have a second image pattern (IP) option in order to obtain a more finegrained grading also covering “in-between”-findings and to reduce intra- and inter-observer variance. 3. Risk scoring for gastrointestinal bleeding should be simplified by a score built by the IP-score for portal fibrosis and the portal vein quotient (PVQ=PV diameter/height). 4. Gallbladder abnormalities including external echogenic wall protuberances, sludge, calculi as well as the result of an ultrasonographic Murphy manoevre should be part of the standard protocol.

Additional investigations should include: 1, Assessment of height-adjusted spleen length and depth should be used for evaluating its relation to portal hypertension and hypersplenism at least in non-malaria endemic areas as well for their regression after therapy. 2. Portal flow and porto-systemic collaterals may be assessed more accurately with use of more sophisticated portable ultrasound machines including Doppler facilities 3. Intestinal lesions might be assessed more accurately with high frequency transducers, and hydrosonography. 4. Gallbladder contractility after a fatty meal may also be assessed sonographically.

All reports and publications on hepatic abnormalities due to schistosomiasis must state if the patients studied have also been screened for co-infections due to HBV, HCV, or HDV.

The nature and dynamics of liver abnormalities, portal circulation and spleen abnormalities encountered in hepatosplenic schistosomiasis can be further explored with other imaging techniques including liver and spleen elastography, contrast ultrasonography, CT or MRI in countries with access to high technology imaging.

Roundtable 10
Clinical and Pathological aspects

Pathogenesis of Schistosomiasis Fibrosis

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Introduction: Schistosomiasis is a major cause of liver fibrosis and portal hypertension worldwide. Although the severe pathologic manifestations of the disease were described by Symmers in 1904 and have been a highly study topic ever since, the mechanisms driving schistosomiasis-associated fibrosis are not fully elucidated. The majority of infected individuals (96%) evolve to an oligosymptomatic chronic phase, the hepatointestinal form of disease. These individuals have only granulomas and minimal liver fibrosis, limited to the granuloma reaction. A small percentage of infected individuals (4%) develop hepatosplenic schistosomiasis mansoni, characterized by severe periportal fibrosis (Symmers clay pipetem fibrosis) and portal hypertension. The reason why only a small percentage of infected individuals develop severe disease remains an open question. This gap in knowledge has consequently limited the development of both effective treatments (especially for the sequela of Schistosoma spp. infection) and of non-invasive biomarkers. Praziquantel treatment is highly efficacious in eliminating the adult worms but, in most severe cases, it is not sufficient to revert the hepatic lesions. Therefore, understanding the host-parasite interactions in Schistosomiasis could help us to understand the mechanisms underlying the pathogenesis of fibrosis and portal hypertension and develop appropriate interventions to ameliorate or even revert the lesions generated in chronic infections. The anatomical picture of the liver in hepatosplenic schistosomiasis was fully characterized by Bogliolo in 1957. His studies demonstrated that the fibrotic lesions are pathognomonic of schistosomiasis and different from liver cirrhosis. Andrade and Cheever (1971) clearly demonstrated the importance of the vascular changes in the pathogenesis of hepatosplenic schistosomiasis, particularly the portal vein obstruction and compensatory hepatic artery hypertrophy. Althought the morphologic and physiologic aspects of Symmers' fibrosis and portal hypertension are well understood, the molecular mechanism is still obscure. Recently the Hedgehog, Osteopontin and IL13 pathways have been implicated in liver fibrosis. Ours aims were to investigate if those pathways also play a role in schistosomiaisis fibrosis and if they could be good candidates for pharmacological intervention and biomarkers of morbidity. **Methods:** Hedgehog/Osteopontin/IL13 pathways were investigated in the plasma and liver biopsies of healthy controls, hepatointestinal and hepatosplenic schistosomiais and in murine models of schistosomiais. Hedgehog and IL13 pathways were manipulated using pharmacological and genetic tools. **Results:** Hedgehog signaling is upregulated in human schistosomiasis and correlates with IL13, fibrosis stage and severity of portal hypertension. Activation of the Hedgehog pathway in schistosomiasis is highly dependent on IL13-mediated signaling. Hepatic and plasma osteopontin strongly correlates with fibrosis stage and severity of portal hypertension in human and murine schistosomiasis and could be a novel morbidity biomarker. **Conclusions:** IL13, Hedgehog and Osteopontin play a central role in the pathogenesis of schistosomiasis fibrosis and portal hypertension and could be novel therapeutic targets and biomarker candidates for hepatosplenic schistosomiasis.

Supported by CNPq, NIH, Duke University and Stanford University.

ORAL PRESENTATIONS

Oral Presentation 1

Epidemiology, Control, and Health Education

SCHISTOSOMIASIS MANSONI: FORESIGHTED FOR SIGHTED AND UNSIGHTED

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Introduction: Schistosomiasis is a parasitic neglected disease with transmission reported in 78 countries. The World Health Organization estimates that at least 206 million people are at risk of infection in endemic areas. It is a notifiable disease; however, there are marked difficulties in obtaining reliable prevalence data due to considerable underreporting. The use of images has great value in the acquisition of information in Biological Sciences and especially in Parasitology. Video-microscopy through the dynamic aspect of moving and real-time images can provide accurate and reliable information that promotes rapid understanding of evolutionary stages of parasites such as *Schistosoma* sp. It is necessary that all population has access to this information, to have *bona fide* social inclusion. However, people with visual impairments remain excluded from a lot of public health information. **Objective:** Preparation of ludic-educational material for people without and with visual, auditory and speech impairment on the *S. mansoni* life cycle. **Methods:** Preparation of palpable biscuit resin models of the different evolutionary forms of parasite and, elaborating videos documenting these stages of the living helminth. In addition to moving images captured by video-microscopy in real time, explanations are being added in LIBRAS. **Results:** Resin pieces and videos representing/demonstrating the evolutionary forms of the parasite were produced. The material produced has been used in health fairs, TV shows and in events for Health Education, for all types of population. The material produced has been used in health fairs, TV shows and in events for Health Education, for all types of population. **Conclusions:** The enthusiastic acceptance by the target public indicates that empowered population may be engaged in prophylactic measures.

Financial support: CNPq.

Oral Presentation 1

Epidemiology, Control, and Health Education

Educational video about the *Schistosoma mansoni* cycle for undergraduate and graduate students

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Introduction: There is an increasingly evident need for the contextualization of teaching relating to social, philosophical, political, economic and ethical issues. The teaching of parasitology in universities is often based on theoretical and practical classes, limited often the reading of the parasite slides. The students of the Universidade Federal Rural do Rio de Janeiro (UFRRJ) discipline of Parasite Evolution, postgraduate course in Veterinary Sciences were encouraged to build an educational video about the medical and veterinary importance of a parasite. The objective of this work is to describe the creative process of production of didactic material on *Schistosoma mansoni* and the students' experience in the process and its importance in the formation of future teachers of parasitology. **Methods**: The video on the biological cycle of the parasite was filmed at the Laboratório de Avaliação e Promoção da Saúde Ambiental (LAPSA) of the Instituto Oswaldo Cruz - Fiocruz. Puppets were produced from the images of the adult parasite (male and female) and the larger-than-real mollusk *Biomphalaria glabrata* to attract attention. Interviews were conducted by students to the characters (adult worms and mollusk). The questions were asked by students. As part of the teaching-learning process, students have empowered teaching-learning process themselves. **Results**: The video made the parasite and the mollusk (intermediate host) the protagonists of the cycle. The biological cycle in this case was described, during the interview by the adult (male and female) and by the mollusk, as puppets. The parasitic couple described the function of their morphology and the long way until they left offspring, the eggs leaving the faeces. The mollusk presented the cycle of the parasite inside itself and its participation to complete the cycle. In addition to the ludic part with interviews with worms and mollusks, the video also presented the stages of maintenance of the cycle under experimental conditions such as “Swiss” mice infection, spontaneous sedimentation, hatching of miracidia, mollusk infection and cercariae shedding. In addition, it included an interview with the researcher responsible for the cycle and specialist in schistosomiasis. The video was prepared exclusively by the students. **Conclusions**: The educational video production process has provided graduate students with a focus on parasitology, to plan, build and create educational materials that can be used by them in their future classes.

Supported by UFRRJ/Fiocruz and PAEF/IOC/Fiocruz

Oral Presentation 1

Epidemiology, Control, and Health Education

Quality of Life of schoolchildren in a community endemic for *Schistosoma mansoni* in the Jequitinhonha Valley, Minas Gerais

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Introduction: Quality of Life (QoL) has been considered as a reliable way to access the burden of a specific condition. In the case of parasitic disease, in particular schistosomiasis, the approaches of QoL have been recognized as a tool to estimate the impact of the disease that considers its chronic characteristics and evaluates the multiple dimensions that may affect the lives of infected individuals. This study aimed to evaluate the quality of life of schoolchildren infected with *S. mansoni*, residents of the endemic area of Ponto dos Volantes municipality, Minas Gerais. **Methods:** cross-sectional study with 242 schoolchildren between 5 and 15 years of age (121 egg-positive for *S. mansoni* and 121 egg-negative). Parasitological surveys with two stool samples and using the Kato-Katz method were carried out in 2014. Demographic, socioeconomic and Quality of Life information were collected using questionnaires. The Pediatric Quality of Life (PedsQL) 4.0 was used to obtain self-report QoL and parents' perceptions of their children's QoL. The questionnaire evaluated 4 dimensions: physical, emotional, social and school. Besides that, blood and anthropometric measurements were collected to determine hemoglobin (Hg) levels and nutritional indicators. The Mann-Whitney test was used in the comparison of quality of life scores between infected and non-infected children. Ordinal logistic regression model was used to evaluate the factors associated with quality of life. **Results:** poor agreement was found in the evaluation between the students self-reports of the QoL and the report of their parents. Egg-positive children had significantly smaller score for QoL as compared to those egg-negative. This difference was statistically significant for the total score and for all dimensions, except social. In the final model, according to both students' self-reports and their parents, age and presence of *S. mansoni* infection influenced QoL. The presence of infection reduces the chance of having a better QoL in 62% according to students' self-report (OR 0.38, CI 95% 0.23-0.60) when compared to egg-negative students, and reduces in 59% (OR 0.41, 95% CI 0.26-0.66) the chance of a better QoL by parents' report. In addition, sex was significantly associated with QoL by parents' report. **Conclusion:** This was the first study that showed the negative impact of *S. mansoni* infection on the quality of life of children in South America. The PedsQL 4.0 questionnaire was considered a good tool to evaluate children's QoL in an endemic area for schistosomiasis mansoni, since the instrument takes into consideration different dimensions related to the individual. Identifying the disability associated with schistosomiasis becomes essential to understanding the real impact caused by the disease on the individual.

Financial support: National Institutes of Health-Tropical Medicine Research Centers (TMRC, Grant number AI098507); CAPES; FAPEMIG; CNPq; INCT-DT.

Oral Presentation 1

Epidemiology, Control, and Health Education

Mortality by schistosomiasis in Pernambuco, Brazil - 2005 to 2016

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Introduction: Approximately 1.5 million people are infected with *Schistosoma mansoni* (Sm) in Brazil, with 541 deaths reported in 2010, mortality coefficient (IC) of 0.28 per 100,000 inhabitants. In Pernambuco state (PE) schistosomiasis has historically been present in approximately 1/3 of the deaths recorded in every country. **Methods:** A cross-sectional study was carried out with the information on mortality available on the DATASUS / Brazilian Ministry of Health website. Deaths with a basic cause of schistosomiasis mansoni, occurring between years 2005 and 2016 were considered. For the calculation of (IC), population data from the Brazilian Institute of Geography and Statistics (IBGE) were used. In estimating the temporal evolution of the mortality coefficients, we used the percentage variation in the analyzed period. In the spatial analysis we use MapInfo. **Results:** In the period, there were 2,096 deaths from schistosomiasis, an average of 174.6 per year. There was a reduction in the mean by analyzing separately the years 2012 to 2015 (average of 144.7 deaths). The (IC) was 2.28 in 2005 and 1.98 in 2016, with the most significant reduction between 2012 and 2015 (1.77, 1.53, 1.51 and 1.50) respectively. Among all the deaths occurred in the period, the age group => 60 years was more frequent with (69.37%). As for the distribution by gender the highest frequency in women (52.25%). The most frequent place of residence was the I Region of Health (Metropolitan State Region) with 1,075 (50.45%) of the deaths. **Conclusion:** There was a significant reduction in mortality in Pernambuco in the period, especially between the years of 2012 and 2015, increasing again in 2016. The reduction in mortality may be related to the control actions that have been developed in the state in the last seven years such as the collective treatment and intensification of surveillance by teams of basic health units. Specific study is needed to identify factors related to falling mortality, as well as the highest IC in 2016. Improvements in socioeconomic and health conditions are the most effective actions to reduce mortality from schistosomiasis and other poverty-related diseases.

Supported by: IHMT-UNL

Oral Presentation 1

Epidemiology, Control, and Health Education

ANALYSIS OF INCOMPLETENESS OF THE MORTALITY INFORMATION SYSTEM RECORDS ON DEATHS DUE TO SCHISTOSOMIASIS IN PERNAMBUCO, BRAZIL, 2000-2014

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Introduction: Schistosomiasis mansoni is a parasitic infectious disease caused by the helminth *Schistosoma mansoni*, of important worldwide prevalence and relevant impact on public health. **Objective:** To analyze the incompleteness of death records due to schistosomiasis of the Mortality Information System in Pernambuco, Brazil, during the period 2000-2014, and to evaluate the medical knowledge about the completion of the death certificate. **Methods:** Descriptive, cross-sectional study with secondary data from the Mortality Information System extracted from the State Department of Health and primary with medical testimonies in public hospitals of Pernambuco. The relative frequencies of medical knowledge on completing the death certificate, the fills with the ignored and / or blank code of the variables available in the Mortality Information System were calculated. The incompleteness scores were evaluated and the percentage variations between the two study periods (2000 - 2007, 2008 - 2014) were calculated. **Results:** In the period from 2000 to 2014 occurred 2636 deaths by basic cause of schistosomiasis in PE, the majority in the 1st Region of Health (72.2%). In the period 2000-2007 the variable 'establishment of residence' showed the greatest incompleteness (71.7%) with a score very bad in all health regions. In 2008-2014 the variable 'place of birth' showed the worst incompleteness (63.4%) with a score very bad in most regions of health. In the two periods of study, it was observed that 'establishment of residence' means 'place of occurrence', 'date of birth', 'address occurrence', 'neighborhood', 'race/color', 'schooling' and 'occurrence' showed the highest negative percentage variations of incompleteness. However, 'place of birth' and 'marital status' showed a growth of Incompleteness (412.4% and 27.0%). Participated in a survey about the knowledge of the completion of the declaration of death 32 doctors, of which 21.9% worked in the Restoration Hospital being 46.7% specialists in medical clinic and 46.9% were residents who met the declaration of death. All reported knowledge to fill, 71.9% reported that the non-fulfillment of some variables was due to lack of information about the patient, 62.5% never participated in a training course on completion of the declaration of death. And 80.6% thought necessary the course. We observed an association between the causes of non-fulfillment of the declaration of death and the desire of doctors in training to fill the same (p-value 0.022). **Conclusions:** Was the reduction of the incompleteness of some important variables, however there was a significant increase of the incompleteness of the variable 'place of birth' which may be misrepresenting the information about the Autochthony of the place where the event occurred. The rationale of physicians - lack of information about patients - is inconsistent, because they are the ones responsible for capturing the information from.
Keywords: Schistosomiasis; Health Information Systems; Death Certificate; Death.

Oral Presentation 1

Epidemiology, Control, and Health Education

Evaluation of *Schistosoma mansoni* infections before and after treatment in a municipality of the Amazon region in Pará state, Brazil

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Introduction: The prevalence of Schistosomiasis in Brazil has been gradually reduced by actions of the Schistosomiasis Control Program, which uses the Kato-Katz technique for detection of *Schistosoma mansoni* infections, as suggested by World Health Organization. However, prevalence variations are observed in the Brazilian territory, ranging from high to medium levels in northeastern and southeastern regions and with low prevalence areas in the north and south of the country. Despite of ongoing control efforts, data on cure rate, reinfection and new cases after treatment in northern Brazil, are rare and often not published. This study aims to verify the occurence of schistosomiasis before and 360 days after treatment in the municipality of Primavera, in Amazon Region, Pará state, Brazil. **Methods:** This is a longitudinal, investigative and descriptive research, with an observational design. After obtaining ethical clearance and completion of the consent form, a total of 358 individuals participated in the study. The *S. mansoni* infection rate was evaluated in two moments, before tretment and one year after treatment. Three different stool samples per participant were analyzed according the Kato-Katz technique to verify the presence of parasite eggs. A total of 12 slides were prepared of the first sample, two slides of the second and two slides of the third sample, totaling 16 slides. Positivity rate, i.e. the number of positives detected over the total population at risk was calculated. One year after treatment all 358 participants were re-examined. The positivity rate, the number of new cases and re-infected participants as well as the cure rate, defined as number of individuals cured after treatment divided by the total number of positives before tretament were calculated. All positive cases were treated orally with Praziquantel according to the guidelines of the Brazilian Ministry of Health. **Results:** The infection prevalence with *S. mansoni* before treatment revealed 11.4%, corresponding to 41 subjects and after treatment 2.7% corresponding to 10 individuals. Out of these 10 positive cases, 9 were indentified as new cases and one was considered as re-infection or treatment failure. **Conclusion:** Our data demonstrate that treatment is effective, revealing a cure rate of 97.5%, considering the one case, which remained positive one year after treatment. Nevertheless, the nine cases of new infections prove that disease transmission in the area is maintained and further studies are required to develop strategies to interrupt the dynamics that drive the propagation of *S. mansoni* infections under these circumstances.

Supported by UEPA, CAPES, CNPq, Instituto Evandro Chagas

Oral Presentation 1

Epidemiology, Control, and Health Education

Development of a natural molluscicide prototype kit (MoluSchall) for the control of schistosomiasis mansoni transmission

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Introduction: Schistosomiasis mansoni is a parasitic disease caused by the trematode *Schistosoma mansoni*. In Brazil, snails *Biomphalaria glabrata, Biomphalaria tenagophila* and *Biomphalaria straminea* are found naturally infected, maintaining disease transmission. The use of a molluscicide is one of the forms of transmission control. *Euphorbia milii* latex has shown promising results enabling its use as an alternative molluscicide. Therefore, the aim of this work is to develop a natural product derived from *Euphorbia milii* latex. **Methods:** The latex was collected, processed, aliquoted, weighed, frozen at -80°C and lyophilized. The lyophilized product was rehydrated with two aqueous solutions ("Diluent 1" and "Diluent 2") and tested, in triplicate, at the dilutions of 1; 2; 4; 8 and 12 µl/L in water against specimens of *B. glabrata, B. tenagophila* and *B. straminea*. As control three groups of 10 snails of each species were placed into beakers only with water and maintained under the same environmental conditions. After proof of concept, a prototype kit was produced according to LD_{100}analyzes for *Biomphalaria* species, being called MoluSchall. Each prototype kit is composed of 10 vials containing lyophilized latex, 10 vials of "Diluent 1" (5 ml) and 10 vials of "Diluent 2" (5 ml). Stability test was carried out using prototype kits stored at temperatures of 2-8°C, 22-26°C and 37°C. After every two months, one vial of a kit stored in the each temperature and its biological activity was evaluated. Toxicological assay was carried out using *Danio rerio* (zebrafish) submitted to the dilutions of 8; 16; 32; 64 and 128 µl/L. In addition, two artificial lakes containing *B. glabrata* were treated with MoluSchall at 8 and 12 µl/L of water. **Results:** The LD_{100} values were 4µl/L for *B. tenagophila* and 8µl/L for *B. glabrata* and *B. straminea.* The MoluSchall presented toxicity for *D. rerio* only in the dilutions 8 (64µl/L) and 16 (128 µl/L) times higher than the LD_{100} defined for *Biomphalaria* spp. The product demonstrated stability and biological activity for 24 months, regardless of storage temperature. In the artificial lakes, the results showed 100% mortality among *B. glabrata* exposed to the 8 and 12 µl/L. **Conclusions:** The prototype kit MoluSchall showed efficiency in the elimination of the three snails species, low toxicity to the *D. rerio* and stability for a period of 24 months. The LD_{100} defined in the laboratory for *B. glabrata* was kept when the product was used in semi-natural conditions. Thus, each prototype kit allows to treat 12,500 liters of water. Therefore, MoluSchall represents a natural molluscicide and a feasible alternative strategy for transmission control of *Schistosoma mansoni*.

Supported by FAPEMIG/IRR/FIOCRUZ

Oral Presentation 1

Epidemiology, Control, and Health Education

Past, present and future of snail control for schistosomiasis elimination

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Introduction: Control strategies to reduce human schistosomiasis have evolved from 'snail-picking' and 'swamp draining' campaigns, more than a century ago, to modern preventive chemotherapy campaigns focused on large-scale delivery (mass drug administration) of praziquantel to people. Unfortunately, despite the rise in mass drug administration in the last several decades, just as many people suffer from schistosomiasis today as did 50 years ago. History has shown us that snail control can complement preventive chemotherapy by reducing the risk of transmission from snails to humans, yet many campaigns today focus solely on medication, in part because of a capacity gap to identify hotspots of schistosomiasis transmission in the environment. New technologies might help to modernize risk mapping and enable more targeted snail control. **Methods:** Here, we present ideas for modernizing and scaling up snail control, focusing on two promising ideas: creative use of natural enemies to control the abundance of infected snails (such as augmenting snail predators) and utilizing computer vision to target control measures to high risk environments, especially in low-income countries with limited capacity. **Results:** Focusing on a case study in northern Senegal, we demonstrated lower human reinfection after praziquantel treatment in an area protected by re-introduced river prawns -- voracious predators of snails -- compared to a control area without prawns. We also provided a proof of concept that computer vision can assist in snail and cercarial identification, with accuracy rivaling well-trained human technicians. **Conclusions:** We conclude that promising new environmental diagnostics and snail control strategies will be useful to achieve the World Health Assembly's stated goal to eliminate schistosomiasis.

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

Immune modulation through maternal *Schistosoma mansoni* infection

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Introduction: Infection with the parasitic helminth *S. mansoni* is characterized by an initial Th1 inflammation, followed by an excessive egg-induced Th2 response. During the course of infection a long term immunosuppression (Reg phase) is established, protecting the host against overwhelming inflammatory responses. This immunosuppression is associated with a reduced host response against parasite antigens as well as bystander antigens, such as allergens. Besides allergy suppressing effects within the infected host, there is recent evidence that schistosomiasis during pregnancy influences the offspring´s allergic responses. **Methodology/principal findings:** We apply transmaternal murine models of chronic schistosomiasis to investigate the effect maternal immune status upon imprinting immune predispositions in offspring. Using experimentally-induced allergic airway inflammation (AAI), exposure to acute, patent maternal schistosomiasis (weeks 8-12 of infection) during gestation increased allergic responsiveness in offspring, which was however strongly suppressed when pregnancy was initiated during pre-patent (weeks 3-5) or late chronic stages (over 16 weeks) of infection. Further, this was associated with infection-phase-specific shifts in placental transcriptional profile as well altered placental cytokine production to schistosome antigens. We further investigated the potential effect of this maternal infection within the immune cell compartments of these offspring, and in our in vivo model began exploring the early sensitization stages of allergic response in these offspring. Culture of T cells has revealed the skewed differentiation of naïve CD4+ T cells from these offspring, with further work revealing this to be associated with the presence of a more activated population of memory-like CD4+ T cells. Our recent work has linked the suppression of AAI in these offspring to an altered response to the earlier sensitization phases of this model, with preliminary results indicating an early shift in cytokine production and responsiveness of CD4+ and CD8+ T cells, associated with an altered character of antibody response to the model allergen. **Conclusion:** We have found that adult offspring from schistosome-infected mothers is strongly modified by the phase of maternal infection. This is associated not only with the profound shifts in systemic immunological profile of these infected mothers as they progress through the sequential (early pre-patent, patent, and late chronic) phases of infection, but with these shifts reflected further as changes at the fetomaternal interface, namely the placenta and associated tissues. Our studies will help to understand the effects of the maternal immune status during pregnancy as a key factor in determining immune outcomes in later life, as we continue investigating the role of maternal immune status during chronic infection in driving underlying steady-state immune alterations in offspring which predispose altered inflammatory and allergic responses.

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

Allergy are positively associated to IL-33 production but inversely associated to high burden of *Schistosoma mansoni* infection and production of IL-10 in endemic area with low parasite burden

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Introduction: Helminth infections and allergies are typical clinical conditions which induce type-2 immune responses. In schistosomiasis, the Th-2 response is triggered by antigens from the parasite eggs and is usually accompanied by the induction of a network of immunoregulatory mechanisms, which contribute to the evolution of less severe schistosomiasis. Moreover, parasite-induced immunomodulatory mechanisms have also been used to justify epidemiological data demonstrating inverse associations between allergy and helminthiasis. However, the modulating effect of helminth infection on allergic diseases is not always observed, and the variations have been attributed to the type of parasite, intensity and chronicity of the infection. In this cross-sectional study, we investigated the relationship between helminth infections and allergy in 257 individuals from an endemic schistosomiasis area, but with infected individuals mostly presenting with low parasite burden. **Methods:** Volunteers from 6 - 75 years of age responded to a socioeconomic and demographic questionnaire and provided fecal samples for the parasitological examinations (Applied methods: spontaneous sedimentation (HPJ), Kato-Katz, Helmintex and saline gradient) and for molecular detection (RT-PCR for *Schistosoma*). Peripheral blood served for a complete hemogram, and for quantification of cytokines (IL-27, TNF-a, IL-1b, IL-10, IL-33, IL-13, IL-6, IL-17, IL-5), chemokines (CXCL-10, CCL-11, CCL-17, CCL-5, CCL-3), as well as for total IgE and specific IgE against house dust mite antigens in obtained serum samples. Multivariable logistic regression models were used to evaluate the relationship between allergic reactivity and covariates, such as immune response and presence of parasites. **Results:** IgE reactivity against dust mite allergens were detected in 47 individuals (23.8%) and 140 individuals (54.4%) were diagnosed with schistosomiasis. However, the majority of schistosome-infected individuals (n=108) presented with very low parasite burden (<12 eggs/g of feces). When compared with non-infected individuals, the intensity of allergic reactivity was significantly lower ($p<0.05$) in *S. mansoni*-infected individuals. Multivariate analysis adjusted by age revealed that allergic reactivity was positively associated with high IL-33 serum concentrations (>100 pg/ml) and negatively with a patent *S. mansoni* infection. However, the modulatory effect of schistosome infection was shown to depend on parasite burden. Infected individuals with low-parasite burden (<12 epg) showed no interference with allergic reactivity, but infected individuals that eliminated more than 12 epg were 6 times less likely to have allergy. Moreover, reduced allergic reactivity was positively associated with IL-10 production. **Conclusions:** Our study indicated that modulation of allergic reactivity by *S. mansoni* infection depends on parasite burden and on IL-10 production.
Supported by CNPq, CAPES and FAPEMIG

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

Identification of genes regulated by SmJNK and Smp38 MAP Kinase pathways and their functional roles in *Schistosoma mansoni*

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Introduction: MAP kinases have been considered promising targets for drug development and, in *S. mansoni*, their important role in the development, reproduction and/or survival of the parasites has already been demonstrated. Due to the data scarcity regarding kinase functions in the parasite, this work has as main motivation to contribute with experimental characterization of SmJNK and Smp38 MAPKs. Here, we explore the potential of SmJNK and Smp38 as candidates for drug development and identify specific genes regulated by them. **Methods:** First, we performed the knockdown of SmJNK and Smp38 in schistosomula by RNA interference and assessed their transcriptional profile through RNASeq. Differentially expressed genes (DEGs) were identified using DESeq2 package in R program by comparing each dsRNA-treated parasite against parasites not exposed to the dsRNA (control). Expression data were then correlated with biological functions through Gene Ontology and KEGG pathway analysis. In addition, we performed SmJNK and Smp38 knockdown in adult worms through electroporation with gene-specific dsRNAs. We assessed the transcript levels using RT-qPCR and evaluated oviposition and movement in these parasites. **Results:** We identified 606 and 1154 DEGs in SmJNK and Smp38 knockdown schistosomula, respectively. A substantial proportion of DEGs (505) encodes proteins with unknown function and there is a great crosstalk between the targets regulated by both MAP kinases. SmJNK and Smp38 seem to regulate the expression of genes related to antioxidant defense, structural composition of ribosomes, spliceosomes, cytoskeleton as well as purine and pyrimidine metabolism pathways. SmJNK knockdown in adult worms resulted in up to 65% reduction in transcription levels on the 6th day after dsRNA exposure. After knockdown, there was up to 86% reduction in oviposition and the oviposition was interrupted after the 6th day. Using the WormAssay software, SmJNK knockdown male parasites showed an 80% movement reduction on the 8th day of dsRNA exposure. Smp38 knockdown in adult worms resulted in up to 80% reduction in transcription levels on the 10th day, with consequent reduction of 86% in oviposition. **Conclusions:** This work allowed a better understanding of SmJNK and Smp38 MAPKs signaling pathways elucidating targets they regulate at the transcriptional level. The genes identified without known function open new possibilities to find parasite-specific genes that can be exploited as drug targets. Additionally, we demonstrated the importance of these kinases in parasite oviposition and survival in vitro, going further in elucidating their functional roles in adult worms.

Supported by European Commission's Seventh Framework Programme for research, CAPES, FAPEMIG, CNPq, Rede de Plataformas Tecnológicas Fiocruz (PDTIS).

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

Analysis of the hematopoietic dynamics during murine *Schistosoma mansoni* infection with a focus on hepatic extramedullary hematopoiesis

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Introduction: The peripheral zone of murine hepatic schistosomal granulomas may present extramedullary hematopoiesis. However, the possible clonal nature of this cellular expansion, the immaturity level of the settlers and the characteristics of this new hematopoietic-favorable microenvironment, are not clear. **Methods:** Five-days old Swiss Webster male mice were percutaneously infected by 70 *Schistosoma mansoni* cercariae (BH strain). They were euthanized in the 40th, 45th, 50th and 60th days post infection (dpi). Liver fragments were collected, fixed in Millonig's formalin, processed and embedded in paraffin. Five micrometers thick sections were stained with hematoxylin and eosin; Sirius Red pH 10,2; Picrosirius Red and Alcian Blue pH 1,0 for brightfield microscopy. Other paraffin sections and cryosections were submitted to immunofluorescence detection of Ki67, MMP9, vWF and CD31 by confocal microscopy. **Results:** By the 40th dpi, it were observed some sparse eggs and large blood vessels surrounded by hematopoietic cells along with inflammatory cells arriving through hepatic sinusoids. Ten days later, myeloid progenitors, both mature and immature eosinophils and neutrophils were seen in the peripheral zone of most granulomas. The presence of some mitotic cells and positivity to Ki67 show that some cells arrive and proliferate in this region, and confirm the occurrence of extramedullary hematopoiesis. It was also observed some groups of few megakaryocytes expressing vWF, indicating that very early progenitors may be arriving in the liver and differentiate there. The presence of acid glycoconjugates, demonstrated by Alcian Blue pH 1,0, and collagen fibers type I and III, demonstrated by Picrosirius Red, suggest that hematopoietic cells reach a favorable microenvironment composed by important key extracellular matrix molecules that attract and maintain the hematopoiesis in the liver. In addition, some hematopoietic cells, most neutrophils are capable of secreting MMP9, which is important to modulate this matrix and act in the mobilization/ homing dynamics. The expression of CD31 demonstrated an increase of blood vessels during the infection, but it was not possible to detect alleged endothelial progenitor and either confirm the occurrence of angiogenesis or vasculogenesis. **Conclusion:** Here, we proposed to deep the studies about the granulomatous-associated extramedullary hematopoiesis during murine *S. mansoni* infection. Our data suggest that progenitors of different levels of differentiation are recruited to the liver of the infected animals, where they find an appropriate environment to myeloid differentiation both in the peripheral zone of the granulomas and around the vessels. The potential participation of endothelial progenitors has to be investigated. Also, it is still necessary to analyze the changes in the bone marrow and to study the degree of maturity of the hematopoietic progenitors which are circulating in the peripheral blood.

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

Schistosoma mansoni SmKI-1 serine protease inhibitor binds to elastase and impairs neutrophil recruitment and inflammation

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Introduction: *Schistosoma mansoni* is one of the main agents of schistosomiasis, which is the most important human helminthic infection in terms of global morbidity and mortality. Although schistosomiasis represents a major public health problem in endemic countries, evidences show that *S. mansoni* downregulates inflammatory responses in many diseases. Fortunately, the control of inflammatory responses is extended to pathogen-derived antigens, leading us to study one *S. mansoni* Kunitz type protease inhibitor (SmKI-1), found in larval and adult phases of the parasite. In this study, we described the role of *Schistosoma mansoni* SmKI-1 serine protease inhibitor in parasite development and as a molecule capable of regulating different models of inflammatory diseases. **Methods and Results:** First, we determine that recombinant (r) SmKI-1 and its Kunitz domain but not the C-terminal region possess inhibitory activity against trypsin and neutrophil elastase (NE). Additionally, rSmKI-1 markedly inhibited the capacity of NE to kill schistosomes. In order to further investigate the role of SmKI-1 in the parasite, we designed specific siRNA to knockdown SmKI-1 in *S. mansoni*. SmKI-1 gene suppression in larval stage of S. mansoni robustly impact in parasite development in vitro and in vivo. To determine the ability of SmKI-1 to interfere with neutrophil migration and function, we tested SmKI-1 anti-inflammatory potential in different murine models of inflammatory diseases. Treatment with SmKI-1 rescued acetaminophen (APAP)-mediated liver damage, with a significant reduction in both neutrophil recruitment and elastase activity. In the model of gout arthritis, this protein reduced neutrophil accumulation, IL-1β secretion, hypernociception, and overall pathological score. Finally, we demonstrated the ability of SmKI-1 to inhibit early events that trigger neutrophil recruitment in pleural cavities of mice in response to carrageenan. **Conclusion:** In conclusion, SmKI-1 is a key protein in *S. mansoni* survival and it has the ability to inhibit neutrophil function as a promising therapeutic molecule against inflammatory diseases.

Supported by CAPES/CNPq/UFMG

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

The proteomic landscape in the spleen during chronic schistosomiasis: a label-free shotgun approach.

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Introduction: The establishment and development of schistosomiasis have been described to be closely associated with an intense immune response induced by the presence of the different stages of the parasite within the infected individual. There is an important immune dynamic between the host and the parasitic antigenic production, that starts with the contact with the cercarie and immature worms, their subsequent sexual maturation and the commencing of oviposition. While the contributions of different cytokines for the establishment of the chronic disease are still discussed, here we proposed the quantitative examination of the spleen proteome in a mice model, in order to evaluate the differential expression of proteins during chronic schistosomiasis. Using label-free shot-gun proteomics, we offer new insights on the host-parasite interactions and its implications in the development of the immune pathology. **Methods:** A mice model was established in order to simulate a chronic infection by *Schistosoma mansoni*. Spleens were collected and treated for isolation of spleen cells and protein extraction. Spleen proteins were digested with trypsin and peptides were separated on an Ultimate 3000 nano UHPLC system (Thermo Scientific). Spectral scans were acquired in a Q-Exactive mass spectrometer (Thermo Scientific). coupled to the nano UHPLC via a nanoelectrospray ion source. The PEAKS 8.5 software was used for data refinement, protein identification, and label-free quantification, Statistical analysis and data visualization were performed using R programming language (3.5.0) running on R Studio (1.1.442). Pathway analysis and protein categorizations, based on Reactome and KEGG databases, were performed on the packages ReactomePA and clusterprofiler. **Results:** The identification analysis showed the expression of 2500 total proteins in the spleen proteome, organized into 1255 protein groups. After label-free quantification, we found ninety-five proteins upregulated in infected individuals and eighteen proteins downregulated in the same conditions. Upregulated proteins were mostly representative of pathways related to the development of adaptive immune response, cell cycle and DNA replication, translation and transduction and cellular response to hypoxia. The downregulated enriched pathways were related to DNA repair, beta-oxidation and DNA fragmentation and apoptosis. **Conclusions:** This study offers new information on the organization of the proteomic landscape in spleen cells during chronic schistosomiasis. With this and further studies, we believe to be enriching the discussion on the contribution of different immune proteins during the establishment of the chronic disease from a complex perspective.

Finalcial support: Fapemig (APQ-03367-16) / UFOP (23109.003209/2016-98)

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

IL13-STAT6 Mediated Activation of Hedgehog Signaling Regulates Schistosomiasis Fibrosis

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Introduction: IL13 and Hedgehog signaling pathways have both been implicated in the pathogenesis of fibrosis. In this study, we investigated if there is cross-talk between IL13 and Hh pathways in an experimental model of IL-13 induced liver fibrosis and in mouse and human schistosomiasis. **Methods:** Hedgehog/IL13 signaling were investigated by qRT-PCR, immunohistochemistry and ELISA in uninfected healthy transplant donors (n=22), infected hepatointestinal schistosomiasis patients (liver granulomas, low fibrosis, n=17), infected hepatosplenic patients (advanced fibrosis and portal hypertension n=72); in *Schistosoma mansoni* infected mice (wild-type, IL13Rα1-/- and TKO (IL-10-/- IL12p40-/- IL13Rα2-/-) treated with anti-IL13 antibody, Hh inhibitors (Vismodegib or AsO3) or vehicle; in mice overexpressing IL13 (plasmid) and in human liver cells stimulated with rIL13 and treated with STAT6 siRNA or Vismodegib. **Results:** Hedgehog signaling is upregulated in human schistosomiasis and correlates with IL13, fibrosis stage and severity of portal hypertension. Overexpression of IL13 (plasmid, infected TKO mice, rIL13) induced Hedgehog production/activation; lack of IL13 signaling (IL13Rα1-/- infected mice, anti-IL13 Ab, STAT6 siRNA) implicated in reduced Hedgehog pathway, indicating that Hedgehog signaling is dependent on IL13. STAT6 ChIP assay further demonstrated that STAT6 directly regulate the transcription of Hedgehog ligands and transcription factors. Smoothened antagonist Vismodegib effectively blocked fibrosis during acute schistosomiasis but failed to inhibit Hh pathway/fibrogenenesis when treatment was initiated in chronic phase due to Smoothened-independent IL13-mediated Gli activation. Gli inhibition with AsO3 in the chronic phase impaired Hedgehog signaling and fibrogenesis. **Conclusion:** Activation of the Hedgehog pathway in schistosomiasis is highly dependent on IL13-mediated signaling. Targeting Hedgehog pathway with Gli antagonists may be a novel therapeutic strategy to treat schistosomiasis fibrosis and related portal hypertension.

Supported by CNPq, NIH, Duke University and Stanford University.

Oral Presentation 2

Immunopathology, Molecular Biology, and Biochemistry

Schistosomal-derived lysophosphatidylcholine triggers M2 polarization of macrophages through PPARγ dependent mechanisms

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Introduction: *Schistosomiasis mansoni*, parasitic endemic typical of the Americas, Asia and Africa, is caused by the infection of *Schistosoma mansoni* trematodes. Infection by this trematode induces the formation of granulomas in the host and a potent polarization of the immune response to the Th2 type. Prolonged infection can also lead to the modulation of cells of the innate immune system to a profile that regulates inflammation. Helminths may be presented at different stages of their life cycle within a human host, and each stage of development may present different combinations of glycoconjugates recognized in different ways by the host. Recent studies in our group have demonstrated that *S. mansoni* lipids, including lysophosphatidylcholine, play an important role in inducing the production of inflammatory mediators in macrophages. In the present work we investigated the role of *Schistosoma mansoni* lipids, especially LPC, in the activation profile of macrophages and the possible mechanisms involved in this process. **Methods:** LPC was obtained from adult worms of Schistosoma mansoni trough TLC technic. After that, peritoneal and bone marrow derived macrophages from C57B/6 mices were stimulated with schistosomal LPC in vitro for 24 hours. Then we analyzed macrophage activation profile by measuring cytokine production, protein and gene expression of M1 and M2 markers. **Results:** We have demonstrated that schistosomic lysophosphatidylcholine (LPC) is able to polarize macrophages to an M2-like profile. We observed that among the other schistosomic lipid fractions, LPC was the only one capable of inducing the expression of M2-like markers, such as Arginase-1, and that this occurs in a manner dependent on the activation of PPARγ. LPC did not induce an increase in the production of nitric oxide (NO) in macrophages, and in high concentrations it was able to inhibit the induction of NO generated by LPS. In addition, we observed that schistosomic LPC induces cell migration and macrophage polarization in vivo and stimulates the formation of lipid corpuscles in peritoneal macrophages in vitro. We also observed that LPC induces lipid body biogenesis in a manner dependent on PPARγ and TLR2. **Conclusion:** Taken together, these results demonstrate an important role of schistosomic LPC in activating macrophages to an M2-like profile, with formation of lipid bodies, through PPARγ-dependent mechanisms.

Oral Presentation 3

Intermediate Hosts

Identification of snail images of *Schistosoma mansoni* vectors through computer vision using real - time flight Drones technology (VANTs).

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Introduction: In Brazil, Schistosomiasis mansoni is endemic but also occurs in a focalized way with persistence of human cases and foci of mollusc vectors. In Pernambuco the disease is endemic in rural areas with foci in coastal areas, high rates of human infection and severe clinical forms, as well as a significant number of deaths, which justifies the need to identify and map areas of risk for the disease. Some aquatic environments where the vector snails live are difficult to access, so the development of a small technological instrument (VANT - Unmanned Aerial Vehicle) as an auxiliary tool in malacological surveys, remotely mapping *Biomphalaria* outbreaks. The VANTs can reach inhospitable places of difficult access and are equipped with environmental sensors and cameras capable of identifying areas by overfly and performing detailed analyzes of aquatic environments: the flora and identification of the snails vectors of schistosomiasis. **Objective:** To identify *Schistosoma mansoni* host snails through images captured by Drones with real-time flights. **Methods**: The study used computer vision techniques to process and classify images obtained by the VANT that is being trained to detect the presence of snails in an artificial aquatic environment using a machine learning algorithm (AdaBoost). The images of snails were collected in laboratory environments for *Biomphalaria* cultivation (artificial channels for breeding and snails reproduction*)* in the schistosomiasis laboratory of the IAM - Fiocruz. A Drone Bebop (4.0.4 video resolution 1920x1080p) was used, equipped with standard manufacturer software. The collected images were processed to detect the presence of snails in computational images. Each flight traveled a 40s trajectory covering the entire length of the channel, returning to the starting point with the camera facing down. The experiment was considered satisfactory since the images generated and processed by the algorithm allowed to identify the presence of snails in the breeding sites and also in those where there were no snails. **Preliminary Results**: 12'10" of videos were produced, and it was possible to extract 2,271 images with 22,000 positive cases and 29,500 negatives (presence and absence of snails in the image). These images are being used in training the computer vision algorithms. Preliminary results indicate the potential use of drones as auxiliary tool in malacological surveys to identify breeding sites of difficult access. **Conclusion**: The future perspective is to optimize the images stabilization although the photos already produced have been satisfactory in the training phase to identify positive and negative cases.

Oral Presentation 3

Intermediate Hosts

Malacological survey in endemic schistosomiasis areas submitted to population collective treatment in Pernambuco state, Brazil, 2011 to 2017.

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INTRODUTION: In the state of Pernambuco (PE) schistosomiasis is endemic in rural areas and presents foci of active transmission in 101 (55%) of the municipalities, located predominantly in the regions of Zona da Mata and Coastal Zone of the State. Between 1999 and 2013, were recorded 2,578 deaths due to schistosomiasis in PE, with an annual average of 200 deaths, 1/3 of the country's deaths. In 2011, the State Department of Health (SES-PE) instituted a Priority Program for Facing Schistosomiasis (SANAR Program), electing 111 hyperendemic localities where the collective treatment (TC) of the population was carried out between 2011 and 2014. **OBJECTIVE:** To carry out a malacological investigation in the localities treated and not treated by SANAR to observe the influence of mass population treatment on the presence of the circulating *Schistosoma mansoni* parasite in host molluscs. **METHODOLOGY:** a malacological research is being done on habitats of *Biomphalaria* snails from the 111 locations where TC was performed and in the same number of places that did not receive treatment. The collected specimens are identified, examined in light and submitted to the molecular diagnosis Nested PCR. **PARTIAL RESULTS**: up to the present date, 3,904 specimens of snails of the genus *Biomphalaria* have been examined, being 3,647 *B.straminea* and 257 *B. glabrata*. Until now, 38 localities where the population did not receive TC and 58 localities where TC was performed in the population (52.2% of the total number of localities treated by SANAR) were surveyed. Preliminary results evidenced the presence of *S. mansoni* DNA in 23% of the lots of snails vectors from localities that received TC by SANAR and in 65.8% of the snails from localities without TC, suggesting a decrease of the circulating parasite in the snails vectors of the treated locations compared to those who did not receive mass population treatment. **CONCLUSION:** the partial data from a descriptive epidemiological analysis suggest that the collective treatment implemented by SANAR in hyperendemic communities of Pernambuco seems to have caused a decrease of the circulating parasite in the schistosomiasis molluscs vectors of the treated areas. At the end of the field collection, analytical epidemiological date should be done to analyze the results.

Oral Presentation 3

Intermediate Hosts

Molluscs of the genus *Biomphalaria* PRESTON 1910 in the Amazon region: update of the distribution and diversity on the state of Pará - Brazil

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Introduction: In Brazil, eleven species and one sub-species of molluscs of the genus *Biomphalaria* are known, being three of them, *B. glabrata, B. tenagophila* and *B. straminea,* intermediate hosts of the trematode *Schistosoma mansoni.* The species *B. peregrina, B. amazonica* and *B. cousini* have been regarded as potential hosts of the parasite. In the Pará state, *B. schrammi, B. kuhniana, B. glabrata* and *B. straminea* have been reported. However, there is a lack of surveys about the distribution of these planorbids. This scarcity of studies is due to the difficulties caused mainly by the vast territorial extension of the region and the great availability of hydrographic basins. In addition, the taxonomy of these snails is hampered by alterations of the fixation process, the size of the specimens and intraspecies variations in the reproductive organs, which may make morphological identification imprecise, justifying the application of molecular biology. Therefore, the objective of this study was to update the geographic distribution of these species of *Biomphalaria* genus in the Pará state. **Methods:** Malacological surveys were carried out between September 2013 and November 2017 in 70 municipalities. After the collection, the snails were taken to the laboratory where they were measured and examined to verify the presence of *S. mansoni* cercariae. Subsequently, five specimens of each collection site were scrified, fixed and the feet removed for subsequent DNA extraction. The mollusc was identified based on the comparison of the characters of the shell, of the excretory organ and female and male reproductive systems. Molecular analysis of the species was carried out by Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) directed to the internal transcribed spacer region of the ribosomal RNA (ITS-rDNA), and cleaved with the enzyme *Dde*I. **Results:** The total number of 22.236 *Biomphalaria* molluscs was obtained, of with 19.133 were analyzed. The presence of the infection with *S. mansoni* was observed in 255 snails (1.3%), all *B. glabrata*. The taxonomic studies identified the species *B. schrammi, B. kuhniana, B. straminea, B. glabrata* and *B. occidentalis.* **Conclusions:** The results so far obtained contributed to increase the knowledge about the dispersion and diversity of the *Biomphalaria* molluscs in the Pará state. It is worth to note, that the presence of the *B. occidentalis* in the Pará state is reported for the first time.

Supported by Instituto Evandro Chagas/SVS/MS

Oral Presentation 3

Intermediate Hosts

Use of multiplex PCR in differentiation of trematodes' larval forms transmitted by *Biomphalaria* snails which distribution in Brazil overlaps to *Schistosoma mansoni*'s

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Introduction: The Trematoda class encompasses helminths whose life cycle is complex passing through different hosts during its development. *Biomphalaria* snails, intermediate hosts of *Schistosoma mansoni*, are important for the maintenance of the biological cycle of this parasite, and also, several other trematodes. It is important to properly recognize and identify trematodes in the larval form that infect *Biomphalaria* since it is the infective stage for the definitive host that is shed by the snail in the environment. The taxonomy of the larval form is limited and methodologically complicated, which highlights the need for new auxiliary techniques capable of identifying these parasites at any evolutionary stage. The morphology of trematodes' larvae is similar between each other, which could lead to incorrect diagnoses of *S.mansoni* and, consequently, unnecessary epidemiological surveillance actions. Therefore, molecular tools appear as an attractive alternative. **Methodology:** This work aims at identifying molecular markers capable of distinguishing four important families of trematodes: Clinostomidae, Echinostomatidae, Schistosomatidae and Strigeidae, all transmitted by *Biomphalaria* snails in the neotropical region. Using the online tool TipMT we designed trematode family-specific primers targeting the ITS region from the rDNA optimized to be used in multiplex PCR, a technique that presents high specificity, low cost and simple execution. DNA samples of the cercariae shed by *Biomphalaria* snails from the Medical Malacology Collection (Fiocruz-CMM) were used as a template for the PCR reactions. **Results:** The panel of primers designed in this study was effective in identifying and distinguishing the four trematodes' families at the same PCR condition. The specificity of the primers was confirmed using DNA from other trematodes' families, nematodes and *Biomphalaria* species present in Brazil, without any unspecific amplification. The primers were sensitive in the range of 0.1 ng to 1 ag of DNA of the parasite. This methodology was also effective for the detection of coinfections. The validation of the technique was made using samples of cercariae shed by *Biomphalaria* snails from Fiocruz-CMM, mostly collected in Minas Gerais-Brazil. Before this step, 69,41% of the samples were unidentified at any taxonomic level. The use of multiplex PCR with the designed primers resulted at the identification for the first time of 43,52% of the samples, 17,64% had its identification confirmed, 2,35% has been corrected and 36,47% remains unidentified highlighting the need of more studies. Schistosomatidae family represents 30,57% of the samples, which 21,16% are *S. mansoni*. **Conclusion:** Through a simple, fast and accurate methodology, it is possible to precisely identify and distinguish four trematodes' families in biological samples in a single PCR reaction. A family level identification provides important information about probable hosts and impacts generated in the affected region, thus allowing the designing of better parasites control strategies.
Financial support: CAPES, Fapemig, CNPq, Fiocruz

Oral Presentation 3

Intermediate Hosts

Accessing genetic diversity of medically important *Biomphalaria* species from a Brazilian living collection

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Introduction: The genus *Biomphalaria* comprises snails that are intermediate hosts of *Schistosoma mansoni*. In Brazil there are 11 species of *Biomphalaria*and three of them are natural intermediate hosts of this parasite, *Biomphalaria glabrata, Biomphalaria straminea* and *Biomphalaria tenagophila*. The two latter ones belong to species complexes that include very closely related species which share genetic and morphological similarities. The presence of intermediate hosts is one of the main factors that indicate the risk of schistosomiasis transmission. Thus, accurate taxonomic identification is crucial. In order to provide more support to the identification, the genetic diversity of these species was investigated using different molecular markers and laboratory-reared Brazilian populations. Here we present preliminary results based on Cytochrome Oxidase I (COI) analysis of twelve populations. **Methods:** The populations were obtained from the living collection of mollusks at the Laboratório de Malacologia – Fiocruz (National Reference for the Health Ministry): *B. glabrata* (Minas Gerais, Bahia, Pernambuco, Rio Grande Norte, Pará), *B. straminea* (Minas Gerais, Piauí and two from Rio Grande Norte) and *B. tenagophila* (São Paulo, Distrito Federal, Bahia). The DNA was amplified using universal primers for the Cytochrome Oxidase I region. DNA sequencing was carried out at the Genomic Platform - RPT01A (Rede de Plataformas Tecnológicas Fiocruz). Genetic variability was analyzed (software: SeqMan, ClustalW, MEGA 7.0) and a preliminary phylogenetic tree based on Neighbour-joining method was built using the sequences generated here and other available in the GenBank. The planorbid*Helisoma trivolis* was used as out-group. **Results**: We obtained four haplotypes of *B. glabrata,* four of *B. straminea* and three of *B. tenagophila*. The amount of new haplotypes was respectively three, three and one. The phylogenetic analysis revealed that each species formed a monophyletic clade well supported by the bootstrap values. Some peculiarities were found in relation to the obtained haplotypes. *Biomphalaria glabrata* populations from Touros/Rio Grande do Norte, Salvador/Bahia and Viseu/Pará shared one haplotype apparently dispersed in different regions of Brazil and in Egypt. Additionally, the Pontezinha/Pernambuco haplotype is more closely related to a haplotype from Puerto Rico than to the Brazilian haplotypes. *Biomphalaria straminea* haplotypes from Tangará/Rio Grande do Norte were more related to populations from São Paulo and Hong Kong than to another haplotype from the same state (Jaguari/Rio Grande do Norte). *Biomphalaria tenagophila* presented the lowest genetic diversity despite the substantial number of sequences available. **Conclusion:** These results contribute for addressing evolutionary, ecological and epidemiological questions, considering that most of the sequences available in databases are from the Southeast region. Increasing the sampling area and molecular markers will contribute to detect more intraspecific variability, supporting accurate identification of Brazilian *Biomphalaria* species of medical importance.

Supported by Faperj/CNPq/Fiocruz

Oral Presentation 3

Intermediate Hosts

Morphological variations of *Biomphalaria straminea* populations from different Brazilian states

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Introduction: Considering the snail transmitters of *Schistosoma mansoni* in Brazil, *Biomphalaria straminea* is the one with the largest geographic distribution. Although the main diagnostic feature of this species is the presence of a series of transverse wrinkles in the dorsal wall of the vagina, the congeneric species*Biomphalaria intermedia* and *B. kuhniana* also present this characteristic, but less markedly, integrating the "*B. straminea* complex". We analyzed four populations from different Brazilian states in the search for other specific morphological relationships other than variations in the degree of vaginal wrinkling. **Methods**: The samples were obtained from the colonies kept in the Laboratory of Malacology (IOC): Paracatu (MG), Picos (PI), Rio de Janeiro (RJ) and Parauapebas (PA). Five specimens from each population were anesthetized and a fragment of tissue from each one was withdrawn for molecular analyzes. The shell was measured (length and width) and the soft part morphology was analyzed. Sixteen parameters were analyzed: length[1] (mm) and width (apex[2] and base[3]) of the prepuce, length[4] and width[5] of the penis sheath, width of the vas deferens[6], number of prostate diverticula[7], length[8] and number of branches[9] of the basal prostatic diverticulum, length of the area of insertion of the prostatic diverticula[10], length of the vagina[11] and of the canal of the spermatheca[12], length[13] and width[14] of the spermatheca, length of the area of the vaginal wrinkle[15] and number of folds of the vaginal wrinkling[16]. **Results**: The mean values for each of the parameters analyzed for Picos, Paracatu, Rio and Parauapebas were, respectively: (1) 2.4; 2.15; 2.45 and 2.44; (2) 0.32; 0.34; 0.34 and 0.29; (3) 0.66; 0.58; 0.52 and 0.76; (4) 3.16; 2.92; 3.22 and 3.33; (5) 0.23; 0.19; 0.20 and 0.16; (6) 0.16; 0.17; 0.17 and 0.19; (7) 11.80; 12.20; 11.60 and 11.80; (8) 0.37; 0.44; 0.36 and 0.47; (9) 4.60; 5.60; 4.60 and 5.80; (10) 1.07; 1.53; 1.35 and 1.51; (11) 0.78; 0.58; 0.70 and 0.52; (12) 0.98; 0.83; 0.95 and 0.71; (13) 1.08; 1.18; 0.93 and 1.01; (14) 0.59; 0.34; 0.44 and 0.42; (15) 0.87; 0.60; 0.76 and 0.76; (16) 3.2; 4.6; 5.4 and 9.2. Regarding the shells, the length and shell width for Picos, Paracatu, Rio and Parauapebas were, respectively: 7.61±0.61 and 2.81±0.21; 6.28±0.65 and 1.98±0.55; 6.39±0.53 and 2.25±0.17; and 6.19±0.89; and 2.37±0.26.**Conclusion:** Values compatible with the description for *B. straminea* for some parameters (presence of wrinkling, number of diverticula and length of penis sheath and prepuce) were observed in all populations. However, there were divergences, i.e., the ratio width of the penis sheath and the vas deferens and between the length of the spermatheca and spermatheca canal. Molecular analyzes (COI-DNAmt and ITS-rRNA) are being carried out to confront the haplotypes with the morphological results in order to extend the current specific parameters, as well as to evaluate speciation.

Oral Presentation 3

Intermediate Hosts

Caracterization of hemolymph and digestive gland neutral lipids of snails *Biomphalaria glabrata* during *Schistosoma mansoni* infection

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Introduction: Schistosomiasis is a neglected tropical disease that affects 78 countries, with about 258 million people infected in the world. This disease is caused by a trematode parasite of the genus *Schistosoma*, which has a heteroxene life cycle, the snail of the genus *Biomphalaria* being the intermediate host and the human as the definitive host In the invertebrate host, we observe the development and multiplication of cercarias, while in the man we have both acute as cronic symptoms of the disease. Lipids are extremely important for the development and reproduction of the parasite *Schistosoma mansoni,* since it does not have complete synthesis pathways and degradation of lipids, making it possible to obtain them by its hosts. Thus, the aim of this work is to determine alterations on lipid profile of in the hemolymph and digestive gland of *B. glabrata* snail during infection with S. mansoni. **Methods:** For this, two groups of snails underwent infection kinetics for 7 weeks. Hemolymph and digestive gland were removed weekly, processed and submitted to protein dosage, lipid extraction and thin layer chromatography. **Results:** The lipids identified during kinetics showed similar results, a peak in the fourth week after infection, except esterified sterol that is the main lipid class and presented a significate reduction. With regard to the digestive gland, we observed that the infection was able to alter the metabolism of lipids, significantly reducing the proportion of triacylglycerol and increasing free fatty acids and an undetermined lipid. Esterified sterols, sterols, monoacylglycerols, diacylglycerols and total phospholipids did not present significant difference in the analyzed groups. In a parallel study approach, the distribution of haemolymphatic proteins was assessed by KBr gradient, polyacrylamide gel electrophoresis, HPLC and TLC. We observed the presence of a lipoprotein, molecular weight of 550 kDa, composed of two subunits (250 and 80 kDa) and the lipid composition consisting of 52.29% esterified sterol, 5.41% triacylglycerols, 9, 78% of an undetermined lipid, 11.62% of fatty acids, 7.56% of sterols, 7.38% of diacylglycerols and 5.95% of phospholipids. **Conclusions:** We concluded that there is modulation of the lipid metabolism in snails infected with *S. mansoni*, both in the hemolymph and in the digestive gland. Alongside this, the biochemical characterization of snail lipoprotein will be of high importance for understanding the effects of *S. mansoni* parasite infection.

Supported by Faperj/ CNPq /UFRJ

Oral Presentation 3

Intermediate Hosts

Toward transgenesis in *Biomphalaria glabrata*

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Introduction: *Biomphalaria glabrata* is the primary model for schistosomiasis-transmitting planorbid snails and is used to study parasite-host relationships, invertebrate immunology, and the evolution of resistance to parasite infections. The recent publication of the *B. glabrata* genome and the adaptation of cutting-edge functional genomic and population genetic techniques open up new avenues for the study of this important vector. However, despite the demonstration of successful transgenesis in mollusks such as *Crepidula fornicata* and *Crassotrea gigas*, the mechanical and molecular tools for such an undertaking in *B. glabrata* remain underdeveloped. **Methods:** We describe the first steps taken to develop transgenesis in the adult and egg stages of *B. glabrata*, as well as in the associated *B. glabrata* embryonic cell line (Bge). Using injection and chemical transfection, we demonstrate how to deliver materials to the tissues of interest. We also describe new assays for tissue visualization and evaluation of fluorescence in *B. glabrata* and Bge, including optical clearance and flow cytometry. **Results:** Injection of the ovotestis of 10-15 mm *B. glabrata* resulted in viable and fertile adults, though some mortality and reduced fecundity was observed. Injection of *B. glabrata* eggs resulted in viable embryos that hatched and developed normally. Finally, transfection of Bge with nanoparticles constructed with mRNA and high amounts of jetPEI resulted in transfection efficiencies of greater than 10%. **Conclusions:** These protocols set the stage for new experimental designs in Bge and the continued development of transgenesis in *B. glabrata*.

Supported by the Global Health Institute, Merck KGaA

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Concomitant infection of *Schistosoma mansoni* and *Helicobacter pylori* promotes promiscuity of antigen experienced cells and primes the liver for a lower fibrotic response

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Introduction: *Helicobacter pylori* chronically persists in the stomachs of infected individuals and is strongly associated with gastric cancer. This has been attributed to the response it generates via the continuous induction of various pro-inflammatory cytokines. Interestingly, certain countries with a high prevalence of H. pylori do not present with comparable high rates of gastric cancer. Epidemiological data has suggested that one of the reasons for this discrepancy may be due to highly prevalent co-infections with helminths, which suppress *H. pylori* associated pro-inflammatory responses. However, there is limited experimental evidence to support this hypothesis and none so far with *S. mansoni,* one of the most common helminths endemic in countries with the highest rate of *H. pylori* prevalence. On the other hand, there is even lesser known on how *H. pylori* may influence *S. mansoni* related hepatic disease. In this study we fill in the basic gaps in the understanding of the immunological mechanisms employed by these pathogens upon co-infection. **Methods:** We infected C57BL/6 mice with *H. pylori* first, followed by *S. mansoni* to mimic the infection course in humans. We analysed the effects of co-infection during the different immune phases (acute Th1 and chronic Th2) of the helminth infection. Here, we investigated *H. pylori* colonization and gastric inflammation as well as schistosome-associated immune responses. We performed flow cytometry analysis, adoptive transfers, immunohistochemistry, RT-PCR, RNAseq, ELISA, *in-vitro* co-cultures and western blots to determine the mechanisms underlying the observed phonotype. **Results:** Surprisingly, we observed increased colonization of *H. pylori* in the stomach of co-infected mice in the Th1 phase of schistosome-infection despite high levels of IFNγ and the fact that this cytokine is known to be responsible for bacterial clearance. Further investigations revealed the occurrence of immune misdirection of antigen experienced T cells, away from gastric tissue, due to strong type 1 chemokine gradients induced by the helminth in the liver. On the other hand, *H. pylori* co-infection altered the liver pathology associated with schistosome infection, resulting in smaller granulomas accompanied by decreased liver-specific alanine aminotransferase (ALT) and total collagen levels. This observation hints towards a “protective” role of *H. pylori* co-infection in *S. mansoni* associated liver disease. **Conclusions:** Our data strongly point towards an immunological interaction of pathogens occupying two anatomically distant organs, eventually resulting in pathological changes that alters the course of both diseases.

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Th2 inflammation activates TGF-β causing *Schistosoma mansoni* induced pulmonary hypertension

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Introduction: A significant complication of *Schistosoma mansoni* infection, occurring in ~5% of those chronically infected is pulmonary hypertension (PH). This disease is fatal and treatment consists mostly of vasodilators. We hypothesized that the disease results from Th-2 mediated activation of TGF-β by thrombospondin-1 (TSP-1). **Methods:** We used a mouse model of intraperitoneal sensitization followed 14 days later by intravenous challenge with *Schistosoma mansoni* eggs, which results one week later in a PH phenotype of elevated right ventricular pressures and pulmonary vascular remodeling in wild-type mice. Pressures were assessed using a PV catheter and active TGF-β levels were measured using a luciferase reporter assay. **Results**: *Schistosoma*-challenged IL4/IL13 double knockout and Rag-1 knockout (B/T cell-deficient) mice were both protected from PH. Adoptive transfer of CD4+ T cells from wildtype but not IL4/IL13 double knockout donors restored PH to Rag-1 knockout mice. *Schistosoma* exposure caused an increase in both TSP-1 and active TGF-β in murine lungs. TSP-1 blockade by bone marrow deficiency or pharmacologic inhibition blocked TGF-β activation and PH. Flow cytometry identified the primary source of TSP-1 is recruited Ly6C+ monocytes; blocking recruitment of these cells by CCR2 deficiency protected against PH.Inhibiting TGF-β signaling also prevented *Schistosoma*-PH. **Conclusions:** Deposition of *Schistosoma mansoni* eggs in pulmonary vessels triggers Th2 inflammation resulting in TGF-β activation by TSP-1, which can cause PH.

Supported by NHLBI (National Heart, Lung and Blood Institute), ATS Foundation, Gilead, Pfizer. PHA and Department of Medicine (University of Colorado).

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Evaluation of morbidity and self-reported symptoms associated with *Schistosoma mansoni* infection in an endemic area of Minas Gerais, Brazil.

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Introduction: The chronic condition of schistosomiasis is the stage where the greatest impact from the infection is observed. Although infected individuals are variably symptomatic, they do not promptly seek for immediate health care, possibly because the chronicity of their condition leads them to perceive the disease as "normal". The less specific signs and symptoms, such as diarrhea, abdominal pain, and anemia may account for most of the burden of schistosomiasis-associated disability among infected individuals in endemic areas who have not developed the advanced forms of the disease. The objective of this study was to assess the association between schistosomiasis and self-reported symptoms in an endemic community for *S. mansoni* in Minas Gerais, Brazil. **Methods:** 577 participants were included in this cross-sectional study. Parasitological survey with two stool samples using the Kato-Katz method was performed. The circulating cathodic antigen (CCA) test was used for the Kato-Katz egg-negative individuals. Demographic information, hemoglobin level, and self-reported symptoms were obtained. Symptoms data were collected using a "Recall Card" questionnaire recorded by each individual, both positive and negative for *S. mansoni* infection. Egg-positive individuals were treated with PZQ. The chi-square test and one-way ANOVA tests were used for analysis. **Results:** The prevalence of *S. mansoni* infection was 33.2% and the mean intensity of infection was 451 eggs/gram of feces (epg). Hemoglobin levels of egg-negative and egg-positive individuals were similar. However, we observed a significant difference in hemoglobin levels according to intensity of infection, with individuals with high-intensity of infection having lower levels of hemoglobin. There was a significant association between all self-reported symptoms and the presence of *S. mansoni* infection ($p<0.001$): for diarrhea (OR: 4.1), blood in the stool (OR: 6.5), abdominal pain (OR: 3.0), fatigue (OR: 2.8), dizziness (OR: 2.8) and headache (OR: 2.1). Overall, women had a higher proportion of self-reported symptoms than men but gender difference was significant only for fatigue and headache. Age did not associate with the presence of self-reported symptoms except for dizziness, which was significantly more prevalent in individuals over 20 years of age. **Conclusion:** Although less specific, the identification of self-reported symptoms by individuals in an endemic community may be an important indicator of disease. Use of symptom scores may contribute to the control of schistosomiasis since it is faster than the traditional diagnostic methods, well accepted, and cost-effective. In addition, it can be used to construct, directly with the community, a perception of the disease that could increase participation in control activities and efforts for the elimination of the disease.

Financial support: National Institutes of Health-Tropical Medicine Research Centers (TMRC, Grant number AI098507); CAPES; FAPEMIG; CNPq; INCT-DT.

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Murine model of neuroschistosomiasis mansoni: clinical, histological and magnetic resonance imagin studies

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Introduction: In the year 2000, an estimated 8 million people were infected with *Schistosoma mansoni* and 30 million were at risk of infection in Brazil. The 'Global Burden of Disease Study 2010' indicated that schistosomiasis is the one hundredth cause of death in Brazil and is responsible for 3.6% of the estimated total of deaths in the world. The central nervous system can also be affected by *S. mansoni* infection. Previous authors have observed helminth eggs in the leptomeningeal, cerebral cortex, basal ganglia, choroid plexus, cerebellum and spinal cord, the latter of these with the highest frequency. In the present study, experimental infection of *S. mansoni* cercariae in mice aims to demonstrate the presence of granulomas formed in the brain and correlate the clinical, histological and magnetic resonance findings. **Methods:** We infected 25 male mice (Mus musculus-Swiss Webster), weighing between 18 and 20 grams, with 50 LE strain larvae subcutaneously, and maintained 25 as controls, without infection. We followed them for 160 days post-infection. Euthanasia was carried out on days 88, 97 and 146. After confirmation of death, brain samples were fixed in formalin. Parasites were recovered using the technique developed by Pellegrino and Siqueira (1956). Images were obtained in the axial, coronal and sagittal planes by magnetic resonance. Following imaging studies, we performed histological studies in order to examine *S. mansoni* eggs, granulomas and inflammatory lesions. **Results:** The characteristic neuromotor behaviors observed was head and chest tilt, paresis, loss of balance reflex, altered muscle tone, ataxia and rotational motion (spinning). Histological findings included the presence of *S. mansoni* eggs disseminated in both hemispheres, in the cerebral, cerebellar and brainstem regions. In all samples, the eggs reached the brain through the arterial system. The granulomatous reaction was more frequent in the leptomeninge and in areas of cerebellar fissures. Edema and leptomeningeal thickening were found. All eggs presented miracidia. Additionally, histological examination demonstrated macrophages, eosinophils, lymphocytes, fibroblasts and collagen in the samples. **Conclusion:** In conclusion, the experimental model of brain schistosomiasis mansoni was developed by injecting cercariae into the subcutaneous tissue. There was a correspondence between the encephalic lesions and the magnetic resonance findings.

KEYWORDS: schistosomiasis, mansoni schistosomiasis, neuroeschistosomiasis murin model, encephalitis, neuroinfection

Supported by FAPEMIG and CNPq

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Evaluation of the therapeutic potential of synthetic aurones on murine model

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Introduction: Legitimate representative of the list of tropical diseases neglected to schistosomiasis - caused by trematodes of the genus *Schistosoma spp.* is an endemic parasite in more than 70 countries. The absence of an effective vaccine enhances the role of chemotherapy in controlling morbidity and transmission, which is still restricted and is threatened by potential resistance to praziquantel. In the last decades efforts in search of new pharmacological entities have been printed, with emphasis on natural products. In this scenario, a group of flavonoids called aurones has interesting biological potential, such as anti-inflammatory, antifungal and antibacterial activity, as well as antiparasitic action against *Plasmodium falciparum* and *Leishmania spp.* Preliminary studies of our group found that aurone analogues - LS26 and LS29 - promoted changes in the integument, reduction of motor activity and death of adult worms of *S. mansoni.* Thus, the aim was to evaluate the therapeutic potential of LS26 and LS29 in the course of schistosomiasis mansoni in *Swiss* mice. **Methods:** The animals were divided into groups (n = 15), treated with LS26 and LS29 in a single dose of 50 mg/kg or 100 mg/kg, 50 days post-infection and groups treated with the drug in two doses given 40 and 50 days after infection, also at doses of 50mg/kg or 100 mg/kg repeatedly, compared to the control group of the vehicle and PZQ. The schistosomicidal action was evaluated by the counting of worms located in the liver and mesentery recovered by perfusion, as well as by the qualitative oogram of the ileocecal junction of the mice. **Results:** The oral 50mg/kg regimen in two doses was the most promising, achieving a significant reduction of 56,20% for LS26 and 57,61% for LS29 of parasitic load and greater hepatic displacement of worms. In addition, LS29 in two doses of 100mg/kg also promoted the same effects with reduction of 37,15% of the global burden of adult worms. **Conclusions:** Finally, it was observed that both drugs seem to interfere in the kinetics of egg development since in all the therapeutic schemes the altered oogram. These results corroborate the data of parasite load reduction and the increase of the partial displacement of the worms of the mesentery, reinforcing the character of active drugs, in vivo, against adult worms of *S. mansoni.*

Keywords: Schistosomiasis. Auronas. Schistosomicidal activity.

Supported by FAPEMIG/CNPQ/UFJF/CAPES/FIOCRUZ.

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Experimental validation of new *Schistosoma mansoni* aspartic proteases inhibitors identified from multi-target virtual screening

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Introduction: Schistosomiasis is an important parasitic disease caused by trematodes of the gender *Schistosoma*. Praziquantel (PZQ) is the drug of choice for the treatment of schistosomiasis. There is, however, a concern with the appearance of isolates resistant to PZQ. *S. mansoni* aspartic proteases (SmAPs) similar to cathepsin D are involved in the host's haemoglobin digestion, being essential in the life cycle of these parasites. One such enzyme, SmCD1, has been investigated for drug development and two others SmCD2 and SmCD3 still need biochemical characterization. Virtual screening (VS) has been widely used in the early stages of drug discovery, because it allows the identification of possible hits, even before they are tested in vitro or in vivo. Several compounds with schistosomicidal activity have been identified in this way, including protease inhibitors. In this work, we want to identify inhibitors of SmAPs, using computational and experimental methods. **Methods:** Initially, a VS of 20,000 compounds belonging to two commercial libraries, Microformat and DIVERSet-EXP, was performed on SmCD1-3 enzymes. The 50 most promising candidates as inhibitors were selected, and subsequently screened on: 1) worms' aqueous extract (WAE); 2) pig pepsin enzyme; and 3) recombinant SmCD1 enzyme, already cloned and expressed by the group in HEK293 cells. The enzymatic assays were performed at pH 3.5 with 5 µM of Abz-AIAF/FSRQ-EDDnp-based fluorescence octapeptide (FRET) substrate (λem: 420 nm, λex: 310 nm), 20 µg extract or 0.5 µg pepsin/SmCD1. **Results:** Overall, most compounds showed highest activity against the digestive enzyme, pepsin, with some compounds presenting IC_{50}values in the mid mM range. Conversely, compounds 10 and 50 preferably inhibited SmCD1 and WAE, respectively. **Conclusions:** VS proved an efficient strategy to identify new SmAP inhibitors. Analogues of the hit compounds will be purchased to carry structure-activity studies aiming to guide structural optimization into potent SmAP inhibitors as candidate antischistosomal drugs.

Supported by CNPq, FAPERJ and Fiocruz

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Taste assessment using rat palatability model of commercial praziquantel tablet for pediatric off-label use

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Introduction: Praziquantel (PZQ) has been the drug of choice for schistosomiasis control, but at the moment, it is widely used off-label to treat preschool-aged children. This treatment has been adapted from the commercial adult formulation and is not adequate for pediatric use. Taste plays an important role in the development of oral pediatric pharmaceutical formulations in relation to patient acceptability and adherence. Human panel is an important method for taste assessment and is an ethical issue challenge, especially when it involves children. Another method is the electronic tongue, but previous works mention that it was not applicable for PZQ analysis due to the non-ionic characteristic and the low solubility of the drug in water. Rat models designed for the evaluation of the palatability showed good correlation to human panels and quite promising for taste assessment of drugs like PZQ. The aim of the present work was the taste assessment using rat palatability model of commercial praziquantel tablet for pediatric off-label use. **Methods:** Sixteen female Wistar rats were used. The rats were maintained on a 12 h/12 h light/dark cycle, housed in plastic cages and received a standard chow and water ad libitum except during the training and testing water restriction conditions as mentioned below. All animal experiments were performed according to the policies and guidelines of the ethics committee of animal use and the study approved by ethics committee. On the first day rats were deprived of water for a period of 22 h to motivate the licking behavior. On the second day water was offered for 30 minutes, and then the consumption was measured. Then water was offered to all animals on demand for 90 minutes. After this period rats were deprived again of water for 22 h. This procedure was repeated one more day, for animals training. On the fourth day, the animals were separated in 4 groups (n=4/group) and different solutions/dispersions kept in recirculation were offered for each group and the consumption measured after 30 minutes: Sweet and Bitter test solutions, PZQ (API) dispersion and water dispersion of the fourth part of the commercial PZQ 600 mg tablet equivalent to the dose of 150 mg of pure PZQ. **Results:** The mean total volume consumed at the end of the 30 min experiment was approximately 12, 14, 8, 9 and 5 mL for water, sweet and bitter solution, API dispersion and tablet dispersion, respectively. Neutral rats' taste perception was observed for water. Rats liked the taste of sweet test solution. Taste aversion behavior was observed such as animal retreating indicating that the rats did not like the taste of PZQ commercial tablets. An unexpected result was obtained for the API because of the poor water solubility of PZQ which did not provide a homogeneous dispersion. **Conclusions:** The rat palatability model used in this work may be a valuable tool for the taste assessment of pediatric pharmaceutical formulations based on bitter-tasting APIs.

Supported by PAPES VII/CNPq/Fiocruz.

Oral Presentation 4

Diagnosis, Treatment, and Clinical aspects

Performance of POC-CCA and Kato-Katz to diagnose Schistosomiasis mansoni in low and moderate endemicity areas of Brazil

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Introduction: Current diagnostic methods for intestinal schistosomiasis (detection of eggs in stool by Kato-Katz (KK)) are limited and may be particularly unrealiable at low levels of infection, as would be expected after several round of treatment or in regions where transmission is low. In order to overcome some of 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. **Methods:** For the KK assay, three stool samples were collected, and 2 KK slides were prepared per sample. In addition was collected one sample of urine from all participants for POC-CCA. **Results:** Up to the present moment, of the 1,223 SAC surveyed in the state of Minas Gerais, we obtained 242 (19.7%) positive individuals, of these samples 17 (7%) were positive only for KK (intensity mean, 20 eggs mg/stool). However, when evaluated the results of the POC-CCA test, 211 (87.2%) samples were positive only for that test, and coincided that 14 (5.8%) samples were positive for both tests in Minas Gerais state. Moreover, of the 552 SAC surveyed until the moment in Sergipe state, 219 (39.67%) samples were positive, being 36 (16.4%) positive samples only for KK (intensity mean, 60 eggs mg/stool) and 89 (40.6%) positive samples only for POC-CCA test, in the total 94 (42.9%) samples were positive for both tests. The POC-CCA test, results trace were considered as positive. After treatment, patients positive for the POC-CCA test became negative or decreased the intensity of the band in the immunochromatographic test. **Conclusion:** Taken together our results from Minas Gerais and Sergipe state indicate that POC-CCA is a more sensitive test than Kato-Katz in areas of low and moderate endemicity. In addition, POC-CCA test appears to be a good test to follow the cure control.

Supported by Medical Research Council (MRC) and FAPEMIG.

POSTER PRESENTATIONS

SESSION:

Epidemiology, Control, and Health Education

Determining factors of the occurrence of schistosomiasis in a neighborhood of São Luís, MA, Brazil

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Introduction: In São Luís, Maranhão, an infrastructure of precarious distribution of water and sewage, together with the socioeconomic conditions of the population, favor the spread of diseases, such as schistosomiasis. It is known that this water-borne disease has been occupying space in neighborhoods such as Vila Embratel, which has favorable conditions for the establishment and spread of parasitosis. Based on this, the present work aimed to describe the factors responsible for the occurrence of the endemic in the area. **Methods**: Data were collected in locu through monitoring, using questionnaire and photographs, in the period from November 2016 to October 2017. **Results**: In the neighborhood under study, there were water collections represented by streams and open sewage, which act as breeding grounds for schistosomiasis-carrying molluscs (intermediate hosts), many of which are located peridomiciliary. The presence of abundant vegetation on the site, which serves as food for the molluscs, allied to the factors mentioned above, contribute to the expansion of parasitosis. **Conclusions**: Taking into account all the conditioning factors found, the peripheral neighborhood Vila Embratel presents itself as an environment conducive to the development and expansion of schistosomiasis, which directly reflects on the health of the residents. Most of them are unaware of the epidemiological implications, so that health education and management assistance to this unprotected population is necessary.

Supported by UFMA

Climate Change and Schistosomiasis: Study of Art

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Introduction: Human made accelerated climate change has altered and may alter the epidemiological profile of schistosomiasis in the world. According to the literature, the increase in temperature, contamination of water resources with metals and pesticides and periodicity of rainfall influence the freshwater environment and consequently the life cycles of intermediate hosts and their respective parasites. The present study presents the state of the art on the relation between schistosomiasis and climatic changes, highlighting the scientific done in endemic areas. **Methods:** A mapping of the scientific production on climate change and the Shistosomiasis between 1945 and 2018 in the ISI / Web of Science database was carried out with further analysis of the information in the VantagePoint software, only using the title, in order to check the total number of all publications on the subject and the first record in the Web of Sciense. **Results:** We found only seven articles in the following subjects. The first record was in 2008 associating climatic changes withpredicted temperature increases in China and possible Schistosomiasis transmission. The countries that publish the most are the Denmark and the Switzerland with four and three articles respectively. Brazil has no articles about the theme. The main scientific journals that publish in the theme is the Tropical Medicine & International Health (three publications). Only one article about subject per year was published from 2008 to 2017. In the years 2011, 2012 and 2014 no record was found. The University of Copenhagen presented the major number of publications (four papers), followed by the Swiss Tropical & Public Health Institute (three papers). These institutions cooperate in only one article. **Conclusions:** We conclude that the theme of this study is neglected. Brazil and African countries do not appear in this survey, with the geographical regions with the highest number of cases. The research group Health and Environmental Education with emphasis on parasitic relationships has as goal to develop studies on schistosomiasis and climatic changes in Brazil.

Supported by PAEF/ LAPSA/ IOC

Chemical profile of *Tithonia diversifolia* extracts in Schistosomiasis control

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INTRODUCTION: The genus *Tithonia* is an important genus, source of various natural products, particularly sesquiterpene lactones, diterpenes, and flavonoids. *Tithonia diversifolia* (TD) is an exotic ornamental species used in Brazil to treat bruises. This species has several activities as, anti-inflammatory, antiespasmodic, cytotoxic, antimalarial, antiviral, antimicrobial, fungicidal, antidiabetic, analgesic, rheumatism and arthritis, antidiarrhoeal, antispasmodic, vasorelaxant, cancer-chemopreventive, bioinsecticide, repellent activities, against intestinal worms and schistosomiasis, urinary and venereal diseases and attenuates withdrawal syndrome in dependents. Chemically, TD contains flavonoids, poliacetilens, terpenes and sesquiterpene lactones (STLs) as their mainly metabolites with great biological and taxonomic importance. Considering the prevalence and morbidity of Schistosomiasis in Brazil and in many other countries of the world, in addition to the fact that other species of *Tithonia* have molluscicidal activity described in the literature, it becomes important the study of TD in the combat and control this great problem of public health. **METHODS:** Extracts from flowers and leaves of *Tithonia diversifolia* (TD) collected in Petrópolis (RJ), were performed by HPLC-UV-DAD and others spectroscopic methods. Chloroformic extract (11.37g) of TD fresh leaves (100g/L), was subjected FTIR, NMR and HPLC-UV-DAD. Using two VLCs in a row, so far, it has been possible to isolate and characterize tagitinins, STLs of great biological importance. **RESULTS & DISCUSSION:** The HPLC-UV-DAD analysis showed a 31.4% area signal, at 210nm and 87.8%, at 255nm, both with Rt 6.23min and maximum UV at 251/330/486/634/602nm, characteristic of Tags. The FTIR analysis showed vC=C signals at 1661cm-1, characteristic of tagitinin C, in addition to the vC-O-C signals at 1149cm-1 of an alkyl ether and a v,F signal at 1732cm-1 representative of a β-lactone, α, β -unsaturated esther group. **CONCLUSIONS:** The CHCl3 rinsing of the foliar surface allowed the extraction of STLs commonly present in glandular trichomes. The NMR analisis allowed the characterization of tagitinin C, STL responsible for others important biological activities of this species. Further chemical studies and biological assays with the identified tagitinin C, in Schistosomiasis control will be performed. Ethanolic extracts will be produced and chemically analyzed to identify other responsible substances for the action in *Biomphalaria glabrata* infected molluscs by *Schistosoma mansoni.*

Supported by CNPq, Farmanguinhos and CIEE.

Current scenario of schistosomiasis mansoni at Espírito Santo state with emphasis at Afonso Claudio city

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Introduction: The objective of this study is to survey the current situation of schistosomiasis mansoni in the state of Espírito Santo, identifying the endemic areas, and emphasizing the Afonso Claudio city. This city carried out in the 90's an intervention project that was based on educational actions in health and implementation of sanitation works in the rural area. This epidemiological analysis of schistosomiasis in the Afonso Cláudio city allowed us to indirectly evaluate the effectiveness of intervention measures in the reduction of post-project cases. **Methods:** It is a descriptive, quantitative, cross-sectional epidemiological study based on secondary data. The number of cases reported for schistosomiasis, distributed by cities and year were collected in specific databases for schistosomiasis mansoni (SISPCE) available in DATASUS from 2005 to 2015. Maps were constructed in three annual scenarios (2005, 2010 and 2015) using the Quantum GIS 2.4 geographic information system and by the tool Join, the maps of positive index were made to the base of the municipal mesh through the code of the municipality. The data referring to the cases of schistosomiasis by locality of the Afonso Claudio city were obtained in the secretariat of epidemiological and sanitary surveillance of Afonso Cláudio city in the period of 2011-2017. **Results:** The highest number of stool examination performed in the State of Espírito Santo for schistosomiasis was in 2007, with 101,282 performed, with a total of 3,475 and a global prevalence of 3.43%. All the cities of the mountainous region presented a positive index for schistosomiasis. The highest number of positives found in the whole period analyzed was in the Afonso Cláudio city with 574 positive results in 5,354 stool examinations performed. The prevalence of localities studied in the alternative model project for the control of schistosomiasis in the Afonso Cláudio city in 1997 continued to decrease over the 18 years after the project, with a current total prevalence of 4%. **Conclusions:** It is believed that the continuity of the reduction of the disease in the post-project studied sites are related to the actions on basis sanitation carried out and the actions of health education with community mobilization.

Supported by PAEF/ IOC/ Fiocruz

Factors related to schistosomiasis in directly affected area by the São Francisco River large-scale water transposition project in the Northeast of Brazil.

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Introduction: The São Francisco River Integration Project with the watersheds of the Northeast foresees the construction of two watercourses: the North Axis that will bring water to the backlands of the states of Pernambuco, Ceará, Paraíba and Rio Grande do Norte, and the East Axis which will benefit Pernambuco anda Paraíba states. The alterations resulting from water constructions can provide eco-bio-social changes that allow the increase and / or the emergence of diseases, like schistosomiasis. The main objective was to determinate the presence of *Schistosoma mansoni* in workers at the construction site and school children, as well as determinate the *Biomphalaria* sp. occurrence in some municipalities of Ceará state. **Methods:** A epidemiological study was conducted from 2015 February to 2016 October based on the diagnostic for schistosomiasis in school children from 7 to 14 years old and workers at the construction site by the Kato-Katz method (3 slides), and mapping the *Biomphalaria* sp. sites based on the collection of snails from water bodies. This study has been realized in municipalities of Aurora, Brejo Santo, Jaguaretama, Jaguaribara, Jati and Mauriti. **Results:** A total of 138 workers participated in this study and according to the Kato-Katz method, 2 (1,44%) were positive for *S. mansoni*. In none of the 604 school children was evidenced the presence of *S. mansoni*'s eggs after interlaboratory re-reading. The malacological survey carried out in 33 water collections evidenced the presence of *Biomphalaria* sp. in 69,70% (23/33). Possibly this study demonstrates an underestimated result of the actual situation of schistosomiasis in this population, considering the number of municipalities studied, and the method diagnostic utilized. Thus, all the necessary elements for the expansion of the transmission of schistosomiasis are already present in these localities, since we have intermediate host, inadequate sanitary infrastructure and carriers of the disease. **Conclusions:** This scenario demonstrates the need for the development and implementation of health policies aimed at control actions and epidemiological surveillance for schistosomiasis in the context to the largest water project under construction in Brazil.

Supported by PPSUS-REDE - MS/CNPq/FUNCAP/SESA

FACTORS ASSOCIATED TO *SCHISTOSOMA MANSONI* INFECTION IN A BRAZILIAN ENDEMIC AREA

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Introduction: *Schistosoma mansoni* infection (SM) remains an important public health problem in many parts of the world. In Brazil, it occurs throughout the coastal region of Northeast, from Rio Grande do Norte (RN) towards the southeast, following the path of important freshwater collections. The objective of this study was to evaluate the association between frequency of use of water collections, food security, demographic, socioeconomic factors and SM infection. **Methods:** The study was carried out in the municipality of Pureza, located in the coastal region of the state of Rio Grande do Norte (RN). A total of 291 individuals (3.0% of the total population) were randomly selected and distributed into three groups: 1) cases (n= 42): positive parasitological diagnosis of SM in the last five years; 2) contacts (n=214): individuals residing in the same region of cases, but negative for SM in the last five years; 3) controls (n=35): composed of individuals who live in localities without water collection, and negative for SM in parasitological analyzes of feces. SM diagnosis occurred through parasitological examination of feces by the Kato-Katz technique. The Brazilian Scale of Food and Nutritional Security (*Escala Brasileira de Segurança Alimentar* - EBIA) was applied to check household food security. Demographic and socioeconomic data were also collected. **Results:** Cases used more frequently water collection than the other two groups: 21.4% used monthly and 23.8% very frequently (daily, 2 to 3 times a week or once a week) (chi-squared, p <0.001). Food insecurity was present in 64.0% of the households. A higher frequency of food insecurity (97.6%) was observed in the case group when compared to the other groups, while food safety was more frequent (68.8%) in the control group (chi-square test, p <0.001). Cases were more frequent (81.0%) in men when compared to the other groups (chi-square test, p <0.001). There was no difference between groups for age, years of education and family income. **Conclusions:** In the endemic area studied, frequent use of water collection, food insecurity and male gender were factors associated with SM.

Supported by CAPES and CNPq.

Eugenia florida leaves extract as an alternative against praziquantel-resistant strains of *Schistosoma mansoni*

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Introduction: Schistosomiasis is a neglected disease occurring in 19 Brazilian states, and affecting about 250 million people in the world. Although the discovery of praziquantel has been a landmark in the treatment of schistosomiasis for almost 30 years ago, and this drug still be the only effective drug against infection caused by *Schistosoma* species and used for its treatment, about 10% of infected people in the world developed the severe form of the disease. Due to the mass treatment with praziquantel and oxamniquine, there are schistosoma strains of praziquantel-reistant and oxamniquine-resistant. Therefore, the search for new schistosomicidal drugs among plant extracts has been justified. Betulinic acid (BA) is a triterpene that occurs in more than 60 plant species and has many biological actions like anti-HIV, anti-malarial, anti-cancerous, hepatoprotective, and much more. Its action against schistosomiasis was demonstrated to promote the reduction of motor activity at low concentrations and death at concentrations between 50 and 200 uM in the different stages of life of *S. mansoni*. In Brazil the BA occurs in several genus, as in the Eugenia genus. Many of them, produce BA and are used in the traditional medicine such as *Eugenia florida*. **Methods**: Considering the natural variation in secondary metabolites content in a plant, it periods. Thus, *Eugenia florida* leaves were collected from Botanical Garden of Rio de Janeiro (Jardim Botânico do Rio de Janeiro - JBRJ- 22º 58' 12.959" S, 43º 13' 26.381" W, 16m altitude) in summer, winter and autumn and were extracted with ethanol by dynamic maceration, in a shaker, at room temperature for 7 days. **Results**: The BA content was confirmed by TLC with standard sample and with HPLC-UV-DAD. These extracts will be standardized and their potential against Schistosomiasis will be evaluated. *E. florida* summer extract presents the major BA content in 5.5%. **Conclusions:** These results stimulate us to evaluate its potential in relation to the *S. mansoni* as an alternative against praziquantel-resistant strains of *S. mansoni*.

Supported by Farmanguinhos - Fiocruz

Experience report on teaching internship in parasitology discipline: preventive actions for schistosomiasis

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Introduction: The teaching internship aim to prepare the post graduate student for teaching practice in the graduate and postgraduate. Student funding programs, as Social Demand of Capes becomes obligatory. The aim of this study was describe my experience as teaching internship, using an emancipating vision applied to parasitology teaching, emphasizing the preventive actions for schistosomiasis. **Methods:** The teaching internship was carried out with the Discipline of Parasitology, Federal University of the State of Rio de Janeiro-UNIRIO. The Teaching Plan and the extension activities were built together by one advisor and me. The strategies, goals and objectives were defined for each activity. The following activities built by me during the teaching internship were: observation, preparation and teaching classes and the orientation of group of the academics in the extension activities whose theme was: "Prevention of Schistosomiasis", for presentation in an extension event, called: Parasitoses Prevention Fair. This event was held in a shelter, involving children from 2 to 11 years. **Results:** The observation of the classes allowed different didactic practice. The orientation of group of academics allowed me to act as a teacher and apply the knowledge of parasitology. Educational strategies were developed and created to prevent schistosomiasis, adapted to children's education. The following educational materials were created: a story book with the cycle of the parasite; a puzzle to assemble the cycle; a giant word-hunting and drawings for paintings. The exchange of experiences with the parasitology teacher was enriching, providing the professional maturity. **Conclusions:** The experience of the teaching internship contributed to the formation of the professional more aware of his/her social, political and citizen role. It was possible to gain knowledge, to learn new methods and ways of to teach and to learn. In addition, reflect and elaborate a new attitude towards graduate teaching activity. Some advisor and students bureaucratically fulfill the teaching experience, even considering a deviation in the research. The report of these activities shows that the experience can be transformative for the formation of teachers, while it is based on the knowledge shared between the different participants of the project.

Supported by CAPES /Fiocruz/IOC/PAEF

Experience Report in the production and development of an extension course for the endemic control agents with emphasis on vector mollusks, schistosomiasis and angiostrongyliasis

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Introduction: The control agent of endemic diseases has two of its attributions focused on the development of educational actions and actions of community mobilization related to the control of the disease, performing joint control actions with the Community Health Agents in the Primary Care team. In this context, frequent training through specific courses, make work more efficient and productive. The objective of this study was to describe the process of production and execution of an extension course for control agents of endemic diseases of the city of São João de Meriti / RJ about the main mollusks of economic, medical and veterinary importance and the diseases transmitted by them, with emphasis on schistosomiasis and angiostrongyliasis. **Methods:** The course was based on the students' needs on the topics, through interviews before the course. The team composed by the researcher, undergraduate and graduate students gathered and defined the objectives to be achieved, strategies and pedagogical assessments. **Results:** The main goals to be achieved by the course were to recognize the biological characteristics of the main mollusc vectors, to identify the ecological and epidemiological characteristics of the parasite transmission areas and to carry out control measures of the vector mollusks. As a pedagogical strategy, the problem-solving method was used and the evaluation process was based on self-assessment, discussion of problem situations and preparation of reports, mainly practical activities. We opted for simple language, with practical and regional examples, illustrative slides and practical activities. The biological cycles of *Schistosoma mansoni* and *Angiostrongylus cantonensis* were also emphasized, due to the presence of mollusk vectors of these parasitosis in the locality. **Conclusions:** The construction and execution of the course was enriching, allowing the personalized creation of a course for a specific reality and the exchange of experiences between the professors of the course (researchers, undergraduate and graduate students) and health professionals with practical experience with the population. All students and teachers taught and learned themselves.

Supported by PAEF/IOC/Fiocruz

Schistosomiasis mansoni urban in the city of Rio de Janeiro

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Introduction: Schistosomiasis is a neglected parasitic disease; in Brazil there are about six million infected, mainly found in the states of the Northeast and Southeast. In the latter region, the states of Minas Gerais and Espírito Santo have the highest number of cases. In the state of Rio de Janeiro, the regions that presented the most number of the cases were north of the state. **Methods:** This is a qualitative research carried out at the Laboratório de Avaliação e Promoção a Saúde Ambiental at the Instituto Oswaldo Cruz. Based on data reported and updated between 2007 to 2018 by Programmatic Areas, Administrative Regions and Neighborhoods of the city of Rio de Janeiro through the Coordination of Epidemiological Surveillance (SINAN – MRJ). According to census in 2010 the city has about 6,320,446 inhabitants, and the compulsory notifications in the city occurred from 2006.**Results:** The city of Rio de Janeiro is not considered an endemic area; studies done through database of the SINAN-MRJ reported 251 positive cases between 2007 - 2018, including 26 deaths. According to the database, the Programmatic Areas (PA) with the most positive cases of schistosomiasis were: PA 4.0 has 19 neighborhoods and involves the regions of Jacarepaguá, Cidade de Deus and Barra da Tijuca, which presents 53 confirmed cases, without reports of deaths; PA 3.1 (28 neighborhoods) that is in the region of Ramos, Penha, Ilha do Governador, Complexo do Alemão and Maré with 47 confirmed cases and 04 deaths; PA 2.1 (18 neighborhoods) located at the regions of Botafogo, Copacabana, Lagoa and Rocinha, with 28 cases without deaths notification and PA 1.0 (14 neighborhoods) located at the regions of Port Zone, Rio Comprido, Centro, São Cristóvão, Paquetá and Santa Tereza with 27 confirmed cases and 03 deaths. **Conclusions:** Although the city of Rio de Janeiro is not considered an endemic area for schistosomiasis, data from the compulsory notification show a constant number of cases in the last ten years. The autochthonous cases need to be investigated, but the presence of mollusks, intermediate hosts, in urban areas and the lack of sanitation ensure the constant possibility of transmission.

Supported by PAEF/ LAPSA/ IOC/Fiocruz

Schistosomiasis Control Program in Picos, Piauí, Brazil: an alert for health surveillance

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Introduction: In Piauí state, the transmission of schistosomiasis was considered focal, limited to the Picos city, with two deaths between 2005 and 2010, without cases of hospitalization. Despite decrease of numbers cases in Picos, there are several indicators to extend attention to avoid installation and upsurge of transmission as: deprived environmental quality and scarce sewage system; an intense people traffic; be located on the boundary with two populous states (Pernambuco and Ceará) as well as crossed by important brazilian highways; a perennial river maintaining intermediate host populations of trematodes; and finally a human population with little or no information about the disease. The aim of this research was to understand the actions of the local Schistosomiasis Control Program (SCP) between 2014 and 2017 and identify difficulties experienced by surveillance agents. **Methods**: This study was developed through a semi-structured interview with the Health Surveillance (HS) and Zoonoses Control Centre (ZCC) staff, in order to identify the history of SCP in Picos city, how actions were planned, how many professionals are currently involved in, what activities and how often they generally develop and comparing the observations with nation-wide publications related to SCP. **Results:** The planned actions developed by SCP, such as epidemiological delimitation and surveillance, coproscopic survey, treatment of positives human cases, control of host mollusc, environmental sanitation procedures, health education and systematic update of the Schistosomiasis System of Information and Control were inefficient and precarious. It was detected variations among different data source, and absence of data sampling systematization mainly due to reduced technical staff (n = 7) and laboratorial infrastructure. According to observed in interviews, depending on demand, these agents also work in neighbouring cities. The follow-up process of positive cases in humans is performed by the Public Basic Health Units, generally closer to the patient's residence and their results, hardly return for SCP to be analysed. In addition to SCP limitations, the effectiveness of schistosomiasis control is compromised because, even if necessary actions were effective, there is no environmental sanitation in these areas which are favourable to spread of disease, as well as health education program for populations at risk of contamination. **Conclusion:** Since September 1977, when occurrence of cases in Picos city was detected, SCP has been practicing vigilance and control in order to eliminate the disease. Currently, the structure of SCP is very critical, demanding to be urgently revitalized. This situation turns attention and investments crucial so that country can achieve the goal of eradicating this disease as established in a treaty by World Health Organization.

Schistosomicidal activity of EF-24, an analog of curcumin

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Introduction: Over the last few year, the search for anti-schistosomicidal hit/lead chemistries has intensified. Curcumin, a keto-enol constituent of tumeric, demonstrates *in vitro* and *in vivo* schistosomicidal and inflamatory activities. However, the *in vivo* potency is low due to poor oral absorption. Studies have been shown that the mono-carbony analog, 3,5-bis[(2-fluorophenyl)metlhylene]-4-piperidione acetate (EF-24), exhibits broad-spectrum biological activities. **Purpose:** The purpose of this study was to evaluate the pre-clinical potential of EF-24 against the Luis Evangelista (LE) and Puerto Rican (PR) isolates of *Schistosoma mansoni.* **Methods:** EF-24 was first screened against schistosomula of *S. mansoni* PR *in vitro* at concentration of 1 and 10 µM followed by a re-evaluation against adult worms at concentration 10 uM. In parallel, the compound was evaluated *in vitro* against adult and juvenile *S. mansoni* LE. Morphological changes were evaluated *in vitro* against adult *S. mansoni* LE followed by cytotoxic studies with mammalian cells. EF-24 also administrated at 400 mg/kg by gavage or intraperitoneally to mice harboring 49-day-old mature *S. mansoni* LE infections. **Results:** After 24 h at 10 µM, EF-24 caused severe degeneration in 80-100% of schistosomula and a hight mortality of adult *S. mansoni* PR. Against *S. mansoni* LE adults and juveniles after 24 h, EF-24 was active with IC_{50} values of 9.6 and 6.7 µM, respectively. Also, tegumental damage was evident with adult *S. mansoni.* EF-24 was 3 times more toxic to adult *S. mansoni* than mammalian cells under *in vitro* conditions. However, EF-24 showed no schistosomicidal activity *in vivo.* **Conclusion:** This is first report regarding the schistosomicidal activity of the curcumin analog EF-24. Considering the results obtained here, it would be of interest to explore the structure-activity relationship of structural analogs. Another possibility would be to use EF-24 in combination with praziquantel which may improve schistosomicidal activity *in vivo.*

Supported by: FAPESP (Grant number: 2016/24456-1; 2018/50011-2)

IN VITRO EVALUATION OF THE EFFECTS OF CURCUMINOIDS AGAINST WORMS AND EGGS OF *SCHISTOSOMA MANSONI* PARASITES

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Introduction: Schistosomiasis is a Neglected Tropical Disease caused by the helminth *Schistosoma mansoni* and estimating about 230 million people affected by this parasitosis worldwide. Researches with natural products have been carried out in order to identify new effective compounds against the disease since the current treatment with Praziquantel (PZQ) has shown low efficacy against young worms stages and drug-resistant strains. Recent studies with Curcumin (CUR) have demonstrated potential schistosomicidal activity *in vitro* leading to further researches with their analogs, curcuminoids. The objective of this work is to analyze 26 CUR analogs against *S. mansoni* Luis Evangelista (LE) strain and identify promising compounds against the parasitosis. **Methods:** Initially, CUR analogs were screened at 50µM against adult worms, selecting the compounds that showed 80-100% of the reduction in parasite viability up to 72 hours, being tested again at concentrations of 3.125µM to 50µM. **Results:** Of the 26 compounds, 8 were selected for tests at lower concentrations and 4 analogs showed higher performance causing the death of 100% of the parasites in 72 hours at concentrations lower than 50µM, 3 causing morphological damage in male and female worms and 4 compounds caused egg damage, causing malformation of the eggs. **Conclusion:** The CUR analogs tested are potential candidates for further studies and investigation of their schistosomicidal activities *in vivo* in order to understand their mechanism of action and optimize their action against the *S. mansoni* parasite.

Supported by FAPESP (2018/04088-3 and 2016/24456-1)

Mortality due to schistosomiasis in Brazil: epidemiological and spatio-temporal patterns, 2000-2015.

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Introduction: Schistosomiasis is still an important cause of death in Brazil. Assessing the burden of schistosomiasis mortality in the country is important to provide useful information for health service planning and to define the locations where control actions need to be intensified. In addition to allowing the monitoring and evaluation of future impacts on schistosomiasis, after the implementation of these new strategies. Thus, the objective of this study is to characterize the epidemiological patterns and the spatio-temporal distribution of mortality related to schistosomiasis in Brazil from 2000 to 2015. **Methods:** Were included all deaths recorded in Brazil. 2000 to 2015, in which schistosomiasis was mentioned in the death certificate as the underlying or related cause of death. We used the municipalities of unit of analysis of geographic data. Standardized and smoothed mortality coefficients were calculated using the Local Empirical Bayesian method (per 100,000 inhabitants). Global and local spatial autocorrelation using the global and local Moran index, based on the LISA (Local Spatial Association Indicators) statistical method. To obtain the spatial analysis, we use the G and G * indices (Genus) of Getis-Ord, revealing spatial agglomeration. The analysis data were distributions in quadrennium. **Results:** During the study period, 17,374,134 deaths were recorded in Brazil. Schistosomiasis was mentioned in 11,587 deaths, 8,216 (70.91%) as the underlying cause and in 3,371 (29.09%) as an associated cause. The mean mortality rate was 0.38 deaths / 100,000 inhabitants. Male (relative risk [RR]: 1.14), age group ≥ 70 years (RR: 72.13) and residents of the Northeast region (RR: 11.35) plus new risks related to schistosomiasis. Municipalities with high mortality rates (> 1.0 deaths / 100,000 inhabitants) were found in all regions. In all quadrennium, high risk clusters were identified in areas of the Northeast and Southeast regions of Brazil. **Conclusions:** Schistosomiasis persists as an important cause of death in Brazil, especially in areas with a high prevalence of the disease. Reinforcing the need for the development of surveillance actions in health and control in the areas of greater risk, with special attention to the male population and the elderly.

Flavonoides from *Chromolaena odorata* in schistosomiasis control

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Introduction: Schistosomiasis is caused by digenetic blood trematodes. The three main species infecting humans are *Schistosoma haematobium, S. japonicum*, and *S. mansoni*. Two other species, localized geographically in African (Congo and Guinea) and Asian continent (Cambodia and Laos), are *S. intercalatum* and *S. mekongi*. *In the current scenario of schistosomiasis control, the disease cycle is interrupted by a synthetic molluscicide indicated by the WHO, Niclosamide (Bayluscide®). This substance, besides being of high cost, is not specific and can causes environmental disasters. Therefore, it is necessary to search and develop new agents more specific to the molluscs vectors and less aggressive to the environment. Plant extracts from medicinal plants have been evaluated for their potential in controlling the schistosomiasis cycle*, such aqueous and ethanolic extracts of leaves of *Chromolaena odorata* as molluscicide against different developmental stages of *Biomphalaria pfeifferi* one of the *Biomphalaria* species in the Old World. According with literature data, flavonoids are also cited as molluscicide and cercaricide agents. By this way, it is important to evaluate the potential of *Chromolaena odorata* extracts as well as their flavonoids against *B. glabrata* and others molluscs that occur in the American continent. **Methodology:** *Chromolaena odorata* collected in Petrópolis (RJ) were separated into leaves and inflorescences, dried and pulverized before extraction. Hydroethanolic extracts from leaves and inflorescences of *C. odorata* were produced in our labor according with Brazilian Pharmacopea and were evaluated chemically by TLC and HPLC-UV-DAD to evaluate their flavonoid content and biologically to investigate their potential against molluscs *B. glabrata*. **Results & Discussion:** The flavonoid content of the hydroethanolic extracted observed through TLC, HPLC and literature data, confirm the flavones as the main flavonoid type in *Chromolaena*extracts. **Conclusions:** *C. odorata* seem to be a promising plant molluscicide and or cercaricide candidate and deserve further studies in order to identify and characterize its responsible componentes, the minimal concentrations to these biological actions to contribute with the necessary parameters to the standardization of its hydroethanolic extracts and the development of a future product in the control of schistosomiasis.

Supported by CNPq, Farmanguinhos and CIEE.

Schistosomiasis mansoni in Belém, Pará state, Brasil: a historical review of disease occurrence of the last decade

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Introduction Schistosomiasis caused by *Schistosoma mansoni* is one of the most neglected tropical diseases. In Brasil it represents an important public health problem in rural areas and urban peripheries of some major cities. The disease is more intensely distributed along the Brazilian northeastern coastal region and also affects in the same scale areas in the south of Bahia and the north of Minas Gerais. In the northern region - especially in the capital of the state of Pará, Belém - schistosomiasis is predominately diagnosed in neighborhoods with precarious sanitation services, large population densities and with tide dependent flooding. This study aims to describe the disease occurrence in Belém during the last ten years and to identify the population groups most affected by the infection. **Methods:** Secondary data from the Information System of the Schistosomiasis Surveillance and Control Program (SISPCE) of the Municipal Health Department of Belém (SESMA) from 2007 to 2017 were used to carry out this study. Data were processed using Epi-Info software, version 2007. In order to analyze the variables gender and age in relation to infection with the parasite, the Pearson chi-square test with a confidence level of 95% was applied and the relative risk was calculated. The SESMA authorized the provision of the data as stated in the official document 161/2017 of the health authorities. **Results:** During the period of 10 years, 85,975 stool samples were examined according to the Kato Katz technique, of which 1,031 (1.19%) proof positive for infection with *S. mansoni*. Of these positive exams, 795 (77.10%) were obtained from males and 236 (22.90%) from females. The analysis of the association between the variables sex and infection revealed that males had a relative risk 3.96 times higher than females in contracting the disease. The age group between 10 to 25 years presented a relative risk 2.56 times higher than the group between 26 to and 46 years. It is worth mentioning that in the ten year long period observed, in 2012 the prevalence exceeded for the first time the 1.00% mark and continued to increase until 2017 reaching 2.46%. **Conclusion:** The population most affected by schistosomiasis in Belém, Pará state is mainly comprised of inhabitants from neighborhoods located in urban wetland areas with insufficient coverage of basic sanitation services, which favors the maintenance of disease transmission. In this context the epidemiological surveillance services are essential for a continuous control and the eventual elimination of this disease as a public health problem.

Supported by PPGEVS/IEC/SESMA

Solidago chilensis hydroethanolic extract: Special metabolites in Schistosomiasis control

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Introduction: Schistosomiasis is caused by trematode helminths of *Schistosoma* genus. Nowadays, this disease affects more than 250 million people in the world. In Brazil, 9 states are endemic and the Northeast and Southeast regions being the most affected. The use of molluscicides extracted from plants becomes a less aggressive option to the habitat, due to the quick degradation of these products in the environment, being less aggressive than those that are chemical. According to with literature data, *Solidago chilensis* Meyen (SCM) is mentioned in the literature as “arnica Brasileira”, and it is externally used to treat wounds, trauma, contusions, as anti-helminth and antidiuretic. This species is included in the 71 plant list of SUS (Brazil’s Unified Health System) and at the Herbal Therapeutic Memento of Rio de Janeiro city. Previous studies show that diterpene and flavonoids are responsible for the anti-inflammatory effect of SCM and also the activity of these extracts against worms. **Methodology**: SCM were collected in two different regions of the Rio de Janeiro state. Its inflorescences were dried, pulverized and extracted by dynamic maceration according to the Brazilian Pharmacopoeia and it was analyzed by HPLC-UV-PDA for its retention time, UV absorption at 310nm and literature data. The obtained extracts from two different collected regions were compared chemically and will be evaluated against their molluscicide potential, especially by their high content of the flavonoid types of quercetin and kaempferol. **Results & Discussion**: The extraction and chemical analysis by TLC and HPLC-UV-PDA showed that the hydroethanolic extract has a flavonoid content in a range of 13.5 to 15.5%. Corresponding to 0.135-152mg/mg in 0.1mL of the tincture. Among the eluted substances were characterized 6 flavonoids already described for the *Solidago* genus and probably related to the pharmacological actions observed for this species. In addition, was also characterized by the chlorogenic acid. **Conclusions**: Considering the previous literature data, these results are important to obtain the validation, standardization and the safety and efficacy of tinctures, determining its chemical and/or their biological markers of the produced SCM extracts and to determine the minimal active concentration to schistosomiasis control.

Supported by CNPq, Farmanguinhos, and CIEE.

THE LIMITS AND POSSIBILITIES OF THE SCHISTOSOMIASIS INFORMATION SYSTEM FOR SURVEILLANCE AND CONTROL ACTIONS IN THE STATE OF RIO DE JANEIRO

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INTRODUCTION: Schistosomiasis is a public health problem in Brazil, where there are still many areas considered medium to high endemicity for the disease. Because of the difficulties for its control and surveillance, it is necessary to understand the dynamics of transmission contemplating the political, economic and social dimensions. Epidemiological data grouped into information systems represent an important instrument for guiding control and surveillance actions. The objective of this study was to analyze the limits and possibilities of the Information System in Schistosomiasis Control Program (SISPCE) for the characterization and surveillance of the disease at the local level. **METHODS:** Data were collected and calculated based on epidemiological indicators such as the coverage and intensity of the Schistosomiasis mansoni program in municipalities with notification for schistosomiasis in the state of Rio de Janeiro, from 1998 to 2016. **RESULTS:** The results point to a low number of municipalities worked and insufficient records in the system, insufficient information provided for the characterization of the endemic and ideal feedback information for the management and development of actions based on the development of the disease. **CONCLUSION:** SISPCE is an advance in the monitoring and surveillance of schistosomiasis, requiring systematic actions by municipalities, maintaining a continuous flow of epidemiological data for the development of efficient management actions. It is justified the need to incorporate the locality as one of the units of analysis, considering its unique aspects for the production of schistosomiasis.

SESSION:

Immunopathology, Molecular Biology, and Biochemistry

Schistosoma mansoni SmKI-1 or its C-terminal Fragment Induces Partial Protection against *Schistosoma mansoni* Infection in Mice

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Introduction: Current schistosomiasis control strategies are mainly based on chemotherapy, but the development of a vaccine against this parasitic disease would contribute to a long-lasting decrease in disease spectrum and transmission. When it comes to vaccine candidates, several genes encoding *Schistosoma mansoni* proteins expressed at the mammalian host-parasite interface have been tested. Among the most promising molecules are the proteins present on the tegument and digestive tract of the parasite. In this study, we evaluated the potential of *Sm*KI-1, the first Kunitz-type protease inhibitor functionally characterized in *S. mansoni*, as a vaccine candidate. **Methods:** Bioinformatic analysis was performed in order to predict relevant epitopes present in *Sm*KI-1 molecule. Then, we prepared vaccines, using the recombinant (r) *Sm*KI-1 and two different fragments: its Kunitz (KI) Domain and its C-terminal tail, all formulated with Freund's adjuvant. After the vaccination, mice were challenged with *S. mansoni* cercariae and the production of specific antibodies, cytokines and the protection efficacy against schistosomiasis were evaluated. Finally, we assessed and compared liver pathology in all groups of vaccinated mice. **Results:** Bioinformatic analysis pointed to the C-terminal fragment as the main region of the molecule responsible for the development of a potential protective immune response induced by*Sm*KI-1. We demonstrated that mice immunized with r*Sm*KI-1 or its fragments, were able to produce IgG specific antibodies. Further, all vaccine formulations tested here also induced a Th1-type of immune response, as suggested by the production of IFN-γ and TNF-α by protein-stimulated cultured splenocytes. However, the protective effect conferred by vaccination was only observed in groups which received r*Sm*KI-1 or C-terminal domain vaccines. Mice administered with r*Sm*KI-1 demonstrated reduction of 47% in worm burden, 36% in egg number in mouse livers and 33% in area of liver granulomas. Additionally, mice injected with C-terminal domain showed reduction of 28% in worm burden, 38% in egg number in liver and 25% in area of liver granulomas. In contrast, KI domain immunization was unable to reduce worm burden and ameliorate liver pathology after challenge infection. **Conclusion:** Taken together, our data demonstrated that *Sm*KI-1 is a potential candidate for use in a vaccine to control schistosomiasis, and its C-terminal tail seems to be the main region of the molecule responsible for protection conferred by this antigen.

Supported by CAPES/CNPq/UFMG

Production of soluble and functional *S. mansoni* recombinant *Sm*KI-1 Kunitz-domain for functional assays and point mutation analysis

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Introduction: The flatworm *Schistosoma mansoni* is a blood parasite that causes schistosomiasis, a debilitating disease that occurs in over 76 countries around the world. Upon migration and feeding, the parasite is targeted by substances from the host's immune system and has to bypass blood coagulation, innate and adaptive immune responses, among other barriers. The SmKI-1 protein, present in both parasite tegument and gut, is intrinsically associated to the parasite survival. Its primary structure presents two domains: (1) a Kunitz-type serine protease inhibitor motif and (2) a disordered C-terminus domain with no homology outside the genera, which seems to be associated to biological membrane cell interaction. The Kunitz-domain (KD) has been proved to play an essential role in neutrophil elastase binding blockage, in neutrophil migration/activation impairment and to be of elevated anti-coagulant and anti-inflammatory biotechnological properties. The KD presents in its tertiary structure an α-helix and a double antiparallel β-sheets, being stabilized by six highly conserved cysteine residues connected in three arrangements of disulfide-bridges, two of them crucial for maintaining native structure conformation. The other disulfide-bridge stabilizes the catalytic domain and the reactive site (P1), the later one being central for its serine protease inhibitory activity due to an arginine residue (18Arg). A mutation on this specific residue or on those that interact with it seems to be the key for enhancing its enzymatic activity, generating a powerful biotechnological product. The correct assembly of the disulfide-bridge arrangements is then of utmost importance for production of functional and properly folded protein. Therefore, this study aimed to produce the SmKI-1 KD domain in its functional form for further point mutation of the Arginine (18Arg) for enzymatic and anti-inflammatory assays. **Methods:** The domain (here termed DKI) was cloned and expressed in a specific system developed in our lab in order to recover a functional and soluble protein after cell lyses. Briefly, the DKI fused to a specific tag for enhancing protein solubility was cloned into an expression plasmid proper for heterologous expression in an E. coli DE3 strain. **Results:** After several strategies used for correctly assembled protein recovery, the functional and folded DKI was obtained. Its structural integrity conformation was checked through circular dichroism and 1D-NMR experiments. Furthermore, we performed structural in silico analyses on the mutated catalytic site in order to predict its structural and interaction changes. **Conclusions:** In summary, our results described an optimized system from cloning to expression of functional proteins containing multiple disulfide bridges for functional assays and biotechnological application.

Keywords: Kunitz inhibitors, point mutation, *Schistosoma mansoni.*

Supported by: Financial support: CNPq, FAPEMIG and CAPES.

Immunopathological aspects of *Schistosoma mansoni* infection in patients with low parasitical load and with history of American Cutaneous Leishmaniasis

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Introduction: Helminths such as *S. mansoni* and protozoa of the genus *Leishmania* are parasites with high prevalences in some tropical regions. Due to wide extension of transmission areas for both parasites, the risk of cocomitant infections are frequent. However, the impart that this type of coinfection and the interactions between different parasites may cause in humans is still poorly studied. Here, we characterized the parasitological, immunological and clinical aspects of patients infected with *S. mansoni* or without a recent history (last 10 years) of American Cutaneous Leishmaniasis (ACL). **Methods:** A total of 257 residents of Brejo do Amparo district, municipality of Januaria, Norther Minas Gerais, an endemic area for socioeconomic questionnaire and provide stool and blood samples. Stool samples were processed to perform spontaneous sedimentation (HPJ), saline gradient, Helmintex® and extensive Kato-Katz techniques for parasitological evaluation. Blood sample were drawn for a hemogram and to measure serum concentration of total IgE, cytokines and chemokines. Individuals with positive parasitological examination for *S. manso*ni were invited for clinical and ultrasonographic examination. The identification of ACL history was obtained through individual socioeconomic, clinical records and through medical records from the Leishmaniasis Treatment and Research Center in Januaria. **Results:** *S. mansoni* infection was the most prevalent parasite in the population (n=119; 46.3%), and 91.6% of the egg-positive individuals eliminated less than 100 eggs/g feces (low parasitic load). In the whole population, 93 individuals (36.2%) have reported ACL, and the prevalence for schistosomiasis in these individuals was significantly higher than in the non-reporting group (p=0.03). individuals with a recent history of ACL and infected with *S. mansoni* (>12eggs/g feces) eliminate fewer eggs. In addition, the reduction of some Th2-type mediators in the peripheral blood was measured in these individuals (decrease in CCL-3, IgE levels and eosinophilia). Opposite to that, schistosomiasis patients with recent ACL history showed increased IL-27 concentrations when compared with individuals only infected with *S. mansoni.* It was also possible to observe a tred in maintaining normal values os hemoglobin and platelets in individuals with a recent history of ACL and infected by *S. mansoni.* The presence of severe fibrosis was also less frequent in this group of patients. **Conclusions:** The data suggest that a recent history of ACL alters the immune response against *S. mansoni* infection, modifying the severity of schistosomiasis in humans.

Supported by FAPEMIG/DECIT/SCTIE/MINISTÉRIO DE SAÚDE/CNPq

Comparative Proteomic Analysis of Murine Serum Proteome on *Schistosoma mansoni* infection

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Introduction: Plasma proteins are a fine source of information towards understanding disease proceses. During schistosomiasis worms regurgitate by-products of their digestion, hence releasing proteins into the bloodstream. Proteomic approaches, can contribute to the understanding of how parasites modify or induce alterations in the host plasma proteome. These approaches might also be useful on the search of novel biomarkers. The objective of this project was the comparative compositional analysis of the plasma proteome from control and *S. mansoni*-infected mice. **Methods:** Plasma samples from non-infected and infected Balb/c mice were collected at 7 weeks post-infection. Protein samples were separated using 1D and 2D SDS PAGE. In parallel, a shotgun spectrometric-based approach was used to assess the compositional analysis and label-free quantification of the investigated samples. **Results:** The 1D SDS PAGE profile revealed dominance of protein bands in both groups. The 2D profiles revealed spots uniquely present in the infected sample. The compositional analysis performed using shotgun mass spectrometric approach revealed the identification of 359 proteins. Of these, only three plasma constituents (albumin, serotransferrin and pregnancy zone protein) corresponded to 85% of the protein content present in the analyzed proteomes. In all, 95 constituents were differentially expressed, of which 81 were upregulated in infected animals. The latter were mostly related to immune functions such as immunoglobulins, complement factors and acute-phase related proteins. **Conclusions:** The presence of parasites living in the host bloodstream modify the composition and abundance of plasma constituents. However, the inherent dominance of a few plasma proteins poses a challenge for the identification of lower abundant ones. Further studies are under way for depletion of major serum components and they should potentially increase the repertoire of molecules associated with *S. mansoni* infection.

Financial Support: Fapemig/UFOP.

FUNCTIONAL CHARACTERIZATION OF POTENTIAL VACCINE ANTIGENS AGAINST SCHISTOSOMIASIS

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Introduction: Schistosomiasis affects more than 200 million people in 78 countries and treatment relies on a single drug, Praziquantel. The emergence of drug resistant parasite strains and its lower effectiveness against the younger parasites point to the need for further intervention methods to control the disease, such as vaccines development. Currently, the availability of parasite genome information and the establishment of advanced methodologies for proteome studies have favored a rational search and the identification of more promising vaccine candidates. Using two-dimensional electrophoresis associated with Western blotting with serum of infected and uninfected individuals from schistosomiasis endemic area we identified some immunoreactive proteins of *Schistosoma mansoni* adult worms. Among them, we selected the gelsolin protein as a potential vaccine target due to its recognition by serum of naturally resistant individuals. The vilin protein was also being studied because it shares significant amino acid sequence similarity to gelsolin. Before evaluate the immunoprotective potential of these proteins against *S. mansoni* infection, we intend to perform a functional characterization of these proteins in schistosomula by RNA interference (RNAi) using double stranded RNAs (dsRNAs). **Methods:** Specific primers to the transcript sequences of the target genes retrieved from the GeneDB database for gelsolin (Smp_008660.1) and vilin (Smp_197860) were designed using *Primer 3* software tool. The amplicons were used for *in vitro* dsRNAs synthesis and schistosomula were exposed to 200nM dsRNAs for each target gene: gelsolin and vilin, separately and simultaneously. The control groups were exposed to nonspecific GFP-dsRNA, and not exposed to any dsRNA (negative control). *In vitro* phenotypic changes in body size, color, shape and viability were followed in schistosomula exposed to dsRNAs up to 30 days. The area (µm2) of the parasites was determined using the *AxionVision 4.8.0.0*program and the viability was assessed with propidium iodide (5µg/mL). **Results:** The results showed that the percentage of knockdown dead schistosomula did not exceed 5% over the 30 days of dsRNA exposure, both in the control and in the experimental groups. By optical microscopy analysis it was observed that dsRNAs exposure did not change the body shape and natural color of the parasites. In order to evaluate other phenotypic changes, the body surface area of the dsRNA exposed schistosomula was measured and was observed a tendency to reduce the area of the worms that were exposed to the dsRNA-vilina when compared to those exposed to the GFP-dsRNA. **Conclusions:** The *S. mansoni* schistosomula exposed to specific gelsolin- and vilin-dsRNAs, separately and simultaneously, did not showed significant changes in the *in vitro* phenotypes investigated in this study.

Supported by FIOCRUZ/CNPq/PROEP-RIPAg, FIOCRUZ-CPqRR, PIBIC-CNPq.

Schistosomiasis in mice co-infected with *Strongyloides venezuelensis* at different stages of *Schistosoma mansoni* infection

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Introduction: Human co-infection by different helminthes is frequent, but their consequences are mostly unknown. *Schistosoma mansoni* and *Strongyloides stercoralis* share common endemic areas, however, this co-infection remains neglected. We investigated if and how these infections might interact to change the pathological and immunological outcomes generally observed when one single infection affects the host. **Methods:** To investigate the impact of coinfection on disease pathology and immune response, we carried out coinfection studies using *S. mansoni* and *S. venezuelensis* in mice. In the current study, female Swiss mice were simultaneously co-infected with *S. mansoni* and *S. venezuelensis* or infected with *S. venezuelensis* 2, 4, or 14 weeks after *S. mansoni*-infection. The animals were anesthetized, the blood collected and the serum frozen for immunoenzymatic assays (ELISA) and to evaluate the pathology. The parasite load was evaluated by the recovery of adult worms and the quantification of the eggs retained in the liver. **Results:** The parasite burden (adult worms and egg retained in liver) for *S. mansoni* was similar in co-infected mice in compared with mono-infected mice. However, the induction of immune response and schistosomiasis associated pathology were different and depend on the time when *S. venezuelensis*-infection occurred. In chronicle phase of schistosomiasis, the simultaneously infected mice showed a decrease in IL-4, IL-17, TGF-β and TNF-α concentration in liver homogenate, comparing to the trematode-infected mice. And, there was a significant increase of IFN-γ. Mice that received the nematode infection after 2 or 4 weeks of the *S. mansoni*-infection showed a concentration of IL-4, IL-13, INF-γ, IL-17, TGF-β and TNF-α higher in liver homogenate than mono-infected mice. When mice were infected with *S. venezuelensis* 14 weeks after *S. mansoni*-infection the level of cytokines in liver homogenate were similar to mono-infected mice. Despite variations in the cytokine profile, there was a significant increase in inflamed liver area of co-infected animals compared to animals infected only by *S. mansoni.* We, indirectly, characterized the levels of macrophages, neutrophils and eosinophils in liver homogenate. And we find an increase of levels of macrophages at chronicle schistosomiasis, and neutrophils, at acute phase, in mice co-infected 2 weeks after *S. mansoni*-infection and infected concomitantly. Mice co-infected 4 weeks after trematode-infection showed increase of eosinophil levels in comparison with mono-infected group. **Conclusions:** This result suggests that the effect of co-infection on host immune responses and morbidity largely depends on the species of parasite and phases of disease.

Supported by CAPES/CNPq.

Phenotype of T CD4+ lymphocytes from patients with periportal fibrosis secondary to *Schistosma mansoni* infection

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Introduction: Schistosomiasis is a parasitic disease that affects about 240 million people around the world. It is estimated that 5-10% of individuals develop into severe forms of the disease. The chronic phase of the disease is associated with Th2 type response, but evidence also suggests the participation of Th1 and Th17 cytokines in the severity of the disease. Our objective was to evaluate the CD4 T lymphocyte profile of patients with the severe forms of schistosomiasis. **Methods:** Peripheral blood mononuclear cells were stimulated with soluble egg *Schistosoma mansoni* antigen (SEA). Expression of surface molecules and cytokines in the lymphocytes were performed by labeling with monoclonal specific antibodies and acquired by flow cytometry. Twelve subjects were recruited for the evaluation of immunology, and 5 subjects were classified without fibrosis and 7 with incipient fibrosis not excluded (WF/IFNE), 10 with periportal fibrosis and 3 with possible periportal fibrosis (PF/PPF), 2 with advanced periportal fibrosis and 2 with advanced periportal fibrosis with portal hypertension (APF/APF+PH). Lymphocytes were obtained by separation of PBMCs and analyzes of the molecules and cytokines were performed by flow cytometry. **Results:** It was observed that the PF/PPF individuals had higher mean fluorescence intensity (MFI) of the IL-4 and IL-5 cytokines compared to the WF/IFNE individuals, as well as, these individuals presented higher MIF of the cytokines IFNγ and IL-17 compared to the SF / FINE group ($p < 0.05$). In addition, the PF/PPF group showed higher MFI of GITR and CTLA-4 molecules compared to the WF/IFNE group ($p < 0.05$). We also observed that MFI of IL-10 expressed by $CD4^{+}CD25^{hi}$ lymphocytes among individuals with APF/APF+PH was higher than individuals with PF/PPF ($p < 0.05$). In addition, the PF/PPF group had a higher frequency of $CD4^{+}CD25^{hi}$ cells compared to WF/IFNE individuals. **Conclusions:** Individuals with periportal fibrosis showed an increase in Th1, Th2 and Th17 cytokines by $CD4^{+}$ T lymphocytes, while the group of patients with advanced periportal fibrosis + pulmonary hypertension presented a regulatory profile associated with production of IL-10 by regulatory T cells.

Financial support: FAPESB Edital Universal 005/2015 (APP 0051/2016)

FUNCTIONAL STUDY OF GELSOLIN FAMILY PROTEINS FROM *SCHISTOSOMA MANSONI*

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Introduction: Reverse genetics techniques, such as RNA interference (RNAi), are powerful tools for gene functional studies. The application of those techniques in the functional characterization of *Schistosoma mansoni* identified genes provides a better understanding of the host-parasite interaction and the identification of vaccines and drugs targets. Immunoreactive proteins to serum of infected and uninfected, or naturally resistant, individuals from schistosomiasis endemic area were identified, among those, proteins of the gelsolin family. In some eukaryotic organisms, gelsolins play roles in regulating actin filaments that participate in the cytoskeleton formation, cellular motility and apoptosis stimulation. As it was also reactive to uninfected individuals sera, the gelsolins were chosen to be functional characterized in *S. mansoni*. **Methods**: Schistosomula were exposed to 200nM specific dsRNA of S. mansoni gelsolin, vilin and gelsolin/vilin, simultaneously, and to unspecific dsRNA-GFP over 6 days and then used to infect mice. The levels of transcripts and proteins were analyzed in the different life stages of *S. mansoni* and also in knockdown schistosomula by quantitative Real Time PCR and Western blotting, respectively. The location of the gelsolins in the parasite was determined and proteins levels were also assessed by immunofluorescence. **Results:** The results evidenced that both genes are more expressed in female adult worms and, the proteins, are present in all life stages of the parasite, being more abundant in cercariae and schistosomule. The gelsolins showed diffuse distribution throughout the schistosomule parenchyma and co-localized with actin filaments, especially in the tegument. Six days after dsRNA exposure, transcriptional levels of knockdown parasites were verified in schistosomula exposed to dsRNA-gelsolin (77%), -vilin (93%) and in combination, -gelsolin/vilin (71% gelsolin and 89% vilin). The analysis of the Western blotting assay showed that schistosomula exposed to dsRNA-gelsolin, -gelsolin/vilin and -vilin presented no decrease in protein levels in comparison to the parasites treated with dsRNA-GFP, although in the immunofluorescence assays, schistosomula knockdown for those genes showed a decrease in fluorescence intensity, respectively: 9.9% (gelsolin), 11.9% (vilin) and 39.1% (gelsolin/vilin). Knockdown schistosomula infected mice were perfused and there was no correlation between number of recovered adult worms and number of eggs retained in the liver and intestine. **Conclusions:** This work presents important knowledge regarding the expression of gelsolin and vilin in the different life stages of the *S. mansoni*, their localization and roles in the parasite development since it is possible that they are involved in mechanisms of cytoskeleton organization. It is necessary to emphasize the importance of gene functional characterization studies in the post-genomic era, since, up to now; there are few characterized *S. mansoni* proteins.

Supported by CAPES, CNPq, FIOCRUZ, FAPEMIG e European Comission, A-PARADDISE.

Neuropeptides participation in the evolution of hepatic granuloma of mice infected by *Schistosoma mansoni*

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Introduction: The genus *Schistosoma* is composed of flatworm, dioecious, parasitic at all stages of development and that always evolve into two hosts. *Schistosoma mansoni* is the only species of the genus described in Brazil, responsible for schistosomiasis. In the mouse, several factors perform the regulation of the immune response to the parasite, including neuropeptides such as P substance and Somatostatin. In this work, we intend to study the presence of somatostatin and P substance during the evolution of peri-ovular hepatic granuloma in *S. mansoni*-infected mice at different times of infection related to the cells that make up the granuloma. **Methods:** Swiss Webster mice infected with *S. mansoni* with 60, 90 and 150 days of infection were killed. Fragments of the liver were fixed in Millonig's Carson formalin for histological processing and staining with Hematoxylin and Eosin, PAS, Alcian blue and Masson's trichrome. Other fragments of the liver were frozen in liquid nitrogen and directed to cryomicrotomy. The frozen sections were fixed in acetone at -20°C and used for immunohistochemistry by incubation with antibodies to Somatostatin and P Substance. Secondary Antibody goat anti-rabbit conjugated to Alexa Fluor 488 was used for visualization of the immunostaining. The sections were stained with Evans blue and DAPI and mounted with anti-fading glycerol under coverslips. Confocal laser scanning microscope (LCSM 710 Zeiss) carried out the visualization. **Results:** In liver of animals with 90 days of infection, few macrophages of exudative granulomas were positive for Somatostatin. The intensity of the fluorescence varied in different granulomas. Sinusoidal capillaries near the granulomas also exhibited positive marking for somatostatin as well as miracidia within the eggs. **Conclusions:** Results indicate that somatostatin expression varies according to granuloma stage, which confirms the regulatory effect of this neuropeptide on the peri-ovular inflammatory response and on stellate cells in hepatic sinusoids. The later stages are necessary to confirm this impression and are currently under analysis.

Supported by: PROVOC/EPSJV/Fiocruz

Evaluation of the reactivity of monoclonal antibodies induced by immunization with antigenic peptide from *Schistosoma mansoni* SmATPDase 1

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Introduction: Schistosomiasis is an endemic disease caused by blood parasites of the genus *Schistosoma*. In the Americas, the infection is transmitted by *S. mansoni* and standard method for the diagnosis, the Kato-Katz, is based in the detection of eggs in stool samples from patients. Among the proteins expressed in *S. mansoni*, SmATPDase 1 is one of the most abundant in the tegument, justifying your chose as candidate antigen for immunodiagnostic of schistosomiasis as well peptides derived from the protein. **Methods:** Peptide was obtained by solid phase synthesis and monoclonal antibodies were produced by the Program for technological Development in Tools for Health – PDTIS FIOCRUZ. Western blots and immunofluorescence were used to confirm identity of the enzyme by antibodies. Reactivity was evaluated by ELISA. **Results:** Here, we developed monoclonal antibodies (mAbs) against a peptide from SmATPDase 1, characterized as IgG1 subclass. The mAbs recognized the enzyme (62 kDa) in the tegument of the parasite by Western blots and positive reactivity was found (0.225±0.009; 1:200; p<0,001) when compared with bovine serum albumin (BSA), ratify the mAbs affinity. Additionally, the reactivity of mAbs was significantly higher that the serum samples from healthy mice at dilution 1:50 (0.327± 0.055; p< 0.05) and 1:100 (0.212± 0.013; p< 0.05). **Conclusion:** These results show the potential application of KDVAKI peptide and their mAbs to study of *S. mansoni* SmATPDases and schistosomiasis diagnosis.

Keywords: Monoclonal antibodies, schistosomiasis, SmATPDase 1 peptide.

Financial support: CNPq, UFJF, FIOCRUZ and CAPES.

Elucidating the roles of SmFES and SmRAF protein kinases in *Schistosoma mansoni*

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Introduction: Praziquantel (PZQ) is the only drug available for schistosomiasis treatment. The identification of drug-resistant parasites demonstrates the urgent need for the establishment of effective and rational strategies for the identification of new therapeutic targets. Protein kinases are considered good drug targets, thus, several inhibitors have already been developed to treat human diseases such as cancer and other parasitic illnesses. Studies suggest that eukaryotic protein kinases (ePK) have an essential role in *Schistosoma mansoni* regulatory mechanisms and, therefore, parasite development and survival. In this study, we propose the characterization and validation of SmFES and SmRAF ePKs as drug targets. **Methods:** In order to elucidate the role of these proteins, schistosomula and adult worms were subjected to SmFES and SmRAF knockdown by RNA interference using specific dsRNAs and the transcript levels were evaluated by RT-qPCR. Schistosomula phenotypic changes were analysed using AxionVision 4.8 software for area measurement while propidium iodide was used for mortality evaluation. Adult worm movement was measured using WormAssay software and the oviposition from paired worms was evaluated. To assess the role of SmFES and SmRAF during the establishment of infection, mice were inoculated with knockdown schistosomula and perfusion was performed after 42 days of infection. Worms and eggs from liver and intestine were counted. Mann-Whitney statistical analysis was performed using GraphPad Prism 5. **Results:** After five days of dsRNA exposure, schistosomula transcript levels were ~88% reduced for SmFES and ~70% for SmRAF. After exposure, SmFES knockdown schistosomula were ~10% smaller than the control group and 14%-28% mortality was observed between the third and tenth day of dsRNA exposure.These results were statistically significant when compared to the control group. Changes in adult worm recovery from mice infected with SmFES knockdown were not observed, while mice infected with SmRAF knockdown schistosomula exhibited 14% less adult worms than the control group. Besides, ~41% of eggs recovered from the final section of the small intestine of mice infected with SmFES knockdown schistosomula were immature. There was no difference in total egg number recovery from liver and intestine. Adult worms were also transfected with SmFES- and SmRAF-dsRNAs. After four days of treatment, the transcript levels decreased by ~43% for SmFES and ~22% for SmRAF. Oviposition in SmFES knocked down worms decreased by approximately 45%. Additionally, male adult worms presented a decrease in movement starting in the first day of SmFES-dsRNA exposure; the highest reduction of ~36% was observed on the fifth day and ~31% for SmRAF-dsRNA treatment on the tenth day, respectively. **Conclusions:** These results suggest that SmRAF may play a role in *S. mansoni* development when in contact with the mammalian host but does not seem to be essential. Besides, SmFES seems to be important for *S. mansoni* reproduction, egg maturation and, schistosomula survival and development *in vitro*. Thus, SmFES could be a potential therapeutic target and could be effective in interfering with oviposition and worm maturation. Supported by: CAPES/CNPq/IRR/FIOCRUZ/Fapemig

The use of gold nanorods as a new vaccine platform against schistosomiasis

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Introduction: Schistosomiasis is an important parasitic disease affecting more than 207 million people in 76 countries around the world and causing approximately 250,000 deaths per year. At present, the main strategy adopted for the control of schistosomiasis is the use of safe chemotherapy, such as praziquantel. However, the high rates of reinfection after treatment restrict the use of this treatment approach and assume the need for other forms of control such as vaccination. Sm29 is a protein that is localized in the *Schistosoma mansoni* tegument of adult worms and schistosomula and is considered a powerful vaccine candidate. Because of the chemical, physical and immunological characteristics of nanoparticles, nanocarriers have received increasing attention. In the field of nanotechnology, gold nanorods are considered potential vaccine carriers. **Methods:** In this study, we bound *S. mansoni* rSm29 protein to gold nanorods either directly or by cysteamine functionalization. We evaluated them as vaccine candidates in a murine model of schistosomiasis. **Results:** When the worm burden was evaluated, the AuNRs-NH_2-rSm29 group of immunized mice showed the best protection levels (42%). Following AuNRs-NH_2-rSm29 immunization, we observed a Th1 immunological response in mice with higher production of IFN-g, mainly by $CD3^+CD4^+$and $CD3^+CD8^+$cells. Furthermore, AuNRs-NH_2-rSm29 could activate dendritic cells *in vitro*, being better uptaked and enhancing MHCII and MHCI expression and the production of IL-1β in a NLRP3-, ASC- and Caspase-1-dependent manner. **Conclusions:** In summary, our findings support the use of nanorods as an immunization strategy in vaccine development against infectious diseases.

Supported by CAPES/CNPq/Fapemig

Cheminformatics identification of *Schistosoma mansoni* Thioredoxin Glutathione Reductase (SmTGR) inhibitors from fragment-bound crystal structures

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Introduction: Since the 1980s, praziquantel (PZQ) is the most used drug to treat schistosomiasis. However, PZQ is ineffective against the larval forms of the parasite, which does not prevent re-infection, and its use in mass administration campaigns raises concerns about the emergence of resistant strains. Thioredoxin glutathione reductase (SmTGR) is an essential antioxidant enzyme of *Schistosoma mansoni* and thus considered as a validated target for development of new schistosomicidal drugs. Recently, our group has been using Fragment-based Drug Discovery (FBDD) technique to identify new SmTGR inhibitors. FBDD employs low size (<300 Da) molecules, called fragments, to interrogate biological targets. Contrary to higher molecular weight compounds, commonly used in traditional screening campaigns, fragments can be grown to increase their affinity in several folds through rational design. Previously, we identified 36 SmTGR ligands in a fragment library (DSPL, Diamond Light Source) using X-ray Crystallography. In this work, we use cheminformatics tools to investigate if these fragments share structural similarity with known SmTGR inhibitors. Moreover, molecular docking simulations are performed to predict the interactions between the fragments/inhibitors and SmTGR. **Methods:** First, we annotated the SMILES code of 10,784 compounds identified as SmTGR inhibitors in a high throughput assay available in PubChem Database (AID: 485364). For each molecule, 17 molecular fingerprints, from chemoinformatic toolkits (OpenBabel, RDKit and CDK), were generated using Cynfony module for Python. Structural similarities between fragments and inhibitors were calculated by Tanimoto Coefficient (Tc). The molecules which shared more than 30% similarity in all fingerprints (consensus model) were ranked and selected for visual analysis. Molecular docking has been carried out by Autodock software using as receptors the crystal structures of fragment-bound SmTGR 3D structures solved by X-ray crystallography (PDB on hold). **Results**: In total, 83 out of 10,784 SmTGR inhibitors were identified by the consensus model with some similarity to the FBDD hits. Among them, 19 showed the highest Tc values, one inhibitor per fragment. These compounds were now submitted to molecular docking calculations. **Conclusions**: Some fragments show structural similarity with known SmTGR inhibitors. Molecular docking studies will reveal the main interactions between the inhibitors and SmTGR and suggest modifications to increase fragments inhibitory activity.

Supported by FAPERJ/CNPq/Fiocruz

Construction of a multiepitope chimeric protein by reverse vaccinology approach and evaluation of its immunogenic potential against infection by *Schistosoma mansoni* in murine model

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Introduction: Schistosomiasis is a serious public health issue in Brazil. Evidence of resistance to the drug and the absence of a fully effective vaccine leaves an open search for a vaccine target. Bioinformatics softwares, combined with genomic databases, allow the theoretical analysis of potential targets. This study aimed to develop a multiepitope chimeric protein whose peptides were rationally selected through computational analysis. Methods: Gene and protein sequences of *S. mansoni* were obtained from a public database (GeneDB). The components of this chimera were predicted by bioinformatics softwares for evaluation of subcellular localization, signal peptide presence, topology, and the presence of epitopes with binding affinity for MHC class II molecules. Four of the chimera components are fifteen amino acid epitopes derived from hypothetical plasma membrane proteins of *S. mansoni*. The fifth component is a peptide derived from the protein Sm14. The multiepitope protein was submitted to molecular modeling in order to create a model to evaluate the stability of its three-dimensional structure. The multiepitope chimeric protein was produced through heterologous expression by *Escherichia coli* (DE3) Rosetta strain and confirmed by western blotting. Protein purification was conducted by nickel immobilized column affinity chromatography. Immunization of C57BL/6 mice with the multiepitope protein was performed in three doses, formulated with complete and incomplete Freund's adjuvant for further cercariae challenge. **Results:** The multiepitope protein has been predicted to have a stable three-dimensional structure. After worm recovery, no protection was observed comparing the multiepitope vaccinated group to control group. Nevertheless, the immunization resulted in the production of significant levels of IgG isotype antibody since the second vaccination, with its levels remaining elevated up to 45 days post challenge. Pathology assays are being conducted to assess the influence of the multiepitope protein on liver damage amelioration. **Conclusion:** Although no protection against *S. mansoni* infection was observed on this study so far, reverse vaccinology has been shown to be a good methodology for the identification of antigenic molecules.

Financial Support: CNPq, FAPEMIG, CAPES

Polyethyleneimine (PEI)-mediated RNA interference in *Schistosoma mansoni* adult worms

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Introduction: RNA interference is up to date the most used and well-established tool for genetic manipulation in several parasites, including *S. mansoni*. Specific double-stranded RNA (dsRNA) delivery methods vary in efficiency depending on the parasite developmental stage or tissue of gene expression. Since most transfection reagents are toxic to the parasite, electroporation is required to achieve efficiency in gene knockdown in *Schistosoma* adult worms, which is labor intensive and may damage the tissue. Successful plasmid transfection using polyethyleneimine (PEI) in *S. mansoni* schistosomula motivated us to test PEI as a dsRNA delivery method in adult worms. **Methods:** Eight adult worm pairs were cultured in RPMI 1640 medium supplemented in 6-well plates. The SmJNK transcript levels were assessed by RT-qPCR and the number of eggs laid were counted every two days. Similarly, eight adult worms (male and females separately) were cultured in RPMI medium supplemented in 24-well plates to assess worm viability for 10 days using WormAssay software. Here, we tested the PEI protocol with dsRNAs specific for the SmJNK MAP kinase transcript and compared to adult worms knocked down for this gene using electroporation. We tested two dsRNA concentrations (3,6 and 7,2 µg/mL) with a PEI nitrogen and DNA phosphate (N/P) ratio of 11:1, this mixture was added once in the second day of culture or exposed the parasites to the PEI/dsRNA mixture at the lowest concentration, every other day for three times. **Results:** In parasites exposed to PEI and dsRNA, SmJNK transcript levels showed a reduction of up to 69% at the lowest concentration and up to 60% at the highest concentration compared to 71% reduction in transcript levels for electroporated parasites. However, there is a large variation among the three biological replicates at both concentrations and methods. After SmJNK knockdown, there was up to 85% reduction in oviposition in both treatments and male parasites presented 80% movement reduction in the 8th day. Comparable results were observed when we used electroporation to knocked-down this gene. **Conclusions:** We successfully established a PEI-mediated dsRNA-SmJNK knockdown in *S. mansoni* adult worms. The optimization of the method with the addition of the PEI/dsRNA mixture in the first three medium exchanges are still under development. The method presented here was as efficient as electroporation, having the advantages of being less time consuming, easier to manipulate avoiding culture contamination and allowing re-exposure of dsRNAs, making possible to perform transfection cost-effectively and efficiently.

Supported by European Commission's Seventh Framework Programme for research, CAPES, FAPEMIG, CNPq, Rede de Plataformas Tecnológicas Fiocruz (PDTIS).

Systemic cytokine and chemokine profiles of individuals with *Schistosoma mansoni* infection and low parasite burden

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Introduction: Intestinal schistosomiasis, caused by the parasitic trematode *Schistosoma mansoni*, is a chronic disease and the prolonged and continuous exposure to *S. mansoni* antigens results in a deviation of the host's immune response. For diagnosis, the Kato-Katz method is recommended, however, this method showed low accuracy in areas of low endemicity. This study aimed to characterize the cytokine and chemokine profile of individuals with an extremely low parasite load, e.g. individuals who were detected by alternative parasitological methods, such as the saline gradient and/or Helmintex®. **Methods:** In order to search for immunological markers of infection, the immunological profile in serum samples of these individuals was compared with patients with a higher parasite load and with individuals repetitively negative by extensive stool exams. The study was conducted in Northern Minas Gerais in a rural area of the Municipality of Januária. Serum samples of a total of 139 parasitologically well-characterized individuals were assessed for the following immunological markers by commercially available immunoassays: TNF-α, IL-1β, IL-6, IL-17A, IL-5, IL-10, IL-13, IL-33, IL-27, CCL3, CCL5, CXCL10, CCL11, and CCL17. **Results:** At pre-treatment, the <4 epg group had higher concentrations of the regulatory marker IL-10, when compared with individuals with epg of 4-99. In the ≥ 100 epg group, both the inflammatory marker TNF-α and the type 2 chemokine CCL-17 showed elevated concentrations. **Conclusion:** In general, infected individuals presented a tendency to decrease the type 1 inflammatory markers and to increase type 2 markers, which could be beneficial for the host in case of reinfection.

Supported by CNPq and Capes

SCHISTOSOMA MANSONI INFECTION COULD ALTER SKIN REACTIVITY TO AEROALLERGENS THROUGHT CCL2 AND IL-10 MODULATION

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Introduction: Infection with *Schistosoma mansoni* triggers immunoregulatory mechanisms inducing a reduction in the anti-allergen Th2 response and in the immediate hypersensitivity reaction positivity. Chemokines influence cellular traffic in the allergy and there are few studies about the relationship between chemokines, cutaneous reactivity and infection by *S. mansoni*. This study investigated the levels of the chemokines CXCL8/IL-8, CCL2, CXCL9, CCL5 and CXCL10 and IL-10 in individuals with schistosomiasis, and also the reactivity of the skin test to aeroallergens (Skin Prick Test – SPT). **Methods**: Individuals from urban areas of Pernambuco, Northeast, Brazil, were grouped according to the presence or absence of *S. mansoni* infection that was assessed using the Kato-Katz and Hoffman, Pons & Janer methods as well the positive or negative SPT (SPT+/S.m+; SPT+/S.m-; SPT-/S.m+; SPT-/S.m-). The positivity for at least one allergen (*Dermatophagoides pteronyssimus, Blomia tropicalis, Periplaneta americana, Blatella germanica, Felis domesticus*, Fungi III (*Aspergillus fumigatus, Penicillium notatum* and *Alternaria alternata*)) (IPI-ASAC® Brazil) was considered SPT+ individuals. Peripheral total blood was cultured with a mitogen (10 µg/mL of Phytohaemagglutinin (PHA); 24h; 37 °C; 5% CO2) and the supernatants were evaluated for chemokines presence by Cytometric Bead Array using a flow cytometer. **Results**: From the 118 selected individuals, 54 were infected (S.m+) (45.7%) and 64 non-infected (S.m-) (54.3%). From the S.m+ individuals, 20.38% (11/54) had positive cutaneous reaction to aeroallergens and 79.62% (43/54) were negative. Among the non-infected, the reactivity to cutaneous reaction was 42.19% (27/64), and 57.81% (37/64) did not react. The levels of CCL2 on the groups S.m+/SPT- and S.m+/SPT+ were lower when compared to the groups S.m-/SPT+ and S.m-/SPT-. Regarding the production of CXCL8, CXCL9, CCL5 and CXCL10, there were no differences between groups. The SPT-/S.m+ group produced more IL-10 when compared to the groups SPT+/S.m+ and SPT-/S.m-. **Conclusions**: The data suggest that the influence of IL-10 may reduce the immediate cutaneous hypersensitivity in individuals with schistosomiasis, which potentially influences the CCL2 chemokine production.

Supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process Number: 478508.2012.3).

Using metabolomics to identify biomarkers in animal models of *Schistosoma mansoni* infection.

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INTRODUCTION: The Schistosomiasis is a neglected disease and very relevant to global public health taking into account the high levels of morbidity caused by this parasite. Traditionally, the diagnosis of schistosomiasis by *S. mansoni* has been performed by direct parasitological techniques, such as the Kato–Katz method. However, in cases of a low infections the Kato-Katz method is not efficient. To improve diagnostic sensitivity over the conventional tests, an emerging area aims diagnostic using biomarkers based on disease-specific metabolites. **OBJECTIVE:** Identify a profile of metabolites for Schistosomiasis when in low parasite load. **METHODOLOGY:** Three mouse study groups were used: high parasite load, low parasite load and a no-infection group. All animals underwent stool examination to determine the parasitic load. Urine samples were collected from each of the study groups to determine the metabolic profile. Gas chromatography coupled to mass spectrometry (GC-MS) was used to determine the identity and the relative quantification of the metabolites present in the samples. **RESULTS:** More them 230 metabolites were listed under the groups, being five of these (Hippuric acid, malonic acid, alanine, glycine and urea) correlated with the parasitological load. While malonic acid and hippuric acid are inversely related to the parasitic load levels of alanine, glycine and urea rise along with the parasitic load. **CONCLUSION:** This study was the first to allowed the identification of metabolites significantly associated with low parasitemia of murine Schistosomiasis.

Suppression of SPO-1 expression in *Schistosoma mansoni* mediated by RNA interference

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Introduction: The study of *Schistosoma*'s gene function is important to understand the biology of the parasite and also to identify new therapeutic targets. In this regard, the use of interference RNA (RNAi) to reduce gene expression has been used as a tool for the systematic analysis of gene function in these organisms. SPO-1, a *Schistosoma mansoni* protein, has been described in the literature to play anti-inflammatory role in the host immune system that might be favoring the establishment and survival of the parasite. Despite this, the effective participation of this protein on the parasite development and survival has not yet been evaluated. **Objective:** To evaluate the impact of reducing the SPO-1 expression on the in vitro phenotypes and survival of the *S. mansoni* schistosomula. **Methods:** Initially, Real-Time quantitative PCR (qPCR) was performed to determine the level of SPO-1 transcript in schistosomula exposed to 200 nM of double-stranded RNAs (dsRNAs) specific for SPO-1 over seven days. As an unspecific control, a GFP (*Green Fluorescent Protein*) dsRNA, was used. Additionally, a negative control group consisted of a non-dsRNA exposed schistosomula was used. Total RNA extraction was performed daily from four thousand schistosomula. This RNA was used as a template to cDNA synthesis used in qPCR. Schistosomula culture were observed every two days using optical microscope to verify phenotypic morphological changes, such as parasite body area. Parasites viability analysis using propidium iodide were also performed. **Results:** Regarding SPO-1 gene silencing, a significative reduction (96%) in SPO-1 transcript expression was observed in schistosomula since the first day after specific dsRNA exposure. Low levels of SPO-1 transcript were observed until the seventh day. Analysis of parasite viability demonstrated that there were no differences between the group exposed to SPO-1-specific dsRNA and the control groups in all time points analyzed. However, during the course of dsRNA exposure there is an increase in the percentage of dead schistosomula in all groups that not correlated with the specific reduction of SPO-1 expression. Significant decrease in schistosomula's body area was observed in parasites exposed to SPO-1 specific dsRNA compared to both control groups ($p = 0,0010$) until the fifth day. In contrast, at the sixth day after dsRNA exposure, a significant increase in the body area of the schistosomula exposed to SPO-1 specific dsRNA was observed when compared to the negative control group ($p=0,0011$) and unspecific control group ($p=0,0014$). **Conclusion:** The use of the interference RNA technology has been shown to be effective in reducing SPO-1 transcripts. SPO-1 silencing had an impact on parasite morphology during the first days after schistosomula transformation, suggesting that this protein plays an important role in recently-transformed schistosomula. Nevertheless, the reduction in the SPO-1 transcript levels had no impact in parasite survival.

Financial Support: Fapemig, CNPq, Capes, CPqRR, Proep/CPqRR

SESSION:

Intermediate Hosts

QR Code Use in the Organization of the Medical Malacology Collection (CMM-Fiocruz)

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Introduction: The Medical Malacology Collection (Fiocruz–CMM) was funded in 1993 and it is placed in the Rene Rachou Institute / IRR - The Oswaldo Cruz Foundation in the State of Minas Gerais, Brazil. The Fiocruz-CMM possesses a collection with around 15,000 limnic snails of medical and veterinary importance, mainly from the *Biomphalaria* genus, which can have epidemiologic relevance in the transmission of schistosomiasis. The information on each sample (species, date, collection point, collector, trematodes' exams results, and the researchers responsible for molecular and morphologic identification) is stored in an electronic Database Index and it is available online in the website of the Center of Reference in Environmental Information (http://splink.cria.org.br/). The modernization of collections is a universal necessity, and this necessity implies in the availability of online data and of easy access. Due to the volume of information in the Fiocruz-CMM, alternative tools that make the access and the use of the collection easier and faster, such as the implementation of QR Codes, are necessary. The QR Codes are 2D barcodes that can contain data in texts, images, or URL formats. These codes can be read by any smartphone through the camera and they ease the access to the information from the Collection. Nowadays, there are free softwares for the creation of QR Codes, already in use by other biological collections. **Methods:** In order to facilitate the access to the Fiocruz-CMM data, the electronic Database Index available in the intranet was written in a script in PHP language especially developed to create a single archive containing information from each specimen. Each one of these archives was stored in the René Rachou's Institute server along with a correspondent QR Code. The reading of this QR Code redirects to the archive with the information from each specimen. Anyone porting a cell phone is capable of accessing the data within the QR Code, as long as one is using the Institute's intranet. **Results:** After the creation of the script that works along with the PHP QR Code library, the QR Codes were created from the information within the Database Index. The next step will be choosing the best code layout to label each flask with the specimens from the Fiocruz-CMM. **Conclusions:** This technology will ease the access to all information contained in the Fiocruz-CMM in a fast and complete way, and it will also enable, in the future, the online access of this information through national and international databases about biodiversity that can be used for epidemiologic surveillance of *Schistosoma mansoni* transmited by *Biomphalaria* snails.

Supported by: Fapemig/CNPq/Fiocruz

Aspects related to biosafety in laboratories that handle mollusks of medical importance in a Research Institute in Rio de Janeiro, RJ

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Introduction: The Biosafety is present in several productive cycles in various economic segments. In this context, including the laboratories that handle mollusks of medical importance, in which the occupational risks related to the collection, packaging, transport and, subsequently, diagnosis or the inclusion of species in a collection. This research aimed to trace the profile of the professionals who handle mollusks in a Research Institute in Rio de Janeiro and analyze their perception with respect to issues related to Biosafety. **Methods:** After approval by the Ethics in Human Beings Research Committee of the IOC/Fiocruz, a survey was conducted with the aim of identifying laboratories carrying out research with mollusks of medical importance. Then the professionals were contacted and they answered a structured questionnaire with open and closed questions. The interviews were analyzed based on the methodological proposal of multirange. **Results:** We identified five laboratories that conduct research with mollusks of medical importance. Nineteen professionals participated in the survey and among them, 68% were female, ranging in age from 30 to 59 years (26%), with more than 20 years of service time (37%) and specialization courses, masters and doctoral degrees (62%). All of them manipulated pathogenic biological agents of risk class 2, with emphasis to *Schistosoma mansoni* (100%), as well as mollusks of the genus *Biomphalaria* (100%). The professionals correlated the term Biosafety with laboratory risks (89%) and showed adherence to the practices and attitudes "Biosafety". In relation to collective protection equipment, the majority informed that they make use of them, but they did not work in a biological safety cabinet to manipulate the biological agents, but on caseworks (100%), although they had this equipment present in the laboratory (84%). All the interviewees participated in training on Biosecurity, but did not feel prepared to deal with an emergency (58%). **Conclusion**: We conclude that the professionals, despite presenting attitudes "Biosafety", are still not fully aware of the risks to which they are exposed, being necessary the intensification of educational processes in the area of Biosafety.

Full destruction of *Schistosoma mansoni* sporocysts by cells in primary cultures derived from the digestive gland of *Biomphalaria tenagophila* Taim

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Introduction: Cell cultures derived from organs/tissues of *Biomphalaria* (Preston, 1910) are important complementary tools for *in vitro* studies of mechanisms involved in *in vitro* interactions between the invertebrate hosts and the parasite in schistosomiasis. So far, *Bg*e – standing for *Biomphalaria glabrata* embryonic cells – has been the only cell line successfully established. With this in mind, we have been working to establish primary cultures from specific tissues of adult *Biomphalaria* and to characterized the morphology and functionality of resulting cells. Presently, we focused on the saccular kidney and digestive gland. *B. glabrata* and *B. tenagophila* Taim have been used as models of susceptibility and absolute resistance against *S. mansoni* infection, respectively. **Methods:** The tissues were prepared by mollusk dissection and the explants were transferred to multi-well plates cells with adequate medium. The growing cells were characterized by optical microscopy. The ability to interact with *S. mansoni* sporocysts was tested using *in vitro* assays. Expression of *aif* – a gene known to stimulate hemocyte activity and proliferation – was monitored after the interaction. **Results:** Cells derived from the digestive gland of *B. tenagophila* Taim were able to fully eliminate *S. mansoni* primary sporocysts. The active cells showed a granulocyte-like profile and *aif* expression increased after the interaction with *S. mansoni*. Cells from the saccular kidney of *B. tenagophila* and homolog cultures from both tissues of *B. glabrata* were functionally inactive. **Conclusions:** Our study suggest, for the first time, that hemocytes from the digestive gland of *B. tenagophila* Taim may play a role in the resistance of this strain to *S. mansoni* infection. This result opens new possibilities to investigate, *in vitro*, the mechanisms involved in interactions between the invertebrate host and the parasite in schistosomiasis.

Supported by CAPES/CNPq/FAPEMIG

The use of Geoprocessing with Instrument for the Monitoring of *Shistosomiasis* in Belém/PA

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Introduction: Public health and the environment are influenced by pattern of the spatial distribution of health problems enables to identify places of risk and to delimit areas that bring vulnerable situations. Infection by different species of *Schistosoma* represents the main public health problem, which affects over 200 million people in the world. In Brazil, *S. mansoni* is the only etiological agent of schistosomiasis affecting approximately eight million Brazilians, and transmission occurs in 19 states. This study determined areas of risk of schistosomiasis transmission in the municipality of Belém/PA using information from the presence of snails of the genus *Biomphalaria* infected by *S. mansoni*. **Methods:** Belém is the capital of the state of Pará, with an estimated population of 1,452,275 inhabitants in 2017, which are distributed between its urban area and its 39 islands. The municipality is known for the active transmission of schistosomiasis, mainly due to its hydrographic characteristics. Data were collected from 3.596 snails of the genus *Biomphalaria* in 2014 of the Laboratory of Intestinal Parasitoses, Schistosomiasis and Malacology (LPIEM) of the Evandro Chagas Institute (IEC). From these, 103 snails(2,86%) were infected by *S. mansoni*, all belonging to *B. glabrata* species. The spatial distribution of the *Biomphalaria* foci was made and the Kernel density estimation (KDE) was applied, with a radius of 500 meters. The KDE identified high density agglomerates of *B. glabrata* infected by *S.mansoni* reaching the neighborhoods of Telégrafo, Sacramenta, Condor and Guamá; and low density agglomerates in Barreiro, Terra Firme and Universitário. Maps of the street arrangement of these neighborhoods were sent to the city's Municipal Department of Health (SESMA) in order to select the sites for Kato-katz (Program of Control of Schistosomiasis-PCE). **Results:** The results of PCE/SESMA were sent to the IEC Geoprocessing Laboratory for spatializatin, and are being analyzed. **Conclusion:** This study showed the potential of the application of geoprocessing to identify the areas of risk for transmission of shistosomiasis and, therefore, facilitated the delineation of strategies for controlling and preventing the cases of the disease in the municipality.

Key-words: Shistosomiasis, SIG, Spatial analysis

Promotion agency: FAPESPA and Evandro Chagas Institute.

Identification of piRNAs in *Biomphalaria glabrata* by high-throughput sequencing

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Introduction: The snail *Biomphalaria glabrata* is the most important intermediate host in the *Schistosoma mansoni* transmission, one of the main causative agents of schistosomiasis, disease which affects millions of people worldwide. The *B. glabrata* snails have a wide geographic distribution in endemic areas like in the Americas showing high susceptibility to infection by *S. mansoni*. The habits of the population and the distribution of the snail, together the absence of basic sanitation, make the schistosomiasis very difficult to be eradicated. After the availability of genome and transcriptome sequences of *B. glabrata* the studies with focus on genetic markers have become even more relevant. Small RNAs and their silencing pathways have been considered very important in several organisms by performing a fine and specific regulation in gene expression, controlling functions in the organism, since cell growth, metabolism to infections and cancer. Some of the most important small RNAs are microRNAs (miRNAs) and PIWI-interacting RNAs (piRNAs), that differ mainly by length size and biogenesis. **Methods:** Using small RNA sequencing and bioinformatics tools we were able to identify an expression number of Bgl-piRNAs. **Results:** We identified 49155 putative Bgl_piRNAs within 195 predicted Bgl-piClusters. These Bgl-piRNAs showed strong and conserved characteristics such as uridine residues at 5' first and adenine at position 10. The overlap with *B. glabrata* genome and transposons showed a high score for adenine at position 10. These results corroborate with previous findings from our group, which showed the existence of a machinery of piRNAs in the snail genome suggesting that these small RNAs are important for *B. glabrata*.**Conclusion:** Our study revealed the presence of conserved piRNAs and their clusters in the genome of snail based on small RNA sequencing data and *in silico* analysis. However, further studies will be needed to confirm the participation of the Bgl-piRNAs in the parasite/host relationship, mainly their effective participation in their target genes. These finding in the genome of the snail will improve the knowledge of the small RNAs in the mollusk and open an avenue to study the roles of piRNAs in the organism.

Financial support: FAPEMIG, CNPq, CAPES, FUNED, UFU and IRR.

Validation of Polyethyleneimine (PEI)-mediated gene knockdown in *Biomphalaria glabrata* and evaluation of genes possibly involved in the resistance phenotype

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Introduction: In Brazil, *Schistosoma mansoni* intermediate hosts are *Biomphalaria glabrata*, *Biomphalaria straminea* and *Biomphalaria tenagophila*. The susceptibility of snails to *S. mansoni* varies between geographic areas, populations in the same area and individuals within the same population. It is known that the mollusk defense system plays important roles in resistance to infections. Here, we intend to standardize the RNA interference (RNAi) approach in *B. glabrata*to elucidate the roles of genes (Allograft Inflammatory Factor-AIF, Matrilin, Molluscan Defense Molecule-MDM and Thioester-containing protein 1.4-TEP1.4) possibly involved in parasite resistance. **Methods:** In order to standardize RNAi assay, 20 *B. glabrata* snails (4mm in diameter, lineage Barreiro) per group were exposed to dsRNA in 48 well plates or 1.5 ml tubes for individual knockdown. Snails were exposed once or four times to different concentrations of dsRNA and Polyethyleneimine (PEI) (0.24ng/µl of dsRNA and 0.19ng/µl of PEI, two and three times this concentration) during all the experiment or only for four days. The analyses of transcripts levels were evaluated by quantitative real time PCR every day after exposure until the 10th day. **Results:** To optimize the technique, *B. glabrata* snails were exposed to TEP1.4-dsRNA, the starting concentration used was (0.24ng/µl of dsRNA and 0.19ng/µl of PEI) as described by KNIGHT et al. 2011, in 48 wells plate. No decrease in the transcript level was observed. However, when the concentration was doubled it was observed a knockdown of 46% when compared to controls on the fourth day. Though, in this format, snails were evading the solution. Then, in order to keep the snails submerse, the experiment was repeated to knockdown (AIF, Matrilin, MDM and TEP1.4) using 1.5ml tubes sealed with parafilm. The transcript levels were assessed every day and the highest reduction of each treatment is as follow: 45% for TEP1.4 on the fourth day, 100% and 14% for AIF and Matrilin on the fifth day, respectively. It was not observed a decrease in transcript level for MDM. The kinetic for Matrilin was repeated, in 1.5ml tubes, by adding the solution (doubled) every day, for the first four days. On the sixth day was observed the lowest transcript level, 30%. Subsequently, an evaluation of knockdown efficiency using a solution of PEI plus TEP1.4-dsRNA three times concentrated was performed. It was possible to observe a reduction in the transcript levels from the forth to the seventh days (40%, 20%, 35% and 15%, respectively) after dsRNA exposure. During the assays no phenotypic change or relevant mortality were observed. **Conclusions:** In the present study it was possible to standardize the use of 1.5ml tubes allowing concordant replicates and between controls. Although the exposure of snails to the most concentrated solution generated a long lasting effect in the knockdown of TEP1.4, new kinetics will be performed in order to obtain a greater reduction in transcripts levels and standardize the assay for the four genes included in this work.

Financial support: FAPEMIG/CAPES/CNPq/FIOCRUZ

Susceptibility of a *Biomphalaria tenagophila* Population to *Schistosoma mansoni* after three and five years of the Introduction Taim Strain (Resistant).

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Introduction: In Brazil, *Biomphalaria tenagophila* (d'Orbigny, 1835) is the second most important species for schistosomiasis transmission. Although many populations of *B. tenagophila* are susceptible to the parasite, a particular population collected at the Taim Biological Reserve in the State of Rio Grande do Sul, Brazil is completely resistant to *Schistosoma mansoni. B. tenagophila* Taim has been used as tool for the biological control of schistosomiasis transmission. This tool includes introducing a large number of *B. tenagophila* (Taim/RS) snails into water bodies where this species is the only transmitter of *S. mansoni.*Although the first results obtained have been promising, it was necessary to evaluate the impact of the Taim strain on the susceptibility of the local snails over time. **Methods:** This study was performed in the Herivelton Martins stream (Bananal-SP) and Snail collections were performed 40 and 64 months after the introduction of the Taim snails. The parameters analyzed were the susceptibility rates and the presence of the typical molecular marker of the Taim lineage (350 bp). **Results:** The group of the offspring from the population collected after 40 months exhibited a significantly reduced infection rate (17.3%) when compared to the group collected prior to the Taim introduction (34.6%). In the second trial, the *S. mansoni* infection rate among the offspring of the original snail population prior to the Taim introduction was 68.08%. However, only 40.54% the of the snail population collected after 64 months post- introduction shed cercariae. In this experiment was observed increase susceptibility rates in both groups. To verify the proportions of infected (i.e., positive) and uninfected (i.e., negative) snails that carried the molecular marker indicative of the Taim strain (i.e., the 350 bp DNA fragment), a sample of the snails that had been exposed to infection was subjected to PCR-RFLP analys. The 350 bp marker was presented only negative snails. The proportions of snails that contained the molecular marker were as follows: 20.59% (after 40 months) and 11.76% (after 64 months). **Conclusions:** After five years, the susceptibility levels *Biomphalaria tenagophila* populations return to their normal values (before introduction). It is possible that the snails coming from the two water sources near the introduction site may be interfering in the transmission of *S. mansoni* resistance from *B. tenagophila* Taim to susceptible snail strains.

Supported by CNPq/ CAPES

Haemocyte changes in *Biomphalaria glabrata* exposed to the sublethal concentration of *Euphorbia milii* latex and after infected with *Schistosoma mansoni*

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Introduction: Schistosomiasis is an endemic disease that affects millions of people worldwide, considered a neglected tropical disease. Efforts to control it have been made in Brazil, but the it still affects 1.5 million people and has expanded to previously non endemic areas. The World Health Organization (WHO) recommends the use of molluscicides as one of the control measures of schistosomiasis. Among the natural molluscicides tested the latex of *Euphorbia milii* presented highlighted in low concentrations as recommended by WHO. This study aims to verify the survival and haemocytary changes of the *B. glabrata* exposed to sublethal concentrations of *E. milii* var. hislopii latex (0.5 mg/L) and subsequently infected with *Schistosoma mansoni*. **Methods:** Four groups of studies were used: Control (G1- unexposed and uninfected); Exposed (G2-exposed to latex and uninfected); Infected (G3-unexposed and infected); Exposed and Infected (G4-exposed to latex and infected three days after exposure / 45 days post exposure). A total of 120 animals were used, ten animals from each group were separated for counting hemocytes in the hemolymph, through the Neubauer chamber, and two observations were performed (in the initial phase of infection and in the patent period). Molluscs were infected with 8 to 10 miracidia and posteriorly shedded cercariae were counted. **Results:** In relation the amount of hemocytes, there was no significant difference between the two observations. In the initial phase individuals of G2 presented a greater amount of hemocytes than the G4 group. In the patent period, the G1 and G2 groups presented difference, the G2 presented more hemocytes than G1. The amount of hemocytes in G4 was higher at 45 days post infection. The elimination of shedded cercariae showed no difference in the different infected groups and there was no correlation between the quantity of hemocytes and the amount of released cercariae. **Conclusions:** These data demonstrate that exposure of molluscs to a sublethal concentration of *E. milii* latex did not interfere in the susceptibility of the animals to *S. mansoni* infection and did not alter the cellular immune response.

Supported by CAPES/Fiocruz/IOC/PAEF

Malacological survey for mapping breeding sites and outbreaks of schistosomiasis vectors in the city of Recife, Pernambuco, Brazil

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Introduction: Schistosomiasis is a neglected disease in Brazil. In the state of Pernambuco the disease is endemic in rural areas and in the metropolitan region, also occurring in a focal way in localities of the state coast. The strong migration of individuals from rural endemic areas seeking work in urban areas resulted in populations agglomerates living in unhealthy conditions without basic sanitation, which facilitates the establishment of the biological cycle of *Schistosoma mansoni* and consequent transmission of the disease. In the city of Recife, a recent study identified 04 breeding sites of *Biomphalaria straminea* with snails carrying *S. mansoni* DNA. The relevance of this project is justified by the need to investigate schistosomiasis transmission in Recife so that the Schistosomiasis Control Program can act to prevent the spread of the disease in this city. The objective of this work was to carry out an extensive malacological survey in natural and artificial freshwater hydric collections of all the neighborhoods of Recife to diagnose and map possible outbreaks of schistosomiasis vector molluscs. **Methods**: The malacological survey began in february 2017 with an active search for natural or artificial (temporary or permanent) breeding sites of vector molluscs in Recife neighborhoods. The identified breeding sites were georeferenced and the molluscs were collected by searching the entire accessible range of each habitat for about 30 minutes. The taxonomic identification of the species was made by dissection of *Biomphalaria* genital apparatus. The infectivity of snails by *S. mansoni* was verified by molluscs light exposure to verify the emission of cercariae and by the molecular technique NESTED PCR for identification of *S. mansoni* DNA. The results were be analyzed by ArcGis 10.1 software that allows the creation of thematic maps showing the location of breeding sites and foci. **Results:** 37 snail breeding sites of schistosomiasis vectors were identified. All molluscs belonged to the genus *B. straminea* and did not eliminate *S. mansoni* cercariae in the light exposure test. The molecular diagnosis NESTED PCR identified 13 snail breeding sites in which the *S. mansoni* DNA was present indicating that the parasite is circulating in these aquatic environment. The breeding sites with mollusks carrying the *S. mansoni* DNA are located in the neighborhoods Apipucos, Ibura and Prado, which deserve special attention as places at risk for establishment of the schistosomiasis transmission. **Conclusion and Perspectives**: As the elimination of *S. mansoni* cercariae in the snails examined was not detected the malacological investigation will continue using the exposure of sentinel mice in those breeding places where snails carry the DNA of the parasite. If confirmed the schistosomiasis infection in the exposed mice, the schistosomiasis transmission may be confirmed in Recife.

Schistosomiasis mansoni in the Amazon region: malacological surveys of intermediate hosts for the identification of disease transmission areas in Belém, Pará - Brazil

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Introduction: In Brazil, schistosomiasis is caused by the ethiological agent *Schistosoma mansoni*, can be found in 19 federative units and is considered a serious public health problem. The presence of molluscs for the genus *Biomphalaria* is a necessary condition for development of the parasite cycle. Among the 11 species and one subespecies found in the country, three are confirmed as intermediate hosts of the parasite, namely *B. glabrata, B. tenagophila* and *B. straminea.* In the state of Pará, the species *B. schrammi, B. kuhniana, B. straminea* and *B. glabrata* have already been identified, with reports of the last two occuring in Belém. The aim of this study was to determine the spacial distribution of the *Biomphalaria* species in Belém, and identifying risk areas for schistosomiasis transmission. **Methods:** Biannual malacological surveys were carried out between September 2013 and October 2017 in 26 districts of Belém and the collection points were georeferenced analyzed according to the Kernel technique. The molluscs, after measurement, were individually placed in glass containers with dechlorinated water and exposed to light to veriry the presence of *S. mansoni* cercariae. Subsequently, five specimens of each collection point were submitted to Hypnol anesthetic, to be sacrificed and fixed in Raillet-Henry for dissection and morphological identification of the species. **Results:** Malacological surveys resulted in a total of 10,803 molluscs collected, of which 9,484 (88.75%) specimens were examined. Among the analyzed molluscs, 5.820 (61.36%) were identified as *B. glabrata* and 3.547 (37,39%) as *B. straminea*. The presence of the parasite was observed in 208 (2.19%) specimens, all *B. glabrata.* Positive molluscs were found in seven districts: Guamá (51 molluscs / 24.5%), Sacramenta (47 molluscs / 22.6%), Telégrafo (47 molluscs / 22.6%), Montese 16.8%), Condor (20 molluscs / 9.6%), Barreiro (7 molluscs / 3.4%) and Terra Firme (1 mollusk / 0.5%). **Conclusion:** Among the intermediate hosts for schistosomiasis, this study reveals that the species *B. glabrata* actively participates in the disease maintenance as intermediate host in Belém. The Kernel technique allowed to analyze the spacial distribution collection sites and consequently to determine possible risk areas of schistosomiasis transmission in Belém. The development of maps identifying sites with schistosomiasis positive snails may support efforts of this municipality by directing activities related to endemic disease control.

Supported by Instituto Evandro Chagas/SVS/MS

Characterization of the activity of the extracts from *Plectranthus grandis* (L.H. Cramer) on *Biomphalaria glabrata*.

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Introduction: The genus *Plectranthus* belongs to the family Lamiaceae, which originates in the Mediterranean countries and Orient, containing approximately 200 genus and 3,200 species. *Plectranthus grandis*, popularly known as Bilberry great, Bilberry leaf-large, false mauve-santa and bilberry Mexican, is used popularly in the treatment of diseases of the digestive tract. The study had as purpose to assess the toxic action of crude extracts in hexane (EHex.), dichloromethane (EDcM), chloroform ($ECCl_3$), ethyl acetate (EAcOEt), acetone (EAceto), ethanol (EEtOH) and methanol (EMeOH) and get the LC_{50} and LC_{90} extracts on *Biomphalaria glabrata*. **Methods**: Extraction for the tests occurred for exhaustion in soxhlet Extractor with solvents. The leaves of *P. grandis* milled and dried at 45°C were processed in LAPSA/IOC. *B. glabrata* (10-12 mm diameter) used in the tests (N=350) were created in the laboratory. The crude extracts were tested in at concentrations of 50, 100, 150, 200, 300, 400 and 500 mg/L in glass cups and groups of 5 snails were put in touch with 200 mL of solutions of extracts. The snails negative control were kept in water and dimethyl sulfoxide (DMSO) 1%. The positive control used was the niclosamida ® in concentration over 1 mg/L. Mortality was observed after 24h, 48h and 72 h of exposure. The tests were with three replicates. As a criterion of death was adopted the retraction of soft part and release of hemolymph. The physical-chemical parameters analyzed were: total hardness (mg/L $CaCO_3$), pH, conductivity (µS/cm) and total alkalinity (mg/L $CaCO_3$). **Results**: The results showed at a concentration of 500 mg/L, in the period of 48 hours, to the EDcM and EHex, mortality of 100%; $ECCl_3$ of 93.4%; EAcOET of 20%; EAceto, EEtOH and EMeOH with 13.4%. The CL_{50} were: EHex.Fo=339.4 mg/L; EDcM.Fo=299.4 mg/L; ECCl3.Fo=364.4 mg/L; EAcOEt.Fo=498.5 mg/L; EAceto.Fo=483.5 mg/L and EEtOH.Fo=398.5 mg/L The physical-chemical analysis at the end of the tests with EHex, EDcM, ECCl3, EAcOEt, EAceto, EEtOH, EMeOH were: total hardness = 288.72±12.46; 273.90±18.74; 305.0±5; 599±69.5; 191.4±20.61; 125.4±29 and 179.85±51.5; pH = 5.41±0.52; 5.04±0.10; 5.8±0.26; 5.33±0.03; 4.96±0.1 and 5.18±0.1; = 2.56±77.47 conductivity; 69.03±2.23; 45.07±1.56; 47.43±20.26; 55.13±3.86; 72.53±8.8 and 142.57±5.1; alkalinity = 64.58±3.61; 70.83±7.22; 37.5±6.25; 41.67±18.04; 31±0; 54.17±18 and 52.50±7.6, respectively. **Conclusion**: It is concluded that extracts Hex, DcM and CCl_3, presented above 90% mortality and Linear Regression analysis were significant in the period of 48 h, suggesting further studies with the fractions of these extracts.

Support: PAEF/CNPq/IOC-FIOCRUZ

Parasitological analysis of *Biomphalaria straminea* (Mollusca: Pulmonata: Planorbidae) from a semi-arid area of Sergipe, Northeast, Brazil

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Introduction: Schistosomiasis remains a public health in Brazil, where three freshwater gastropods are the transmitters of *Schistosoma mansoni*: *Biomphalaria glabrata, B. straminea and B. tenagophila.* The first two species are responsible for transmitting the parasite in the northeast, the most prevalent region of Brazil. Sergipe is one of the states with the highest index of individuals infected with schistosomiasis. The aim of this study was to analyze the positivity of *Biomphalaria straminea* to *S. mansoni* and other cercaria types in a semi-arid area of Sergipe, Brazil. **Methods:** A malacological evaluation was carried out from March 2016 to November 2017 in Nossa Senhora de Lourdes municipality, Sergipe, Brazil (10° 04' 46" S e 37° 03'25" W). The mollusks were collected and sent to the Laboratory of Entomology and Tropical Parasitology, Federal University of Sergipe, São Cristóvão, SE, Brazil; and to the Laboratory of Malacology of the Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil, where they were processed for identification and for parasitological analyzes (by light and darkness exposure techniques). **Results:** From the 34 hydric collections analyzed, 12 (35%) had *Biomphalaria* spp. In all, 1264 mollusks of this genus were collected, analyzed and identified as *Biomphalaria straminea* No specimens released any *S. mansoni* larvae. A specimen of *B. straminea* collected from São Francisco river (10º 01'774'' S and 036º 57'495'' W), released Vivax cercaria when analyzed after darkness exposure. **Conclusion:** Although no positive snails to *S. mansoni* were found, epidemiological surveillance of snail vectors is recommended in the region since there are reports of individuals who died with schistosomiasis in the community.

Survey and monitoring of schistosomiasis mansoni vectos in Itainópolis, Piauí, Brazil: increasing knowledge and establishment of new risk areas

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Introduction: In Brazil, schistosomiasis is a serious public health problem and considered as an important neglected water-borne disease. Caused by the parasite *Schistosoma mansoni*, its transmission occurs through limnic molluscs of *Biomphalaria* genus. The establishment of new risk areas of schistosomiasis mansoni in Brazilian regions is only possible through knowledge of the geographical distribution of the three mainly host species of the parasite:*B. glabrata*, *B. tenagophila* and *B. straminea*. The aim of this work was to survey and monitor the molluscs of the genus *Biomphalaria* in Itaim River, in Itainópolis city, state of Piauí, and investigate the presence of *S. mansoni* cercariae in molluscs. **Methods**: The samples were collected monthly between June 2017 and April 2018, using a metal mesh, at five points in the urban perimeter of the river. For the investigation of *S. mansoni* cercariae, molluscs were exposed to artificial light from incandescent lamps for a period of four hours, during three consecutive days. **Results**: A total of 462 specimens of *Biomphalaria* genus were sampled. *Biomphalaria straminea* species was the only species identified during the period, without positive cases for *S. mansoni*cercariae. However, it was found Apharyngeal Brevifurcate Distome cercariae in the snails collected in November 2017. Months with highest number of snails collected were coincident with dry season, in which rainfall index was zero (June, July and August of 2017). Conversely, the period with highest amount of precipitation (January and March 2018) were months in which there were smallest abundance of molluscs. Regarding the collection points, Points 3 and 4 were highlighted by a higher number of molluscs collected (N = 176 and 174, respectively). These points also reveal largest human activity like fishing, leisure and washing clothes and automobiles. **Conclusion**: Although there was no occurrence of *S. mansoni* cercariae in straminea tested in this research, the permanent monitoring of molluscs in Itainópolis city is important considering that intermediate host of trematode was sampled in the region. This fact can be characterized as a risk factor for occurrence of schistosomiasis mansoni, further aggravated by vicinity of investigated area with Picos city, known as endemic in Piauí State according to National Survey of Prevalence of Schistosomiasis and Geohelminth Infections. The distance between these two cities is only 53.3km and people traffic between cities is permanent due to banking, educational, medical and trade services. Consequently, the risk of the establishment and expansion of schistosomiasis in investigated city is imminent and should be controlled.

BIONFKEY - Electronic tool for identification and control of schistosomiasis transmitting molluscs

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Introduction: According to the World Health Organization, schistosomiasis is the second parasite that most affects humans because of its socioeconomic importance. In Brazil, schistosomiasis is still considered a serious public health problem. Snail vectors represent an important link in the epidemiology of the transmission of schistosomiasis and their study is important so that the role they play in the transmission of parasitosis can be correctly analyzed and the appropriate control measures can be guided to each locality. The correct identification of the transmitting molluscs requires training in the discrimination of the diagnostic characters, as well as in the processing and dissection techniques, which is not always possible due to the excess of diseases to be worked by the municipalities, after the decentralization of health in Brazil. Therefore, this work aims to develop a web and mobile tool for identification of Brazilian*Biomphalaria* species (BIONFKEY). **Methods:** This tool has different sections: illustrated identification guide of the transmitting molluscs (shell and morphological characteristics); information on diseases associated with snails (life cycle, breeding sites, forms of infection, prevention and control). The interactivity of the tool will be done through an appropriate place where the user can upload photos, geographical coordinates and description of the points with snail focus. A mobile and web application is being developed using web technologies such as HTML5, CSS3 and JavaScript as a basic strategy for software development. **Results:** This work is part of a larger project: MALACOKEY - an electronic guide to identify molluscs of medical-veterinary-economic interest, still under development. Preliminary results are the elaboration of the sections with information about the disease, forms of control and prevention, as well as an easy-to-use identification guide of the schistosomiasis transmitter species in Brazil (*Biomphalaria tenagophila, B. straminea* and *B. glabrata*). **Conclusion:** BIONFKEY will first be tested by health agents from the municipality of Rio de Janeiro and then made available on the Fiocruz website. We believe that this tool will support the health services, so that control measures can be executed in a more agile and effective way and contribute to the epidemiological surveillance of schistosomiasis in Brazil.

Supported by Fiocruz

Biomphalaria straminea and associated trematodes from Picos, Piauí, Brazil

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Introduction: In Piauí state, Brazil, notification of positive cases of schistosomiasis has been decreasing every year, but in Picos city, in southeastern region of state, the disease is still considered endemic, having as main intermediate vector snails *Biomphalaria straminea*, different from other regions of Brazil in which *B. glabrata* and *B. tenagophila* are more important. Therefore, the aim of this study was to survey *B. straminea* in Picos city and investigate infection by *Schistosoma mansoni*, in order to obtain data that support knowledge of the ecological and parasitological aspects of this snail. **Methods**: Snail collection occurred monthly between July 2017 and April 2018 in five stations distributed along an urban stretch of the river Guaribas. All live *B. straminea* specimens were submitted to light exposure for three consecutive days, during four hours, with a temperature ranging from 35-38^{o}C, in order to stimulate releasement of cercariae. **Results**: A total of 4,378 live snails were sampled. Which 1,574 individuals (35.9% of the sample) corresponded to *B. straminea*. The month with the greatest abundance was October/2017 (n = 583) and the lowest was February/2018 (n = 14). There was a significant difference in the abundance of this species among the sampled months (X^2 = 35.31, g.l. = 9, P≤0.05). Station 2 showed greatest abundance (n = 713) and Station 3 the lowest sampling (n = 52). Abundance of *B. straminea* among studied stations was also significantly different (X^2 = 27.24, g.l = 4, P = 0.05). No cercariae of *S. mansoni* were found, but cercarian types Pharyngeal Longifurcate Distome, Pharyngeal Brevifurcate Distome and Echinostome were observed. October/2017 was the month that presented Pharyngeal Brevifurcate Distome and Pharyngeal Longifurcate Distome, whereas in November/2017 and April/2018, the release only of Echinostome was observed. **Conclusion**: This study draws attention to abundance of *B. straminea* in Guaribas river, it being evident that lack of environmental sanitation and intense predatory human activity on river banks provide ideal conditions for development of these animals and, consequently, installation and maintenance of outbreaks of disease if there is contamination by trematodes. Although no cercariae of *S. mansoni* were found in present study, other cercaria types that may be associated with the minor infection in humans were identified. Thus, policies for improvements in basic sanitation and also in population health education are of paramount importance for Picos city that offers all conditions for maintenance of this disease.

In vitro haemocyte characterization response of *Biomphalaria straminea* from Sousa (PB-Brazil) strain exposed to *Schistosoma mansoni*

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Introduction: *In vitro* studies have shown that the susceptibility of *Biomphalaria straminea*, intermediate host of *Schistosoma mansoni*, relates to the presence of lectins. As an important carrier of Schistosomiasis in Brazil, *B. straminea* presents low infection rates under laboratory conditions. Despite that, there are few studies regarding the morphological features and *in vitro* behavior of this molluscs's haemocytes challenged by *S. mansoni* miracidia. The purpose of this work is to ascertain the haemocyte respose of *B. straminea* (Sousa-PB strain) challenged by the parasites. **Methods:** In order to confirm the infectivity of the parasite strain, there were positive controls of *Biomphalaria glabrata* molluscs. The hosts have been massively exposed to the parasites and have been killed at 24, 48, 72 hours and 30 days post exposure (dpe); uninfected molluscs were used as negative control. All molluscs had their haemolymph drawn; put into Eppendorf tubes and the haemocytes stained in Trypan Blue and counted in Neubauer's chamber. After counting, 1000 haemocytes have been placed into cell culture plates in order to observe the interaction between the cells and the parasites. Haemocytes were also stained by Acid Phosphatase and marked by*Griffonia simplicifolia* and *Lens culinaris* FITC conjugated lectins. The organs of all molluscs were fixed in Carson's Millonig Formalin, embedded in parafin and stained with Hematoxylin and Eosin. Microscope AxioObserver with McR5 camera from Zeiss made the images. **Results:** In the uninfected group of *B. glabrata*, haemocytes were spotted circling the mother sporocysts rather than interacting with them, whilst the haemocytes of 30 dpe molluscs attached to the parasite's tegument. For 30 dpe *B. straminea*, some cells were attached to the anterior end of the sporocyst. The only point analyzed for acid phosphatase was 72 hours post exposure in *B. straminea*'s haemocytes and the cells were posotive for the enzyme. Some *B. straminea*'s haemocytes presented lectins staining on their insides. Most cells observed were blast cells and their diameter was 6µm for *B. straminea* e 7µm for *B. glabrata*. In the histological analysis, no parasite as well as cell reactions were found as the result of the exposure in *B. straminea*'s tissues at both 24 hours and 30 dpe, whilst *B. glabrata* showed reactions only at 30 dpe. **Conclusions:** 1) The activity of the acid phosphatase as well as haemocytes with lectins-positive structures on their insides state important effective elements of *B. straminea*'s response to *S. mansoni*; 2) The parasite's death, right after its penetration, might be the main aspect that features the resistance of *B. straminea* Sousa-PB strain to *S. mansoni*.

Supported by CNPq/Fiocruz

Effect of the LC_{50} of original Roundup® on the reproductive biology and duration of action in *Biomphalaria glabrata.*

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Introduction: *Biomphalaria glabrata* shows irrefutable epidemiological importance due its role on schistosomiasis mansoni transmission in Brazil. Nowadays there is general awareness that environmental factors can have a significant impact on the dynamics of the parasite-host relationship. The widespread use of herbicide in agriculture has caused a series of environmental alterations, including contamination of communities of living things and accumulation in abiotic segments of ecosystems. In particular, the presence of herbicide in the biotopes in which snails live can have direct action on their reproductive biology and survival. The objective of this work test the duration of action of original Roundup® on the snail *Biomphalaria glabrata* by observing the mortality and reproductive biology when infected by *Schistosoma mansoni*. **Methods:** The snails were submitted for 30 days to aqueous solutions of original Roundup at concentrations of 0.10, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.018, 0.016, 0.014, 0.012 and 0.01 (%) to determine the LC_{50}. To analyze the reproductive biology of *B. glabrata*, four groups were formed: infected and exposed to the agrochemical; infected and not exposed; not infected and exposed; and not infected and not exposed. **Results:** The LC_{50} (0.012% aqueous solution of original Roundup) caused mortality of at most 95% for 24, 48 and 72 hours during the experimental period (30 days), and lost effect totally after 27 days. The reproductive activity of the non-infected and exposed snails was 30.3±20.5 eggs in 2.7±1.2 egg masses, and there was a reduction of 51% in the average number of hatched eggs (11.5±7.5) in comparison to the control group (23.9±18.0) during four weeks (p=0.003). **Conclusions:** The results indicate that the LC_{50} of the aqueous solution of original Roundup has significant action on *Biomphalaria glabrata* for up to 27 days. Roundup significantly diminished the reproductive biology of infected and exposed snails in comparison with the control group.

Supported by PAPES/CNPq/Fiocruz, Ciee.

Prevalence of infection of *Biomphalaria glabrata* by *Schistosoma mansoni* in the water collections of Salvador, Bahia, Brazil

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Introduction: Schistosomiasis is a neglected parasitic tropical disease (NTD) transmitted by contact with fresh water contaminated with larvae of the trematode parasite of the genus *Schistosoma.* Schistosomiasis is transmitted by gastropod snails of the genus *Biomphalaria*, with *B. glabrata* considered the main species that transmits schistosomiasis in Brazil. The present study aimed to evaluate the populations of *B. glabrata* in water collections in the city of Salvador for their distribution, to identify the foci of transmission, to determine the prevalence of *S. mansoni* infection and to characterize the populations of cercariae. **Methods:** The malacological surveys were carried out between June and December 2017 in 17 water collections distributed by Sanitary Districts of Salvador, using the method of Oliver and Schneiderman (1956). Morphological identification of snail species was performed observing characteristics of the shell and mantle. Snails were evaluated for *S. mansoni* infection by exposure to light weekly over a period of 30 days. In a sub-group of snails selected randomly, quantitative real time polymerase chain reaction (qPCR) using *S. mansoni*-specific primers that for 18S rRNA subunit was also performed. **Results**: We collected 1,403 snails. All were identified as *B. glabrata*. Five snails (0.4%) were positive by classical cercarial shedding. Besides the low general positivity, higher infection rates were found in Lagoa do IAT (1.9%) and Horta de Saramandaia (5.5%). Non-*Schistosoma* larvae were observed in 3.2% of the snails, these being Xiphidiocercaria, Strigeidae, Spirorchiidae and Clinostomidae. qPCR analysis, detected *S. mansoni* DNA in 6.2% (CI 95% 4.5% – 8.4%) (39/626) of snails. All cercaria-shedding snails were positive in qPCR. Based on the qPCR results the positivity rate varied between 2% and 43.4%, depending on the water collection. The highest positivity was observed in Dique do Cabrito (43,4%) and de lowest in Horta de São Bartolomeu (2%). **Conclusion:** *B.glabrata* is widely distributed in the city of Salvador, with at least 7 areas of transmission risk for schistosomiasis. The qPCR technique had greater sensitivity for detecting potential risk of transmission and can be used to complement the light exposure method. It is worth noting that estimating the prevalence of *S. mansoni* in snails by only taking into account the classical elimination may underestimate the problem.

Supported by CAPES/Fiocruz

Near-infrared spectroscopy applied to medical malacology: preliminary results

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Introduction: Near Infrared Spectroscopy (NIRS) is a vibrational spectroscopy technique that studies the energy levels of atoms of different molecules with a wavelength of 780 to 2500 nm or 14290-4000 cm^{-1}. The spectral information obtained comes from the interaction between elements that form covalent bonds among each other, such as: -CH, -NH, -OH and C = O, which demonstrates the possibility of the technique being applied to several types of samples. NIR spectroscopy can contribute in complex ecological studies based on changes in spectra, which act as physical-chemical fingerprints. The objective of this work was to describe the process of standardization of the use of NIR for species of *Biomphalaria*. Preliminary results related to the construction of the model of species discrimination using shells exclusively and live animals are presented. **Methods:** Specimens of the three intermediate host species of schistosomiasis mansoni in Brazil were used from different populations kept in the Laboratory of Malacology (IOC / Fiocruz). The spectra were collected in the NIRS (LAPSA / IOC / Fiocruz) using 50 scans per samples and 16 cm^{-1} resolution (Shells spectra from the three snail vectors: *Biomphalaria glabrata, B. tenagophila* and *B. straminea*). For live animals, we used populations of *B. tenagophila* and *B. glabrata*. The chemometric analyzes were performed using the Unscrambler software, where the spectra were preprocessed using the Savitzky-Golay first-derivative (21-point window and 2nd-order polynomial). For the construction of the classification model it will be used the linear discriminant analysis (LDA). **Results:** The spectra collected from the shells were standardized for drying time, due to the interference of water in the spectra. After the removal of the visceral mass, the samples dried at room temperature (25° to 28°C), showed to be the minimum ideal drying time of the shells from the 15th day. Samples of live specimens were also standardized, with the following procedure stipulated: before being individually placed in a glass vial for analysis, the surface of the shell was dried on absorbent paper and the left side was placed on the laser. Preliminary results demonstrated the possible separation of *B. glabrata, B. tenagophila* and *B. straminea* through the shells exclusively, but a larger number of samples were required to construct a more complete model. In relation to the live animals, it was possible to differentiate the species with 60% of correct answers. **Conclusions:** The Near Infrared Spectrophotometer is a possible easy-to-use modern technique of species differentiation in the near future. The model of species differentiation using only the shells needs adjustments in the number of samples.

Supported by PAEF/ LAPSA/IOC/ Fiocruz

SESSION:

Diagnosis, Treatment, and Clinical aspects

Production of the recombinant MEA protein and use in the ELISA test to determine the kinetics of schistosomiasis mansoni infection from murine samples

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Introduction: Schistosomiasis, a parasitic disease caused by the *Schistosoma mansoni* trematode, remains a serious public health problem in many parts of the world. Only in 2016, more than 19,000 cases of the disease were registered in Brazil. Recent data published by the DATA research group have demonstrated that the immune response directed at specific antigens of the evolutionary stages of the parasite has been related to the clinical form of the disease. Therefore, the use of a specific marker, such as the recombinant Major Egg Antigen (MEA) protein, may lead to an efficient diagnosis, especially in patients with low parasite load. From the production of the protein, it became possible to determine the kinetics of infection through the ELISA, which allows the evaluation of the immune response of the host at specific times, allowing a greater understanding of schistosomiasis and helping in the development of a new diagnostic method. **Methods**: Recombinant protein production was performed using the Gateway® cloning method. For the determination of the infection curve, Swiss mice were first infected subcutaneously with 25 cercariae and blood samples were collected on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 65, 90 and 120 days of infection and 15, 30, 60 and 90 days after treatment. These samples were used in the ELISA test previously sensitized with MEAr to determine the infection curve. **Results**: The production of the MEAr protein and its purification were standardized and demonstrated immunogenic potential. The infection curve was characterized using the anti-total IgG, IgG1, IgM and murine IgA conjugate. **Conclusions**: The production of the recombinant MEA protein is of relevant importance for future use as a potential biomarker in the diagnosis of schistosomiasis mansoni. The determination of the infection curve is relevant for a better characterization of the disease and future use in the development of new diagnostic tests.

Supported by CNPq/Fiocruz

Effect of treatment on school-aged children's hemoglobin levels in a *Schistosoma mansoni* endemic area of Minas Gerais, Brazil.

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Introduction: Schistosomiasis is an important public health problem in Brazil and still represents a significant segment of the global burden of illness, mainly in children 5 to15 years of age. The relationship between anaemia and *Schistosoma* infection has been investigated in recent years and is now recognized as an important contributor to the disability adjusted life year (DALY) estimates for schistosomiasis. Therefore, the objective of this study was to assess the association between schistosomiasis and soil-transmitted helminths (STH) infection and hemoglobin (Hb) levels of school aged children residing in areas in the Jequitinhonha Valley, northeastern Minas Gerais, Brazil. **Methods:** A cohort of 387 school-aged children positive for *Schistosoma mansoni* eggs were included in the study. Parasitological, socioeconomic, demographic, and hemoglobin were analyzed in all children infected or coinfected with *S. mansoni,* hookworm, and/or *Ascaris lumbricoides,* aged 6 to 15 years, in eight endemic areas. Eligible individuals were treated with PZQ and/or ALB until they were determined to be negative for the presence of *S. mansoni* or STH eggs in the feces on two consecutive days of Kato-Katz thick fecal smear testing. These individuals were surveyed again 12 and 24 months from the date of successful treatment with PZQ and treated again if positive. Hemoglobin levels before and after treatment were analyzed by multifactorial analysis of variance, with the predictors reinfection group, (co-)infection, time of observation, age, and the interaction between them. To evaluate the effect of antiparasitic treatment on hemoglobin levels, values before and after treatments were analyzed by Student's paired t test. Repeated measures ANOVA was used to assess the association between hemoglobin levels with different participant characteristics. **Results:** We observed that the mean levels of hemoglobin in the first (13.1 g/dL) and second (13.3 g/dL) year post-treatment were significantly higher than at baseline (12.8 g/dL). At baseline, the hemoglobin levels of the co-infected individuals were significantly lower in relation to the non-co-infected individuals. However, in reinfected and non-reinfected groups this association was not statistically different in any follow up time point. Longitudinal analysis of hemoglobin level showed an increase over time, independent of age and reinfection by *S. mansoni.* **Conclusion:** The data suggest that treatment elevated Hb levels in this population, especially in the group of *S. mansoni*-infected children co-infected with STHs.

Financial support: National Institutes of Health-Tropical Medicine Research Centers (TMRC, Grant number AI098507); CAPES; FAPEMIG; CNPq; INCT-DT.

Optimized extraction methods of *Schistosoma mansoni* tegument surface proteins for rational immunoscreening of potential antigens for vaccine and diagnostic development

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Introduction: Repeated treatment with praziquantel (PZQ) has resulted in a lasting improvement with respect to the pathology associated with schistosomiasis, and in many areas the control strategy is shifting from targeting morbidity to elimination of the infection. However, the use of PZQ presents several limitations and new interventions are needed to achieve schistosomiasis elimination, such as the development of vaccines and other diagnostic methods. By immunoscreening of *Schistosoma mansoni* adult worm proteome using serum of individuals from a schistosomiasis endemic area, we had recently identified 47 immunoreactive cytoplasmic proteins. Since the apical membrane of tegument is the parasite interface to host's immune system, we aimed to optimize the protein extraction protocols in order to enrich our extracts with parasite's surface proteins to be used in another immunoscreening assays. **Methods:** In the first protocol, the *S. mansoni* (LE) adult worms were lysed using the 2-D Fractionation Kit (GE Healthcare) and centrifuged according to the manufacturer instructions. Briefly, the supernatant was discarded and the insoluble proteins present in the pellet were solubilized and separated by two-dimensional electrophoresis. Proteins were then transferred to PVDF membranes and exposed, separately, to serum of infected and naturally resistant individuals from a schistosomiasis endemic area (the rural community Virgem das Graças, Brazil); to serum of uninfected individuals from a non-endemic area; and to a polyclonal antibody against Sm29. Immunoreactive proteins were identified by mass spectrometry. The second protocol was performed with *S. mansoni* (LE) adult worms and schistosomula. Firstly, the tegument was detached by freeze/thaw/vortex method. Then, the surface membranes were enriched by low-speed centrifugation. The proteins were solubilized using the mentioned kit and identified by shotgun proteomics. **Results:** In these new preparations, the presence of proteins that have already been described in *S. mansoni* tegument surface was demonstrated by detection of Sm29 by western blotting and also by shotgun proteomics identification. Preliminary two-dimensional western blotting experiments using serum of individuals from a schistosomiasis endemic area resulted in a different spots recognition profile for each group. The protein content of some immunoreactive spots was identified by mass spectrometry and is being evaluated as potential targets for schistosomiasis vaccine or diagnostic development. **Conclusions:** The use of these optimized protocols has allowed us to obtain a high quality tegument preparation to perform the screening of *S. mansoni* potential antigens.

Supported by: FIOCRUZ, IRR, CNPq, FAPEMIG.

A novel cell-free method to culture *Schistosoma mansoni* from cercariae to juvenile worm stages for in vitro drug-testing

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Introduction: The arsenal in anthelminthic treatment against schistosomiasis is limited and relies almost exclusively on a single drug, praziquantel (PZQ). Thus, resistance to PZQ could constitute a major threat, especially considering the widespread use in mass drug administrations. Even though PZQ is potent in killing adult worms it has been shown to be limited in its activity against earlier developmental stages. Current in vitro screening strategies for new drugs depend on newly transformed schistosomulae (NTS) for initial hit identification, thereby limiting sensitivity to new compounds predominantly active on later developmental stages. The aim of this study was, therefore, to establish a highly standardized, straightforward and reliable culture method to generate and maintain advanced larval stages in vitro. We present here how this method can be a valuable tool to test drug efficacy at each discrete intermediate larval stage, reducing the reliance on animal use (3Rs). **Methodology/principal findings:** Cercariae were mechanically transformed into skin stage schistosomulae and successfully cultured under serum-free and cell-independent conditions for up to four weeks with no loss in viability. Under these conditions, larval development halted at the lung stage. Addition of human serum propelled further development into juvenile worms within eight weeks. Skin and lung stages, as well as juvenile worms, were submitted to 96-well format drug screening assays using known compounds with anti-schistosomal properties such as PZQ, oxamniquine, the drug of choice for treatment of *S. mansoni* before the advent of PZQ, mefloquine and artemether, both antimalarial drugs that have been shown to exert schistosomicidal activity. Our findings showed stage-dependent differences in larval susceptibility to the tested drugs. **Conclusion:** With this robust and highly standardized in vitro assay important developmental stages of *S. mansoni* up to juvenile worms can be generated and maintained over prolonged periods of time. The phenotype of juvenile worms, when exposed to reference drugs, was comparable to previously published works for ex vivo harvested adult worms. Allowing the detection of stage-specific differences in drug susceptibility. Therefore, this in vitro assay can help reduce reliance on animal experiments in the search for new anti-schistosomal drugs and provide a platform for the investigation of the cell type- or host protein-specific influence on the parasite's development.

Combination of silibin nanoparticles and spermidine in reducing liver fibrosis in the acute phase of schistosomiasis

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Introduction: Schistosomiasis is a disease that has high prevalence and morbidity in tropical and subtropical areas. In Brazil, the etiologic agent is the *Schistosoma mansoni*. This helminth inhabits the mesenteric intestinal vessels, where the female will release the eggs. The more relevant sequel caused by this parasite is the hepatic fibrosis due to a granulomatous inflammatory reaction from embolized eggs to liver tissue. The treatment of choice for this disease is the praziquantel. However, this medication is not enough for the treatment of sequel from *S. mansoni* infection, mainly the liver fibrosis. One of the candidates who can reduce the sequelae of infection is the silibin. Silibin presents antifibrotic action, antioxidant, anti-inflammatory and the immune response modulation as already demonstrated in the literature. The other candidate is the spermidine. The mechanism of action involves your increased uptake and degradation of fibronectin by hepatocyte. The aim of this study is to evaluate the combination of silibin nanoparticles and spermidine as reducing agents of hepatic fibrosis in the acute phase of schistosomiasis. **Methods:** In this study, we used mice of BALB/c strain infected with 60 *S. mansoni* cercariae from BH strain. These animals, after 60 days of infection were treated with praziquantel (500 mg/kg per day for two consecutive days). After this, the treatment with spermidine solution in intraperitoneal route (50 mg/kg/day) and nanoparticles of ε-polycaprolactone (PCL) containing silibin (10 mg/kg/3-3 days, oral route), both for 25 days began. After the treatment period, the animals were euthanized. **Results:** The evaluation of collagen by Picrosirus red area, showed a reduction in the groups treated with silibin and with the silybin and spermidine combination. In addition, the values of aspartate aminotransferase in treated groups were reduced to the basal values, except for the infected group treated with the combination that showed a high value of this marker. **Conclusions:** The results of these evaluations indicate a reduction of granulomas in the groups treated with silibin but did not show an effect when spermidine was used. With the combination was observed an effect in reducing fibrosis, although present a possible toxic effect.

Schistosoma mansoni infection in humans: comparison of prevalences obtained by the Kato-Katz method and by rapid urine test (POC-CCA).

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Introduction: Schistosomiasis is considered a chronic disease with 200 million infected people worldwide. In Brazil, the disease is considered a public health problem, with approximately 1.5 million infected individuals and 25 million people living at risk of infection. However, the Brazilian Schistosomiasis Control Program (SCP) has changed the situation of the disease, with the decrease of medium to high endemicity areas and the increase of low endemicity areas. As a consequence of control measures, the parasite burden in the endemic population has been reduced, which hinders the correct detection of *Schistosoma mansoni* infection by common parasitological methods. The method of choice for the diagnosis of schistosomiasis and geohelminths is the Kato-Katz method, as recommended by the World Health Organization (WHO). However, in areas of low endemicity and in individuals with low parasite load this method has low sensitivity and does not provide enough accuracy for diagnosis, thus requiring complementary methods. This study aimed to determine the prevalence of *S. mansoni* infection with parasitological and immunological methods in an area of low endemicity within the Municipality of Januaria, Northern Minas Gerais. **Methods:** The performance of parasitological results from up to three fecal samples with a total of six Kato-Katz slides were compared with the result from one urine sample, which detected circulating cathodic antigen from *S. mansoni* (POC-CCA). Of a total of 216 individuals three fecal samples (S1, S2, and S3) were collected on consecutive days and two thick smears were prepared from each sample. Additionally, about 10-15 ml of first morning urine was collected on the first day and analyzed by POC-CCA, according to the manufacturer's instructions. The test was classified as negative or positive for active *S. mansoni* infection, with the following classification: trace or weak reagent, +1, +2, +3. From initially 216 individuals (S1), subsequent fecal matters were collected from 168 (S2) and 94 (S3) individuals. **Results:** The overall prevalence obtained by parasitological examinations of three fecal samples (S1, S2, S3) was 4,3% (n=94). In comparison, the prevalence, as observed by the POC-CCA test, was 52,1% (n=94), including trace results as positive reaction. If trace results were excluded, a prevalence of 5,3% resulted. **Conclusion**: The applicability of POC-CCA in low prevalence areas is discussed and further comparison with additional indirect serological methods is provided.

Keywords: Schistosomiasis; *Schistosoma mansoni*; prevalence; diagnosis.

Cost of treatment with Praziquantel by the Schistosomiasis Control Program /Brazil among 2008-2017.

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Introduction: Schistosomiasis is one of the most important neglected diseases. In Brazil, there are approximately .1.5 million infected people distributed in all regions of the country. Praziquantel is currently the drug of choice for the treatment of schistosomiasis in Brazil for all clinical forms of the disease. According to the Program for Control of Schistosomiasis in Brazil (PCE), it is recommended as a strategy the treatment of the population according to the prevalence of the disease in the region. In areas with a prevalence greater than 25%, treatment should be performed on the entire local population. In areas with a prevalence of 15 to 25%, treat positive cases and their respective cohabitants and prevalence of less than 15% and in focal areas, treatment is only for positive cases. The aim of this study is to evaluate the cost of praziquantel therapy in areas of medium and high prevalence of schistosomiasis in Brazil. **Methods**: Information on the cost of treatment with Praziquantel in Brazil between 2008-2017 was requested from the Ministry of Health, emphasizing those states that historically presented higher prevalence, such as: Maranhão, Pernambuco, Alagoas, Minas Gerais and Espírito Santo. At the same time, the DATASUS/PCE platform was searched, using the same period, in the variables "performed tests", "positive (cases)", "positivity rate", "treated", "contraindication", "refusal" and "absence". **Results**: Praziquantel is acquired by the Ministry of Health and passed on to the states according to the demand for cases in each locality. From 2008 until 2017 R$2,478,669.10 (9,767,216 tablets) were spent in the purchase of this drug, with 68% in the states analyzed in this study. There was an increase in the cost of the drug, related to the increase of cases in the years of 2012, 2013 and 2015. Regarding the variable "contraindication", Minas Gerais was the state that presented the most cases, reaching 93% of cases that could not be treated with Praziquantel in 2016. **Conclusions**: The low cost of Praziquantel reinforces the recommended strategies by the Ministry of Health, which until now has managed to reduce the number of cases and hospitalizations, but the transmission of the disease is still continuous in these regions. The increasing number of patients who cannot be treated with praziquantel reflects the need for further research and investment in the development of new drugs or even new formulations of praziquantel for the treatment of schistosomiasis.

Supported by PAEF (LAPSA e LITEB/ IOC/ Fiocruz), CAPES e Fiocruz

The use of silybin nanoparticles as complementary treatment of schistosomiasis infection

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Introduction: Schistosomiasis is an important neglected tropical disease. This disease present high prevalence and morbidity in Africa, America and Asia. The praziquantel is indicated for treatment of the parasites, but not treated sequels of schistosomiasis. Thus, new proposals have been tested in the effective search for reduces fibrosis. Silybin has been extensively studied because of its antifibrotic, antioxidant, anti-inflammatory, immunomodulatory capacity. However, silybin has low oral bioavailability, and it is necessary to propose new formulations. Thus, the present study investigates the effect of silybin ε-polycaprolactone (PCL) nanoparticles in reduction of liver fibrosis caused by *Schistosoma mansoni* in chronic phase. **Methods:** For this study, BALB/c mice infected with 60 cercariae of *Schistosoma mansoni* strain BH (Belo Horizonte) were used. These animals, after 90 days of infection, were treated with praziquantel (500 mg/kg/day for two consecutive days). After this, the treatment with nanoparticles of PCL containing silibin (10 mg/kg /7-7 days, oral route) for 60 days began. **Results:** Results indicated prolonged release of silybin in PCL with encapsulation efficiency 97.8/98.1%. Hepatic fibrosis was not reversed probably due to the slow release of the formulation which inhibited the biological action of silybin. The preparation showed a certain toxicity leading to the increase of aspartate amino transferase in the blood. **Conclusions**: New pharmaceuticals formulations of silybin will be proposed to evaluate the use of silybin as a liver fibrosis reduction agent in schistosomiasis.

Validation of three cathepsin D-like aspartyl proteases as potential therapeutic targets against *Schistosoma mansoni* by RNA interference

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Introduction: To date, Praziquantel is the only available drug to treat schistosomiasis and its therapeutic action is still unknown. Potential drug targets have been described in the literature over the years, among these distinct classes of peptidases play important roles in the parasite development and infection maintenance. An aspartyl protease (AP) similar to cathepsin D (SmCD1) was described in *Schistosoma mansoni* and has been shown to be involved in the initial degradation of hemoglobin from host erythrocytes and was validated as a potential therapeutic target by RNA interference.Two other *S. mansoni* APs (SmAPs) were identified later, herein referred as SmCD2 and SmCD3. This work aims at characterizing the three SmAPs in the different parasite life stages and validating those genes as therapeutic targets. **Methods:** For this purpose, primers were designed and standardized for target amplification, expression assessment by RT-qPCR and subsequent dsRNA synthesis. To characterize the function of each SmAP, mechanically transformed schistosomula were exposed to 200 nM of specific dsRNAs for each gene (SmCD1, SmCD2 and SmCD3) or in combination (SmCD1/SmCD2) for 7 days. The expression levels were assessed by RT-qPCR and mortality was verified using propidium iodide. Knocked-down parasites were subsequently used to infect Swiss mice. After 45 days, mice were euthanized, and adult worms were recovered by perfusion. Egg numbers were counted in the intestine and liver. **Results:** After dsRNA exposure, a 99.9% reduction in transcript levels was observed for SmCD1 and SmCD2 on fifth day, while no relevant silencing was observed for SmCD3 during the same period. Statistically significant mortality (6.6%) was observed for the group exposed to SmCD2-dsRNA on the fifth day after exposure. Parasite exposed to SmCD1 and SmCD2 dsRNAs, in combination, resulted in transcript levels reduction of 50% and 99.1%, respectively. We have observed an increase in underdeveloped adult females knocked-down for SmCD1, SmCD2 and in combination recovered by perfusion. However, in the ex vivo experiments, no significant changes were observed for parasite or egg recovery. Also, we verified that exists two SmCD1 isoforms and, one of them, represents 68.8% of transcripts in the schistosomula stage and carries a C-terminal extension. The proportion of this isoform in the other parasite developmental stages is still unknown. **Conclusions:** The results suggest that these SmAPs play an important role in parasite development.

Supported by FAPERJ, FAPEMIG; European Comission, 7th Framework (A-PARADDISE-anti-parasitic drug discovery in epigenetics); CAPES/University of Nottingham, Drug Discovery; FIOCRUZ;

Evaluation of a *Schistosoma mansoni* antigen selected by immunoproteomics to compose a new method of schistosomiasis immunodiagnosis

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Introduction: New interventions are a priority to achieve schistosomiasis elimination, since the current adopted measures, essentially based on chemotherapy, have been demonstrated inefficient to achieve this goal. Once the parasitological methods currently used fails to detect *Schistosoma mansoni* infection, especially in areas of low endemicity and in individuals with low intensity of infection, improvement in the diagnosis of the disease is desired. Therefore, it is still necessary to develop other methods, such as new serological tests for the diagnosis of schistosomiasis, as well as to prospect parasite antigens to be used in these tests. An immunoproteomic study conducted by our group identified some proteins with potential to be used in schistosomiasis diagnosis. These proteins were reactive exclusively to serum antibodies from infected individuals living in an endemic area for schistosomiasis. One of these proteins, named by us as PPE, showed promising preliminary results. In view of these results, the aim of the present study is to evaluate the potential of PPE as a target to compose a new method of schistosomiasis immunodiagnosis. **Methods**: To obtain PPE to be used in the immune diagnosis of schistosomiasis, the experimental strategy applied was the heterologous expression of the recombinant PPE (rPPE) fused to 6 histidine residues at the carboxyl terminus in a prokaryotic system. After the separation of the soluble fraction from the total bacterial protein extract, the recombinant protein was purified using nickel columns. The PPE reactivity to sera of individuals from schistosomiasis endemic area was tested in an ELISA assay. **Results:** The rPPE showed the expected molecular mass of approximately 40kDa and it was expressed 2 hours after IPTG addition, although its expression has increased gradually after 4 and 16 hours. The rPPE was more abundant in the soluble fraction of the bacterial extract. During the purification step, most of the recombinant protein was retained on the nickel column and a small amount was detected in the flow-through. Large amount of rPPE with satisfactory level of purity was obtained in the elution fraction. The preliminary ELISA assay demonstrated a higher reactivity against rPPE of IgG antibodies in a pool of serum from infected individuals when compared to uninfected individuals from schistosomiasis endemic area. **Conclusions:** The experimental conditions for expression, induction and purification of the recombinant protein were favorable, yielding large amount of rPPE with satisfactory level of purity to be tested in a new schistosomiasis immunodiagnosis test.

Supported by CAPES, FIOCRUZ/CNPq/PROEP-RIPAg, FAPEMIG.

Investigation of death in infected mice with *Schistosoma mansoni*

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Introduction: Mesenteric ischemia is a syndrome due to vascular insufficiency that causes a supply decrease of nutrients to the intestinal viscera. Since schistosomiasis is a disease which the parasite inhabits the hepatointestinal circulatory system and exist high death rate of the *Schistosoma mansoni* infected animals kept in the IAM-FIOCRUZ experiment facility, this study aimed to investigate the main cause of death of these animals. **Methods:** A total of 220 animals infected from October 2016 to May 2017 were analyzed, 110 of the BH strain and 110 of the LE strain with parasite load of 120 cercariae. The animals that presented a decrease in the exploratory act, melena, increased abdominal volume or any sign that elucidated suffering were euthanized for clinical investigation and a longitudinal incision was made in the abdomen to expose the abdominal viscera, allowing the visualization of areas in the intestine. The feces were collected and analyzed through the reaction with hydrogen peroxide for occult blood research. Liver fragments were removed and these were submitted to morphometric analysis of the percentage of collagen, number, diameter, density and area of the granuloma, in addition to number of inflammatory cells. A Nikon Eclipse E200 microscope was used in magnifications of 10x and 40x. Death data were analyzed using Graph Pad Prisma V6. **Results:** During of 8 months, 70 deaths were recorded, of these 42 animals had mesenteric ischemia. We found that the percentage of death due to mesenteric ischemia was 35.9%, compared to the percentage of unknown causes of death which is a difference of approximately 10% greater for mesenteric ischemia. Deaths started between 31 and 60 days post-infection with most episodes occurring between 91 and 120 days for mesenteric ischemia and between 121 and 150 days for unknown causes. The morphometric study revealed that livers of animals with mesenteric ischemia presented a higher percentage of collagen, with granulomas of greater diameter and consequently larger area when compared to the animals that died due to unknown causes ($p < 0.05$), however, the number of inflammatory cells of these animals was lower. **Conclusion:** It is necessary to reduce the parasitic load of both strains analyzed to increase the survival of the animals and maintain the artificial cycle of *S. mansoni.*

Keywords: Mesenteric Vascular Occlusion, Ischemia, *Schistosoma mansoni*, Mice.

Supported by Fiocruz

Ectopic forms of schistosomiasis mansoni: a literature review

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Introduction: Schistosomiasis mansoni is a disease that can have multiple clinical manifestations. The clinical ectopic form occurs when the parasite is located far from its usual site (the portal system). This manifestation is uncommon and there is little information in the literature. In this context, the objective of the study was to review the ectopic forms of schistosomiasis reported in the literature. **Methods**: A literature review was carried out at the Scielo and PubMed databases, in consultation with published articles from 2001 to 2017. **Results**: The search has found 22 articles. Most of them were case reports and described that the ectopic occurrences of schistosomiasis are associated with chronic manifestations, severe and difficult to diagnose. Experts report that about 15% of patients with the hepatosplenic form may evolve to a renal ectopic form, characterized by glomerular lesion. Transverse myelitis is the most common neural ectopic form. Research on neuroschistosomiasis showed predominance in males and the factors attributed to the greater possibility of contagion in men were socioeconomic and a greater physical effort performed by them, which is responsible for the increase of the intra-abdominal pressure and the carrying of *S. mansoni* eggs to the central nervous system. Other forms found are schistosomiasis in the urogenital system, myocardium, stomach, thyroid, adrenal, skin, or even in any human tissue or organ. Studies have shown that cutaneous manifestations usually occur in the anogenital region and few extragenital cases have been described. In a study carried out in the State of Sergipe, Brazil, on patients with cutaneous schistosomiasis, the clinical manifestations ranged from asymptomatic to painful. Several authors have pointed out an important manifestation of the infection: endocervical schistosomiasis, which is associated with a great diversity of clinical symptoms. The clinical presentation presents with hypogastric pain, dyspareunia and dysmenorrhea, hemorrhage or leucorrhoea. In 2017, a case report of a woman with suspected ovarian neoplasia was published. Histopathological examination revealed the presence of granulomatous inflammatory processes around viable and calcified *S. mansoni* eggs, with no evidence of neoplastic tissue. **Conclusion**: The study reported several ectopic forms of schistosomiasis mansoni and the difficulty of correct diagnosis. Therefore, further studies are extremely important, so that they may help in the knowledge of the epidemiological dynamics and help in the early detection, differential diagnosis and prevention of the occurrence of severe forms of the disease.

Supported by UFMA

Protein kinases inhibitor screening for the identification of potential therapeutic candidates against *Schistosoma mansoni*

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Introduction: Although praziquantel is an effective drug for the treatment of schistosomiasis, cases of resistance have been reported, which shows that a new effective alternative is necessary. *Schistosoma* sp. parasites interact with extracellular stimuli and generate appropriate cellular responses to allow development and survival. In this context, signal transduction involving eukaryotic protein kinases (ePK) has an essential role in regulatory mechanisms. Recently, we demonstrated that SmERK1/SmERK2, Smp38 and SmJNK are involved in parasite reproduction and development since parasites depleted for those genes presented significantly lower parasite recovery in vivo, egg production and/or underdeveloped ovaries. Thus, the aim of the project is to assess *S. mansoni* kinases (SmERK1, SmERK2, Smp38, SmJNK and SmRaf) as a new drug target by elucidating and comparing host and parasite protein structures and performing compounds screening using ePKs inhibitors. **Methods:** Initially, the sequences of protein kinases were compared to those from the human host. Genes were commercially synthesized, cloned into pCold-GST vector and expressed in *E. coli* to obtain recombinant proteins. The expressed proteins were purified by affinity chromatography using a GSTrap column. Once the sufficient amount of purified proteins is achieved, crystallography will be carried out. Additionally, the purified proteins will be used in inhibitor screening to identify modulating compounds to *S. mansoni* ePKs. To identify candidate inhibitors that specifically target kinases an assay based on ATP binding site interrogation using fluorescence polarisation detection has been developed to test recombinant ePKs activity. For this, we used the BODIPY® FL ATP-γ-S dye–labelled nucleotides and validated this probe with the purified recombinant ePKs and a commercial Protein kinase A. **Results:** Using bioinformatic tools we observed that the sequences identity of *S. mansoni* ePKs with those proteins in human were 68% for SmERK1/SmERK2; 70% for SmJNK; 55% for Smp38 and 26% SmRaf. GST tag proteins (-SmERK1, -SmERK2, -SmJNK, and -SmRaf) were obtained with the expected size and SmJNK and SmERK1 were further purified by affinity chromatography. Moreover, the protein activity assay was used to validate the recombinant ePKs binding capacity and considerable binding specificity was achieved. Our results indicate that the BODIPY® FL ATP-γ-S is applicable to verify the activity of recombinant ePKs for compounds screening. **Conclusion:** Here, we present the development of a new affordable assay to evaluate pan-kinase activity to propel studies towards the identification of new effective schistosomiasis therapeutics.

Support: CNPq, FAPEMIG, CAPES/Drug Discovery Program, IRR/FIOCRUZ.

Identification of new promising antischistosomal hits from screening of the MMV Pathogen Box on *Schistosoma mansoni* juvenile worms

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Introduction: Schistosomiasis is a helminth infection caused by parasitic *Schistosoma* sp. worms spread over the tropical regions of the world with more than 200 million people affected and an estimated 800 million at risk. The parasite juvenile stage is highly relevant for finding new schistosomicidal compounds because it is less susceptible to praziquantel (PZQ), the single drug currently used in chemotherapy. **Methods:** Tetrazolium salt (XTT) biochemical viability assay was employed on *S. mansoni* juvenile perfused worms to screen the 400 compounds in the pathogen box (PB) from Medicines for Malaria Venture (MMV). **Results:** Assay standardization demonstrated signal robustness (Z') of 0.40 using 48 hours of incubation with XTT. At the screening concentration of 10 µM through 72 hours of drug incubation, 37 compounds were identified as hits with eight most-promising compounds presenting 7-23% residual viability and 29 compounds bringing residual viability to 23-43%. **Conclusions:** Considering original activity annotation in the PB, 35% acts on tuberculosis, 19% kinetoplastids, 14% schistosomiasis and 13% on malaria. From the schistosomiasis disease set of 12 compounds, only 7 were considered hits in this anti-juvenile screening. From 20 reference compounds (known anti-infectives) tested, 4 were hits using XTT assay: PZQ, mefloquine, posaconazole and benznidazole. Until now, the last 2 drugs have never been described as anti-schistosomal compounds. Finally, the most active compound belongs to the anti-onchocercarial set and it's structure displays a N,N-disubstituted carboxamide carrying hydrophobic 4-methoxyphenyl and 2-(6-methylpyridin-2-yl)ethyl moieties. All 8 hits and 60 analogue compounds were already provided by MMV to allow for hit validation on secondary and orthogonal assays. Confirmed hits will be further studied on dose-response curves, cytotoxicity and in vivo experimental chemotherapy also available on our anti-schistosomal drug discovery platform.

Supported by CNPq/Fiocruz

Computational fragment-based drug design of new *Schistosoma mansoni* thioredoxin glutathione reductase inhibitors

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Introduction: Fragment-based drug design (FBDD) is a powerful approach to identify low molecular weight compounds (typically less than 300 Da) that bind to the macromolecular target of interest. Because of their lower molecular weight, fragments are less likely to contain portions that could restrain binding and are more properly functional motifs that match the target requirements. Usually, the fragment hits can be optimized through molecular growing, with the aim of finding new hits with higher affinity and improved intermolecular interactions with the target. Here, a computational FBDD approach was performed to design novel ligands for *S. mansoni* thioredoxin glutathione reductase (*Sm*TGR), an essential enzyme for redox homeostasis and survival of schistosome worms. **Methods:** Initially, a subset of 13 co-crystallized fragments in ten different binding sites of *Sm*TGR were discovered from a screening campaign of a library of 776 compounds. The X-ray structures were then used as molecular basis for the computational fragment-to-hit optimization, as follows: (i) prioritization of the most promising pockets, based on pocket druggability (PockDrug and DoGSiteScorer); (ii) substructure-based fragment growing with the eMolecules commercial database; (iii) activity prediction of selected hits using QSAR models developed for *Sm*TGR; and (iv) docking studies of selected hits and *Sm*TGR and molecular dynamics simulations. **Results:** For the druggability analysis, we used the previously discovered fragment hits co-crystralized *Sm*TGR. According to our analysis, three out of the 10 pockets were selected as the most druggable. Seven fragments co-crystallized in these pockets were optimized by growing method and 570 analogues were selected from eMolecules database, for rapid acquisition without the concerns of synthesis. Then, we applied a previously developed QSAR model for prediction of inhibition of *Sm*TGR. This analysis allowed us to select six compounds that were further analyzed by molecular docking and molecular dynamics simulations. The computational binding modes of the most promising compounds are under investigation. **Conclusions:** A fragment-based drug discovery workflow using robust computational strategies guided the identification and optimization of new *Sm*TGR candidate inhibitors. Future experimental assays will be necessary to validate the computational results.

Supported by: CAPES, FAPEG and CNPq

Preliminary Results of the Comparison of the Real-Time PCR Technique, POC-CCA® Rapid Test and Coproscopical Tests for the Diagnosis of Schistosomiasis in Clinical Samples from a Low Endemicity Area of Northern Brazil

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Introduction: In countries considered endemic for schistosomiasis, coproscopy remains the diagnosis of choice because it is a quick, easy and inexpensive method for the identification of *Schistosoma mansoni* eggs in feces. However, repeated sample analysis is needed to improve sensitivity, especially in cases of low parasite load. Molecular techniques for DNA detection of this parasite in fecal samples, and rapid tests for urine antigen screening have been proposed as alternative methods for the diagnosis. Therefore, the objective of this study was to compare the real-time PCR (qPCR), Point-of-Care (POC-CCA®) rapid test and coproscopical tests for the diagnosis of schistosomiasis in clinical samples from low endemic areas of northern Brazil. **Methods:** Stool and urine samples were collected from 20 patients from Turiaçu, Maranhão, and divided into Group A: 10 patients diagnosed positive by the Kato-Katz technique examining 16 slides and/or Helmintex method (composing the reference standard), and Group B: 10 patients with negative samples by the two coproscopical techniques. Subsequently, the same 20 fecal samples were analyzed by the qPCR method, and urine samples from the same patients were analyzed by the POC-CCA® rapid test. **Results:** Compared to the reference standard the qPCR demonstrated a sensitivity (S.) and specificity (E.) of 90%, the POC-CCA® test a S. of 60% and E. of 70%, the Kato-Katz technique a S. of 60% and E. of 100% and the Helmintex a S. of 80% and E. of 100%. Concordance using the Kappa index was better for the qPCR and Helmintex indicating a value of 0.8, followed by the Kato-Katz with 0.6, and the POC-CCA® with 0.3. **Conclusions:** The qPCR showed better sensitivity than other methods indicating a great potential to become a method of choice for the diagnosis of schistosomiasis, especially in areas of low parasitic load. POC-CCA®, however, did not show a good performance in the samples evaluated. However, further studies are needed to better evaluate these new techniques in samples with low parasite load.

Supported by IEC/SVS/MS & DECIT/ MCTI/CNPq/MS/SCTIE

Role of IL-33 / ST2 activation pathway in *Schistosoma*-induced granuloma formation and modulation.

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Introduction: Chronic schistosomiasis morbidity has been associated with the intensity of the type-2 granulomatous inflammatory response induced by egg-secreting antigens that become trapped in capillary venules of the host tissues, especially liver and intestine. Egg-induced granulomatous inflammation participates in host protection confining the cytotoxic effect of egg antigens and remodeling damaged host tissue, but this response may also generate fibrosis associated with severe cases of the disease. IL-33, an alarmin mainly produced by endothelial, epithelial and fibroblast and secreted after cell damage, binds to its receptor (ST2) expressed in innate immune cells, such as ILC2, and stimulates the early production of IL-13 and IL-5 that leads to eosinophil infiltration and activation of Th2 response. More recently, experimental work using bleomycin-induced lung inflammation and fibrosis demonstrated that IL-33/ST2 activation is important for M2-macrophage differentiation and to the evolution of the disease. However, the role of IL-33/ST2 activation on *Schistosoma*-induced granuloma formation and modulation is mostly unknown. In the current work we comparatively evaluated egg-induced granuloma and their pathologic outcome in wild-type Balb/c (WT) and Balb/c mice genetically deficient in the production of the IL-33 receptor ($ST2^{-/-}$) experimentally infected by *Schistosoma mansoni*. **Methods:** $ST2^{-/-}$ and WT mice were infected with 50 cercariae of *S. mansoni* and their liver were comparatively evaluated for parasite burden, immune response and fibrosis at acute and chronical schistosomiasis. Concentration of immune mediators and fibrosis markers were estimated by RT-PCR and/or ELISA-assay and the overall inflammatory response was evaluated by histopathological analysis. **Results:** Preliminary data showed that the number of circulating worms and eggs retained in the liver were similar in WT and $ST2^{-/-}$ infected mice. Interestingly, the concentration of type-2 cytokine in liver homogenate has also similar in infected mice of both experimental groups, but $ST2^{-/-}$ infected mice showed significantly higher concentration of IL-17 during acute schistosomiasis and lower IL-10 at chronicle phase. During acute schistosomiasis the mRNA level of Arg-1 and TGF-β was higher and type III collagen and NOS-2 was significantly lower in $ST2^{-/-}$ than in WT. In contrast, liver from chronicle $ST2^{-/-}$ infected mice had increased levels of NOS-2 mRNA compared to WT. The changes in the response profile detected in $ST2^{-/-}$ infected mice affected granuloma modulation; the histopathological analysis of infected liver showed granulomas with an intense cellular infiltrate and exudative appearance in the two experimental groups at the acute phase, but the modulation observed in WT animals did not occur in $ST2^{-/-}$, these animals showed disorganized granulomas, rich in eosinophils, neutrophils, and decreased extracellular matrix deposition. **Conclusion:** The absence of the IL-33/ST2 pathway was not essential for *Schistosoma*-induced Th2 response; however, this pathway activation is necessary for granuloma modulation during the chronic phase of *S. mansoni* infection.

Supported by CNPq

Contribution of monocytes in the pathogenesis of periportal fibrosis secondary to schistosomiasis

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Introduction: Approximately 5-10% of individuals with *Schistosoma mansoni* infection develop the severe form of the disease, which is characterized by portal hypertension and liver fibrosis, pulmonary hypertension, esophageal varices, and a variety of clinical manifestations caused by the host immune response to egg antigens. Some studies have pointed to the involvement of monocytes in the pathogenesis of liver fibrosis. The aim of this study was to characterize the subsets of monocytes in individuals with different degrees of periportal fibrosis secondary to schistosomiasis. **Methods and results:** The study included thirty-one subjects from an area in Brazil where schistosomiasis is endemic. Whitin the study, 13 patients presented without fibrosis (WF). 14 patients presented with periportal fibrosis (PF), and 4 patients presented with advanced periportal fibrosis (APF). Ultrasound analyses were performed using Niamey Protocol. Peripheral blood mononuclear cells (PBMCs) were cultured in the presence of soluble egg antigen (SEA, 10µg/mL) for 16 hours. The expression of surface markers (CCR5, MARCO, IL-13R), metalloproteinases (MMP-2 and MMP-9), and cytokines (TNF, IL-6, TGF-β) on monocytes were evaluated by flow cytometry. The oxidative burst capacity was measured as dihydrorhodamine-123 MFI in monocytes from different groups. The results were expressed in mean fluorescence intensity (MFI). It was observed that MFI of both MMP-2 and MMP-9 were higher in the monocytes from individuals with periportal fibrosis [57.4 (33.3 - 113); 68.8 (33.3 - 113), respectively] compared to monocytes from individuals without fibrosis [26.2 (17.6 - 36.6); 34.4 (24.4 - 57.3)]. Similarly, the expression of CCR5, and the scavenger receptor protein MARCO was higher in monocytes from individuals with periportal fibrosis [66 (14.8 - 146) 69.1; (36.9 - 96.4), respectively] as compared to monocytes from individuals without fibrosis [25 (16 - 38.1); 39 (38.1 - 43)]. The expression of TNF, IL-13R and TGFβ did not differ among all groups evaluated. The oxidative burst capacity was also higher in the PF group [33.9 (6.9 - 52.6)] compared to WF group [4.8 (2.6 - 42.7)]. **Conclusion:** There is an increase in monocytic expression of molecules associated with fibrogenesis in individuals with periportal fibrosis as compared to individuals without periportal fibrosis.

Supported by: FAPESB Edital Universal 005/2015 (APP 0051/2016)

Normal mode analysis and virtual screening for discovering inhibitors of Schistosoma mansoni thioredoxin-glutathione reductase

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Introduction: Schistosomiasis is considered a neglected disease because it does not attract private interest in the development for a new treatment. Schistosoma mansoni thioredoxin-glutathione reductase (SmTGR) is a key enzyme in the redox biochemistry of this helminth that infects people in the tropical world's most impoverished areas. SmTGR is a dimeric enzyme and there is evidence pointing that its catalytic activity depends on the approximation of its N- and C-terminal domains: glutaredoxin (Grx; residues 1 to 106) and thioredoxin reductase (TR; residues 586-593), respectively. Therefore, any ligand that impairs this movement would be a potential SmTGR inhibitor and antischistosomal drug. To study collective motions in proteins Normal Mode Analysis (NMA) is a powerful method that reveals natural vibrations of domains. In this work we have applied NMA on SmTGR to identify hinge residues that can be used as alternative binding sites for virtual screening (VS) of small molecule databases in order to find new allosteric inhibitors. **Methods**: The 3D structure of free SmTGR obtained by x-ray crystallography by our group was submitted to NMA (anisotropic network model, cutoff = 15.0) study with the Prody program. All rigid body movements were disregarded (the first 6 modes), leaving another 10 modes to be investigated. On the site of interest for VS it was centered a box with dimensions of (20x20x20)Å3 for molecular docking of small molecule ligands with Autodock-Vina.1.1.2. The ligand library was composed by a subset of ZINC15 database filtered by criteria: 350< MW <500, -1< LogP <4.5, pH≈7.4, “only clean”. This subset comprised 535,364 compounds. Top scored ligands were filtered in SwissAdme for no alerts and no violations of drug-likeness and medicinal chemistry criteria. **Results:** From 10 modes obtained in NMA, the sixth was the most relevant since it was consistent with the hinge region (residues 106-110) between Grx and TR domains. Docking at this new alternative binding site for VS resulted in 164 compounds with relevant binding affinities (superior to Vina’s error range). After filtering with SwissAdme, 66 compounds remained as promising candidates for further consideration. **Conclusions**: This work has prospected 66 potential drug-like inhibitors to a new allosteric binding site on SmTGR enzyme through VS. These hits will be further accessed computationally to predict binding affinities with higher accuracy methods such as Molecular Mechanics-Poison-Boltzman/ Solvent Surface Area (PB/SA). After rescoring, the compounds predicted as the most potent SmTGR inhibitors will be purchased for experimental validation.

Financial Support: CNPq, CAPES, FAPERJ, Newton Fund.

Presentation category: P (poster)

Evaluation of recombinant Cathodic Circulant Antigen (rCCA) as candidate to detect individuals with low *Schistosoma mansoni* parasite burden by Dot-blot analyses

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Introduction: Schistosomiasis is a serious public health problem worldwide. The World Health Organization (WHO) estimates the elimination of the disease by 2021. Based in this estimate is critical to develop an efficient diagnosis method for the treatment of the true positives. For the diagnosis, WHO recommends the holding of one or two slides of Kato-Katz. However, in Brazil the infection is characterized by individuals with low parasite burden (> 99 eggs/gram of feces) and Kato-Katz, although specific, has shown low sensitivity for these individuals. Therefore, it is essential to develop effective diagnostic methods. Immunoassays based on the detection of antigens and antibodies, has been in evidence due to their high sensitivity. In this scenario, our work proposes the use of the CCA recombinant protein for the detection of specific antibodies in human sera samples from individuals with low parasite burden by Dot-blot methodology (Dot-CCAr). **Methods:** The CCAr was produced from recombinant vector acquired from Life Technologies. Bacteria *E. coli* BL21 were transformed with recombinant vector and the production was induced by the addition of 1 mM Isopropyl-D-tiogalactose (IPTG) in Luria-Bertani (LB). Purified protein was obtained from a nickel column in native conditions. For Dot-CCAr, a PVDF membrane was sensitized with CCAr, incubated with human sera and after washing steps was incubated with anti-IgG human-peroxidase conjugate and finally revealed. The work was approved by the Ethics Committee of the René Rachou Research Center (CEUA). **Results:** The protein was successfully produced and purified and, demonstrated significant potential for positive and negative samples discrimination when used in Dot-CCAr, demonstrating antigenic ability even in the recombinant form. **Conclusions:** Innovative and efficient methods are necessary to determine the real prevalence and to achieve control interventions. The development of a Dot-blot test with CCAr may constitute a promising tool in the elimination of the disease, currently deficient. In addition, this new method will meet the criteria of Point of care. Finally, the goal is to increase efficiency by using exclusively the peptide bone of CCA instead of using whole glycoprotein.

Supported by Universal CNPq and Oswaldo Cruz Foundation.

Signal of glomerular damage associated with podocyte injury in schistosomiasis patients one year after treatment in a low endemic area in northeast of Brazil

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Introduction: Kidney involvement is poorly investigated in schistosomiasis, being schistosomal glomerulopathy the main findings (incidence ranging from 5-6%, increasing to 15% in severe hepatosplenic form). The long-term impacts of initial infection have not yet been investigated and glomerular involvement may be decisive for the development of chronic kidney disease (CKD), contributing to a poor patient prognosis. The aim was investigating glomerular damage using urinary biomarkers associated with podocyte injury in schistosomiasis patients followed-up at one year post-treatment. **Methods:** Samples of feces and urine were collected from individuals from the Bananeiras community - Capistrano municipality in the State of Ceará - Brazil at baseline. The Kato-Katz method and the up-converting phosphor-lateral flow circulating anodic antigen (UCP-LF CAA) assay (urine) were used for *Schistosoma mansoni* diagnosis. Two groups were enrolled: Positive (PG: n=38) and negative (NG: n=17) ones, and one year after treatment, samples of urine were collected from these same individuals. Regarding urinary biomarker, was quantified albuminuria by immunoturbidimetry (Cobas C111, ROCHE ®) and proteinuria by colorimetric method (Labtest ®). Moreover, urinary oxidative stress was assessed using determination of urinary malonaldehyde (MDA) that reacts with thiobarbituric acid (TBARS). Finally, urinary biomarkers of podocyte injury VEGF e TGF-β1 were determined by immunoassay (ELISA, R&D Systems ®). All urinary biomarkers were expressed by urinary creatinine ratio. **Results:** Recruited patients had mean age of 39±14 (minimum 15 and maximum 71 years) and 27 (49%) were male. All groups had no individuals with signals of clinical renal disease. However, using urinary biomarkers was observed increased levels of albuminuria (PG: median=4,59 and interquartile range of 2,92 – 8,97 vs NG: 2,61 (2,03 – 4,14) mg/g-Cr, p=0,015), urinary MDA (PG: median= 5,0 (4,3 – 6,2) vs NG: 4,0 (3,6 – 5,3) µmol/mg-Cr, p=0,030), and also in VEGF levels, a podocyte associated urinary biomarker (PG: median=30,8 (9,3 – 124) vs NG: 2,2 (1,05 – 4,67), p=0,009). Also, in a correlation analysis, urinary VEGF had significant correlation with albuminuria (rho de Spearman= 0,864, p=0,001) and urinary MDA (rho=0,691, p=0,019). **Conclusions:** The schistosomiasis' patients one year after treatment had no important clinical renal diseases, however presented increased signals of glomerular damage that may be associated with podocyte injury.

Supported by MCTI/CNPq number 01/2016

USE OF POC-CCA FOR SCHISTOSOMIASIS PREVALENCE STUDIES IN LOW ENDEMICITY AREAS

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Introduction: Schistosomiasis remains as a major public health issue present in several locations of Brazil, especially in the northeast region. This disease, however, is likely to be underestimated mainly because of issues involving the diagnosis of the parasite. To overcome these problems a Point of Care urine test based on immunochromatography for parasite Circulating Cathodic Antigen (POC-CCA) was developed. For some African countries, research has pointed to good results, with high sensitivity and specificity, using the POC-CCA test. In this work, our objective was to evaluate the performance of POC-CCA ECO version sold in Brazil in areas of low endemicity for schistosomiasis. **Methods:** Two areas were selected: one in the city of Salvador – BA, in the Dique do Cabrito (DC) region; and the other in the village of Jenipapo (JEN), in the municipality of Ubaíra. The selected areas have previous histories of repeated community-wide treatment for schistosomiasis. Urine analysis was performed immediately after collection and parasitological survey was performed by the Kato-Katz method using three stool samples collected on different days. We compared the results of the POC-CCA ECO version to the Kato-Katz for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). **Results:** Using POC-CCA ECO version, we found a positivity of 10.0 (11/110) and 22.5% (27/120) for DC and JEN, respectively. Parasitological surveys indicated that only 2.5% (31/1264) of the population evaluated were infected with *Schistosoma mansoni* in DC and 5.9% (43/729) in JEN. For the urban region, relative to the Kato-Katz, the POC-CCA ECO version demonstrated a sensitivity of 0% with a specificity of 92%, PPV 0% and NPV 96%. In the rural area, the rapid test presented a sensitivity of 44%, specificity of 85%, PPV of 52% and NPV of 73%. **Conclusions:** The results indicate that POC-CCA ECO should not be used for the diagnosis of schistosomiasis in populations of low endemicity because it poorly identifies known infections with schistosomiasis based on direct observation of parasite eggs.

Suported by NIH (R01AI121330)

Impact of malnutrition at infection by *Schistosoma mansoni*

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Introduction: Protein-energy malnutrition and schistosomiasis are important public health problems that coexist in Brazil and other countries around the world. Clinical and epidemiological studies carried out in endemic areas for schistosomiasis correlated this disease with the nutritional status of the host. From a clinical, anthropometric and pathological perspective, associations between malnutrition and some manifestations of schistosomiasis have been reported in both human and experimental models. However, there are few studies evaluating the impact of inadequate nutritional status on parasite survival and development. Therefore, it is necessary to further explore how malnutrition impacts *S. mansoni* development in the host as well as to understand the mechanisms that mediate this relationship. **Objective:** To evaluate the impact of protein-energy malnutrition on *Schistosoma mansoni* infection in C57BL/6 mice. **Material and Methods:** The animals were divided into three groups: control (fed with diet containing 14% casein), low protein 3% and low protein 8% (3% and 8% of casein, respectively as well as iron and zinc deficiency). After malnutrition establishment (evaluated through the weekly measurement of total body weight and determination of biochemical parameters: concentration of total proteins, albumin and hemoglobin), mice were challenged by the percutaneous route with 100 cercariae. Fifty days after infection, the animals were euthanized and the worms recovered from the hepatic portal system by perfusion of the mesenteric veins. Fragments of liver and intestine were removed and digested with 10% KOH to determine the number of eggs present in these organs. **Results:** Low protein 3%-fed mice showed significative weight loss and decreased serum levels of total proteins ($p=0,0012$), albumin ($p<0,0030$) and hemoglobin ($p=0,0043$), compared to the control group. Differences in the mean number of worms recovered by perfusion and eggs present in the liver was not observed between groups. However, a significant reduction in the number of eggs crossing the intestinal wall was observed in low protein 3%-fed mice, in comparison to the control group. To evaluate whether this reduction was a result of a decrease in female fecundity, the number of eggs/gram of intestine/couple of worms was calculated. The results indicate that malnutrition has an impact in egg oviposition by females. **Conclusion:** We demonstrate in this study the establishment of an experimental model of malnutrion in mice that can be further used to evaluate the impact of malnutrition in resistance and susceptibility of diseases. Our data showed, in the case of schistosomiasis, that malnutrition does not impact in parasite survival, but promotes a reduction in fecundity, probably due to the lower supply of essential nutrients available in the host. Further experiments still need to be performed to assess the impact of malnutrion in the host immune system.

Financial Support: Fapemig, CNPq, Capes, CPqRR

Standardization of the Dot Blot-MEAr immunological method for the diagnosis of individuals infected with *Schistosoma mansoni* in the endemic area of Montes Claros-MG

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Introduction: Preliminary analyses using the MEAr protein have demonstrated a high immunogenic potential for the detection of *Schistosoma mansoni* infection. Considering the need for rapid, high sensitivity and low cost methodologies, this study standardized the Dot Blot- MEAr. **Methods:** A total of 50 human serum samples collected in 2017 in the rural district of Tabuas, an endemic area of Montes Claros-MG and previously tested by Kato-Katz methods and saline gradient in other projects, were used to standardize the immunoassay. A PVDF membrane was sensitized with the MEAr protein and incubated with a blocking buffer. Then, the samples were added in duplicates separated by spots. The membrane was incubated with blocking buffer solution with human anti-human IgG-HRP antibody. The reaction revealed in ECL kit and the images were captured by LAS 4000. Each spot was analyzed by reaction intensity in Image J software with cut-off determination in the GraphPad Prism software. **Results:** The method indicated 33 positive samples in acute phase and 17 negative with cut-off of 77% of sensitivity and 50% of specificity. **Conclusion:** Considering the profile of low parasite load in infected individuals in Brazil, a fact that induces the difficulty of detection and diagnosis of the disease, the standardization of the method demonstrated a high sensitivity index, reducing false negative results. This can be usual, efficient and inexpensive method that can be introduced to aid or replace current techniques that require more time to execute.

Support: CNPq / Fiocruz

Real-time PCR performance (q-PCR) for the diagnosis of schistosomiasis mansoni in feces samples from infected individuals in endemic areas with low parasite load

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Introduction: *Schistosoma mansoni* infection is an important public health problem in Brazil. However, due to chemotherapeutic interventions in endemic areas, there has been a change in the epidemiological profile of the infection during the last decade. Currently, a growing number of individuals have low parasite load, which detection difficult by the Kato-Katz (KK) method. **Methods:** The objective of this study was to compare the performance of the Reference Test established and q-PCR technique for the diagnosis of *S. mansoni* schistosomiasis in an endemic area with low parasite load. Three faecal samples from 257 individuals from the Brejo do Amparo district, Januária, MG, were collected during three consecutive days. The statistical analysis determined the prevalence, sensitivity, specificity of the q-PCR technique in relation to the Reference Test (all positive individuals in any of the parasitological methods applied: 18 K-K, Gradiente Salino and Helmintex slides). Samples of 500mg of feces were used for extraction of *Schistosoma mansoni* DNA using the Fast QIAamp DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany), and following DNA Isolation from Large Amounts of Stool protocols, Isolation of DNA from Stool for Pathogen Detection ". In the PCR reaction sequences of the primers specific for *S. mansoni* sense 5'-CCG ACC AAC CGT TCT ATG A-3 ', anti-sense 5'-CAC GCT CTC GCA AAT AAT CTA AA-3', and probe 5 And the primers specific for the human β-actin gene (H.sapiens ACTB) sense 5'-CCA TCT ACG AGG GGT ATG '- / 56-FAM / TCG TTG TAT CTC CGA AAC CAC TGG ACG / 3BHQ_1 / 3 ', anti-sense 3'-GGT GAG GAT CTT CAT GAG GTA-5', and the probe 5 '- / 56-JOE / CCT GCG TCT GGA CCT GGC TG / 3BHQ_1 / -3'. A amplification reaction was conducted on the StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific Inc., USA) under the universal cycling program with 45 cycles and annealing temperature of 60 ° C. **Results:** The prevalence of the infection defined by the Reference Test was 45.9%, while the KK analysis of two slides identified only 20.4%. The q-PCR had a prevalence of 54.4%, sensitivity 91.3% (95% CI 84.2-95.3) and specificity 80.2 (CI95% 71.8-86.5). **Conclusion:** In conclusion, we showed that the use of more sensitive techniques such as q-PCR contributes to a more accurate diagnosis of schistosomiasis in areas of low parasitic load.

Keywords: schistosomiasis, PCR, diagnosis, parasitic load

Supported by PAPES CNPq/ FAPEMIG/ WHO

Dilemma with "Traces": The evaluation of *Point-of-Care Circulating Cathodic Antigen* (POC-CCA) performance with Traces classified as negative results.

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Introduction: The POC-CCA is a rapid immunochromatographic test that uses schistosoma antigen for the diagnosis of *Schistosoma mansoni*. The rapid test has been introduced in Brazil recently, and the performance of this test is still under evaluation, mainly because of inconclusive test results, or so called weak positive results. In this context the current study aims to verify the POC-CCA test performance, classifying "traces" as negative results in a low prevalence setting. **Methods:** This cross-sectional, investigative and descriptive study was carried out among 607 participants, 235 individuals living in Turiaçú, Maranhão state and 372 in Primavera, Pará state, both municipalities located in Amazon Region. After obtaining ethical clearance and completion of the consent form, three stool samples and one urine sample were collected from each participant. The Kato-Katz technique, compiling 12 slides of the first stool sample, two slides of the second and two of the third sample, totaling 16 slides was used as Reference Standard for comparisons. The POC-CCA test was applied according to Rapid Medical Diagnosis recommendations. Positivity rate was calculated for both techniques by the number of positives detected over the total population at risk. All positive cases were treated according to the guidelines of the Brazilian Ministry of Health. **Results:** Examining two Kato Katz slides 29 schistosomiasis positive and 578 negative individuals were identified, indicating a positivity rate of 4,7%. The Reference Standard diagnosed the presence of 67 positive and 540 negative participants, with a positivity rate of 11,0%. Test results of the POC-CCA showed the presence of 55 positive, 434 negative individuals and 118 "traces" (Considering the 55 POC-CCA positives, a rate of 9,1% could be established). Comparing the Kato-Katz technique, using two slides with the POC-CCA results and classifying "traces" as negatives, it became evident that six "traces" were confirmed as true positives. Furthermore, comparing with the Reference Standard, a total of 24 "traces" were detected as true positives. Out of the 55 positive POC-CCA results, 24 were confirmed as true positive in comparison to the Reference Standard. These data reveal a total of 24 positives indicating a positivity rate of 3,96%, when traces are classified as negative results, and 48 positives with a rate of 7.91%, including traces as positive results. **Conclusion:** The study demonstrates that classifying "traces" as positive results identified 50% more positives and the detection rate is superior than that of two Kato Katz slides.

Supported by CAPES, CNPQ, FAPESPA, Instituto Evandro Chagas.

Sensitivity of Kato-Katz and Ritchie methods in communities with high and low prevalence for schistosomiasis

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Introduction: The Kato-Katz (KK) method is recommended by the World Health Organization for the diagnosis of soil transmitted helminths and schistosomiasis. Although this method has a higher sensitivity than direct microscopy, it is dependent on multiple samples to maximize the sensitivity. In addition, this technique is not suitable for diarrheal samples, as well as for the identification of intestinal protozoa, which is a disadvantage. This study aimed to compare the sensitivity of KK and Ritchie coproparasitological methods for the diagnosis of helminths in communities with high and low prevalence for schistosomiasis in Sergipe state, Brazil. **Methods**: After signing the Free and Clarified Consent Term, 132 students were selected to participate in the study. In the period from March to April 2018, the single fecal samples provided by the participants were submitted to the Ritchie and KK methods. Fisher's exact test was used to estimate the sensitivity of the methods. **Results**: Of the 132 participants, 22.7% were positive for *Schistosoma mansoni* by KK and 28.8% by Ritchie; for *Trichuris trichiura* the positivity was 1.5% by KK and 2.3% by Ritchie and for *Enterobius vermicularis* was 0.76% by both. There was a statistically significant difference ($p < 0.05$) among the methods for the diagnosis of the mentioned parasitic infections and also when the students of the community with high endemicity for *S. mansoni* were distinguished. In the community with low endemicity there was a significant difference between the methods only for *S. mansoni* and *T. trichiura*, again being the Ritchie's method the most sensitive. In addition, other intestinal parasites were identified by Ritchie, among which the most prevalent were *Blastocystis hominis* (37.8%), *Entamoeba histolytica/E. dispar* (29.6%),*Endolimax nana* (14.4%), *Entamoeba coli* (15.9%) and hookworms (3.0%). **Conclusions**: Ritchie's method showed higher sensitivity than the KK for the diagnosis of helminths in single fecal samples. In addition, due to the decrease in the prevalence of helminthes and the increase of protozoan infections, a more sensitive and comprehensive technique is necessary, particularly in areas with lower parasite intensity.

Female Genital Schistosomiasis Evidence from Indirect Disease Markers: Neopterin and sIgA in vaginal lavage, eggs in PAP smear and urine.

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Introduction: Female genital schistosomiasis is characterized by the presence of schistosome eggs/worms in the upper or lower genital tract. Existing community-based prevalence studies indicate that FGS of the lower genital tract is a common manifestation of the infection with *S.haematobium*. Community based studies have reported an association of FGS with ulcers on the cervix, vagina, vaginal discharge, menstrual disorders, ectopic pregnancy and miscarriages. Bleeding, painful intercourse as well as primary and secondary sterility have also been indicated as common complains in FGS infections. FGS is also hypothesised as a potential risk factor for HIV transmission. While FGS has such far-reaching consequences, there are no routine diagnostic methods. **Methods:** To assess the potential use of indirect disease markers for FGS, neopterin and sIgA levels were measured in vaginal lavage obtained from Zimbabwean reproductive women aged between 15 and 49 years. FGS was diagnosed from the study population using biopsy and genital smears, including PAP smears. The women were also diagnosed for urinary schistosomiasis. **Results:** Secretory IgA was high in women with FGS, 40 ng/ml compared to 14.02 ng/ml among endemic control group. The prevalence of urinary schistosomiasis was 59 % at baseline and this declined to 8 % and 6 % at 3 and 15 months examination, respectively. There was a higher mean neopterin level in women with FGS, 68.03 ng/ml compared to the endemic control group, 16.53 ng/ml, p=0.027. Mean neopterin levels declined at 3 months post treatment of infected individuals. The diagnostic values on sensitivity and specificity for neopterin test were 65 % and 84 % respectively. There was high correlation of FGS and PAP smear at all follow-up time points R=0.913, 0.872 and 0.881 at baseline, 3 and 15 months, respectively $p < 0.001$. There was a high correlation between neopterin and PAP smear at all times R=0.923, P<0.001. Women with heavy infection at baseline had eggs examined in their PAP smear samples. Finding high levels of sIgA and neopterin was significantly associated with finding *S.haematobium* ova in the genitals by PAP smear examination ($p< 0.001$). **Conclusion:** Our results indicate that FGS causes inflammatory immune response that results in increased neopterin and secretory IgA levels in genital fluid. Treatment of schistosomiasis results in regression of pathology and a decline in the inflammatory markers. FGS is still a neglected disease in Zimbabwe and affects the majority of rural women. Diagnosis is possible by using PAP smear examination regularly would help eliminate the disease which pre-exposes women to some secondary infections including HIV. Considering the cost of the diagnostic procedure and sample collection method, PAP smear would assist in the rapid diagnosis of the disease and can be carried out in very remote rural areas.

Finacial support: UZ Research Fund and WHO

Experimental model of schistosomiasis mansoni: ocular findings

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Introduction: Schistosomiasis is considered a public health problem, affecting more than 200 million people in the world, occurring in Africa, Asia and South America. The 'Global Burden of Disease Study 2010' indicated that schistosomiasis is the one hundredth cause of death in Brazil, and is responsible for 3.6% of the estimated total of deaths in the world. Ectopic forms occur when eggs, worms or antigen-antibody immune complexes are found outside the portal circulation. Eye and adnexa are rarely affected by schistosomiasis mansoni. Aguiar *et al.* (2009) found a retinal granuloma in the eye of one female Swiss mice, among 25 infected mice, by exposing the tails into a suspension of 40 cercariae. The pathology in the eye still remain obscure. Animal models may be instruments to comprehension of the pathogenesis of the disease. **Methods:** In the present study, experimental infection of *S. mansoni* cercariae in mice, aimed to demonstrate the presence of granulomata formed in the brain. We infected 50 male mice (*Mus musculus-Swiss Webster*), weighing between 18 and 20 grams, with 50 LE strain cercarie subcutaneously, and maintained 25 as controls without infection. We followed them for 160 days post-infection. Euthanasia was carried out on days 88, 97 and 109 post-infection. After confirmation of death, the heads were taken in blocks, carefully fixed in 10% formalin and submitted to paraffin sections, alternating at 50 µm, to stained with hematoxilin-eosin. Histological studies were performed in order to examine *S. mansoni* eggs, granulomas and inflammatory lesions. Parasites were recovered using the technique developed by Pellegrino and Siqueira (1956). **Results:** Three out of 50 infected mice (12%) presented mature eggs, shells and granulomatous reactions in bulbar conjunctiva, lacrimal gland, sclera, sclera/choroid. **Conclusion:** To the best of our knowledge, this is the first description of ocular involvement, on the topography mentioned, in an experimental model of schistosomiasis mansoni.

Supported by CNPq/Brazil; Centro de Pesquisas René Rachou-FIOCRUZ/BH and Faculdade de Medicina da UFMG.

Evaluation of the Pharmacokinetic-Pharmacodynamic Relationship of Praziquantel in the *Schistosoma mansoni* Mouse Model

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Introduction: 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. **Methods:** 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. **Results:** 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%. **Conclusion:** 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.

This work was supported by Merck KGaA, the European Research Council (Grant: ERC-2013-CoG 614739-A_HERO) to JK, and the German Federal Ministry of Education and Research (Grant: BMBF-PTJ 031577) to HH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Neurological involvement of *Schistosoma mansoni* infected mice.

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Introduction: Although the digestive system is the first system to be affected by the eggs of the parasite *Schistosoma mansoni,* these can reach other systems causing the ectopic forms of schistosomiasis. The present study aims to identify the presence of *S. mansoni* DNA in the central nervous system (CNS) in infected mice and to characterize the motor and sensory alterations in infected mice. **Methods:** It was an experimental, controlled study and 32 male Balb/c mice were divided into groups according to physical effort (n = 16) or rest (n = 16). After confirmation of positivity for *S. mansoni,* the physical effort test was applied to the animals of the "physical effort" groups three times a week, and the tactile, thermal, strength and motor performance of all animals were investigated at the frequency of once per week. After 13 weeks post confirmation of infection, the animals were led to euthanasia with overdose of anesthetics, the perfusion was performed and the spinal cord and encephalon were removed for detection of the parasite DNA through the conventional Nested PCR. The neurofunctional evaluation was analyzed through the Anova test of Friedman followed by the Dunn test ($p < 0.05$). **Results:** Of the 16 infected animals, 4 died and the 12 remaining, 3 presented *S. mansoni* DNA in the spinal cord. Neurofunctional analysis revealed oscillations of muscle strength, decreased motor performance and hyposensitivity and hypersensitivity between the infected and control groups when comparing isolated weeks, however, along the timeline no clinical signs were found that suggested a possible CNS injury by presence of parasite egg. **Conclusions:** In this study, the presence of *S. mansoni* DNA in the CNS did not appear to impair the sensory and motor responses of the animals so that a pattern or tendency could be observed by the tests, especially in the last weeks, as well as in the signs and symptoms of the animals. The results suggest that the animals may have presented the asymptomatic form of the disease, or that the mechanism of neural plasticity did not allow for evident changes in the clinical evaluation performed.

Keywords: Neuroschistosomiasis; *Schistosoma mansoni;* Models, Animal; Pain Measurement; Polymerase Chain Reaction

Supported by Fiocruz

A Luminol-H_2O_2 system for detection of *Schistosoma mansoni* eggs in feces sediment produced by the Helmintex method

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Introduction: *Schistosoma mansoni* is the only etiological agent of schistosomiasis in Brazil, occuring in 19 states. Kato-Katz (KK) method has been recommended by WHO, however, it lacks sensitivity in areas with low infection intensity. The method of detection of schistosomal antigen in the urine (POC-CCA) has been proposed as an alternative to KK. Helmintex (HTX) method is based on magnetic isolation of eggs, resulting in a very sensitive egg-detection procedure. Although HTX is still time consuming and labor intensive, recent modifications have significantly improved it. Our aim was to optimize the detection step by using a chemiluminescent reaction triggered by *S. mansoni* eggs. **Methods:** Samples containing 50, 25 and 10 *S. mansoni* eggs were obtained from different sources (*in vitro* culture, liver and naturally infected human feces), 100 µL of ten final HTX samples obtained from naturally infected patients and controls (1x PBS, blood human, paramagnetic particles, ethyl acetate and a pool of human sediment free from *S. mansoni* infection) were incubated with a solution of luminol-H_2O_2. Light was detected both visually and measured in a spectrophotometer (431nm) for 15 minutes at 27^{o}C. **Results:** A bluish color was observed during 3 minutes in eggs obtained from human feces and for 15 minutes in eggs cultured *in vitro* and liver infected mice. The quantification of chemiluminescence revealed a significantly higher intensity of the light emitted by samples containing eggs when compared to controls. In addition, egg-positive fecal samples from endemic area also presented higher chemiluminescent peaks when compared to negative samples. **Conclusion:** It is possible to detect egg-positive fecal sediments through a luminol-H_2O_2 induced chemiluminescent reaction. This modification may considerably reduce time for the HTX examination and opens the possibility of future developments to make this novel, highly sensitive and confirmatory method to be applied in routine of schistosomiasis diagnosis both in laboratory and field conditions.

Supported by CAPES/CNPq