Documentation related to Session 2 of the meeting
Click on the links below to see the pre reads and presentations

Malaria Policy Advisory Group (MPAG) Meeting

13 – 15 April 2021 *(CEST time zone)*
Virtual meeting

PROVISIONAL PROGRAMME*

Tuesday, 13 April 2021			
	Session 1	**Open**	
12:00 – 12:05	Welcome by the ADG, UCN	Dr Ren Minghui	
12:05 – 12:15	Welcome by the Chair, MPAG	Dr Dyann Wirth	
12:15 – 13:00	Report from the Director, GMP	Dr Pedro Alonso	
13:00 – 13:30	Partner Perspective, US President's Malaria Initiative	Dr Raj Panjabi	
13:30 – 14:00	Rethinking malaria	Dr Rose Leke & Dr Alastair Robb	**For guidance**
14:00 – 14:15	*Coffee break*		
	Session 2	**Open**	
14:15 – 15:00	Clinical malaria – parasite density thresholds in different transmission settings and implications for use of RDTs	Dr Jane Cunningham	**For guidance**
15:00 – 15:30	Update on the situation of antimalarial drug efficacy and resistance in Africa	Dr Pascal Ringwald	
15:30 – 16:00	Proposed technical consultation to stage *P. knowlesi* along the continuum between zoonosis and human pathogen: Background / Annexes / Presentation	Dr Kim Lindblade	
16:00	*End of day*		

Wednesday, 14 April 2021			
	Session 3	**Open**	
12:00 – 12:45	HRP2 gene deletions – a focus on horn of Africa region	Dr Jane Cunningham	**For decision**
12:45 – 13:30	Proposed technical consultation on urban malaria	Dr Abdisalan Noor	**For guidance**
13:30 – 13:45	*Coffee break*		
	Session 4	**Open**	
13:45 – 14:15	Update on guidance for severe malaria	Dr Peter Olumese	**For decision**

Documentation related to Session 1 of the meeting
Click on the links below to see the pre reads and presentations

14:15 – 14:45	Update on the classification of insecticide-treated net products – annual update as requested by MPAG	Dr Jan Kolaczinski & Dr Marion Law	**For guidance**
14:45 – 15:15	Update on digital solutions for malaria elimination surveillance	Dr Abdisalan Noor & Ms Mwalenga Nghipumbwa	
15:15	*End of day*		

Thursday, 15 April 2021			
	Session 5	**Closed**	
12:00 – 15:00	Finalization of wording of recommendations	Dr Dyann Wirth	**For guidance**

** Provisional programme and may be subject to change*

Clinical malaria: Parasite density analysis & implications for diagnostic test specifications

John Aponte, Jane Cunningham
WHO/GMP
MPAG April 2021

Global **Malaria** Programme

World Health Organization

Introduction

In malaria endemic areas, asymptomatic carriage of malaria parasites occurs frequently and the detection of malaria parasites in blood films (or antigens on RDTs) from a febrile individual does not necessarily indicate clinical malaria.

In clinical trials case definitions for symptomatic malaria require the presence of fever together with a parasite density above a specific cutoff.

In clinical settings the cut-offs are effectively based on the limits of detection of the diagnostic modality – ie microscopy. RDTs, PCR

Introduction

A. Limits of detection (LOD) of tests ?

- PCR – 0.02 -2 parasites/ µL
- Microscopy, variable – experts- 20 parasites/µL ; in-reality - ≈ 150-200 p/µL
- Ag-RDTs – detect antigens not parasites - comparable – 100-200 parasites/µL; *high sensitive RDT ≈ 10 parasites/ µL*

B. Limits of fever ≈ pyrogenic threshold - ? p/µL

P. falciparum

- Luxemburger et al. (WThailand, low transmission) – 1400 p/µL [327-6516]
- Dicko et al. (Mali, seasonal transmission) – 200 – 3200 p/µL
- Gatton & Cheng (malaria therapy data) – 650 – 3500 p/µL
- Karyana et al. (Papua Indonesia, perennial) – 1734 p/µL

P. vivax

- Luxemburger et al. (WThailand, low transmission) – 180 p/µL [45-734]
- Karyana et al. (Papua Indonesia, perennial) – 310 p/µL

Diagnostic test LOD should be at least equal to the lowest pyrogenic threshold to capture all clinical malaria

World Health Organization

Objective

To evaluate different thresholds of parasitemia density that defines clinical malaria.

- To describe the distribution of parasitemia density on patients with malaria disease present to the health facility, in different epidemiological settings and age groups.

- To describe the distribution of the parasitemia density in symptomatic and asymptomatic subjects in cross-sectionals, in different epidemiological settings and age groups

- To determine the attributable fraction of fever due to malaria and sensitivity and specificity of different parasitemia cut-off points and implications for use of existing diagnostic tools

We're not addressing:

- relevance of asymptomatic parasitemia to disease transmission
- health impact or natural history of undetected and/or asymptomatic parasitemia
- *P. falciparum* outside of Africa or *P.vivax*

Attributable fraction

- AF: Proportion of cases that are attributable to a risk factor (cases of fever due to malaria)
- As malaria parasites could be found in asymptomatic people, the presence of parasites in a person with symptoms, could be cause by other reason and not necessarily by the malaria infection.
- The asociation between parasitaemia and fever is not linear and the standard methology could lead to even negative estimates of the AF (as parasite are found in asymptomatics)
- The AF could be used to estimate the true number of disease cause by malaria in the mixture of fever cases caused by malaria and by other diseases

Methodology

From Cross-sectionals:

Attributable fraction of clinical disease (fever) due to malaria

Estimate probabilistically the TRUE number of fever cases due to malaria

Based on the TRUE number of cases estimate SENSITIVITY and SPECIFICITY of a given cut-off point

- Using an exponential logistic model of the risk of fever associated with the density

  (Smith, T, J A Schellenberg, y R Hayes. «Attributable fraction estimates and case definitions for malaria in endemic areas». *Statistics in Medicine* 13, n.º 22 (30/11/1994): 2345-58.)

- Using a bayesian latent class model to estimate the proportion of fever cases due to malaria based on the distribution of densities on non-febrile cases

  (Vounatsou, P., Smith, T., Smith, A.F.M., 1998. Bayesian analysis of two-component mixture distributions applied to estimating malaria attributable fractions. Journal of the Royal Statistical Society: Series C (Applied Statistics) 47, 575–587. https://doi.org/10.1111/1467-9876.00129)

Both methods have advantages and disadvantages:

Exponential logistic

- Estimated by maximum likelihood
- Sensible to outliers of very high density values
- Estimation of all possible cut-off points
- No confidence intervals for AF, Sensitivity or specificity
- Could estimate biased values of AF if the association between density and fever is not adequately fitted

Bayesian latent class

- Bayesian estimation
- Robust to outliers but require categorization of densities
- Depends on the categorization of the data.
- Provide confidence intervals for AF, Sensitivity and Specificity
- Robust to the partition function that separate the classes. i.e non biased estimation AF

Diagnositic characteristics using AF (example)

Source data and model results

Cut-off	1000 (density)
Total fevers (N)	656 Data
Fevers with density >= cut-off (Nc)	327 Data
Attributable fraction (λ)	43.7% Model
PPV for density >= cut-off (λc)	85.9% Model

Synthetic 2x2 table created from data and the model

Fever classification according cut-off	True Malaria	True No Malaria	Total
Malaria Fever (density >= cut off)	281 (Nc x λc)	46	327 (Nc)
Non Malaria fever	5	324	329
Total fevers	286 (N x λ)	370	656 (N)

Note:
To estimate the model it is necessary to have data on the distribution of parasitemia density in both symptomatic asymptomatics, but
To estimate diagnostic characteristics, only data from the symptomatic population is used.

'Performance' of the " test" with a given cutoff point

Sensitivity	281 / 286	98.1%
Specificity	324 / 370	87.5%
Positive predictive value	281 / 327	85.9%
Negative predictive value	324 / 329	98.3%

Exponential-logistic model

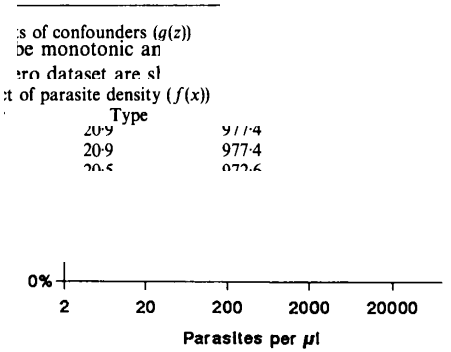


Figure 5. Sensitivities, specificities and positive predictive values of malaria case definitions

ATTRIBUTABLE FRACTION ESTIMATES AND CASE DEFINITIONS FOR MALARIA

Table I. Distribution of *Plasmodium falciparum* parasite densities in blood slides taken fro children (febrile and afebrile) in Kilombero District, Tanzania, 1989–1991

Parasite density (parasites/µl)	Number of observations Febrile	Afebrile	% febrile	Odds ratio
0	16	160	9·1	1·00
1–499	18	328	5·2	0·55
500–4999	49	846	5·5	0·58
5000–49999	47	507	8·5	0·93
+	7	18	28·0	3·89
	137	1859	6·9	

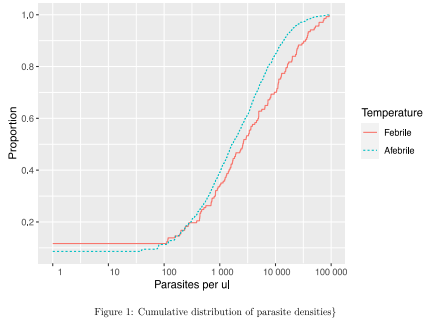


Figure 1: Cumulative distribution of parasite densities}

DISCUSSION

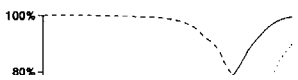
investigated a number of approaches to the estimation of malaria-attributable ases in areas of high endemicity. We have shown that classical approaches on of the overall prevalence of parasitaemia in fever cases and controls are u

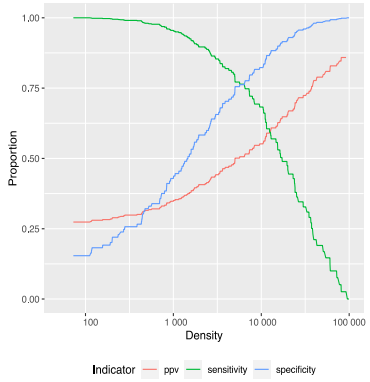
Kilombero dataset
AF in the paper: 0.216
AF in the code: 0.246

T. SMITH, J. ARMSTRONG SCHELLENBERG AND R. HAYES

Table V. To estimate the sensitivity and specificity of a case definition a 2 × 2 frequency table is constructed. The estimates obtained are then sensitivity: $n_c\lambda_c/N\lambda$, specificity: $1 - n_c(1-\lambda_c)/N(1-\lambda)$

Diagnosis	True aetiology Malaria	Other	All
Malaria	$n_c\lambda_c$	$n_c(1-\lambda_c)$	n_c
Other	$N\lambda - n_c\lambda_c$	$N(1-\lambda) - n_c(1-\lambda_c)$	$N - n_c$
All	$N\lambda$	$N(1-\lambda)$	N





*Smith, T, J A Schellenberg, y R Hayes. «Attributable fraction estimates and case definitions for malaria in endemic areas». *Statistics in Medicine* 13, n.º 22 (30/11/1994): 2345-58.)

Bayesian latent class model

	Wet season		
Category	Parasite level	m_i	n_i
1	0	43	60
2	3251	40	58
3	9673	3	14
4	16095	3	13
5	22518	2	10
6	28940	1	8
7	35362	0	7
8	41785	1	6
9	48207	1	6
10	225685	0	69
Total		94	251

AF Paper: 0.444
AF code: 0.472

$$\theta_s(k) = \Lambda\theta_m(k) + (1-\Lambda)\theta_c(k), \qquad (1)$$

$$\lambda(k) = \frac{\Lambda\theta_m(k)}{\theta_s(k)}, \qquad (2)$$

phi[i]

p0[i]

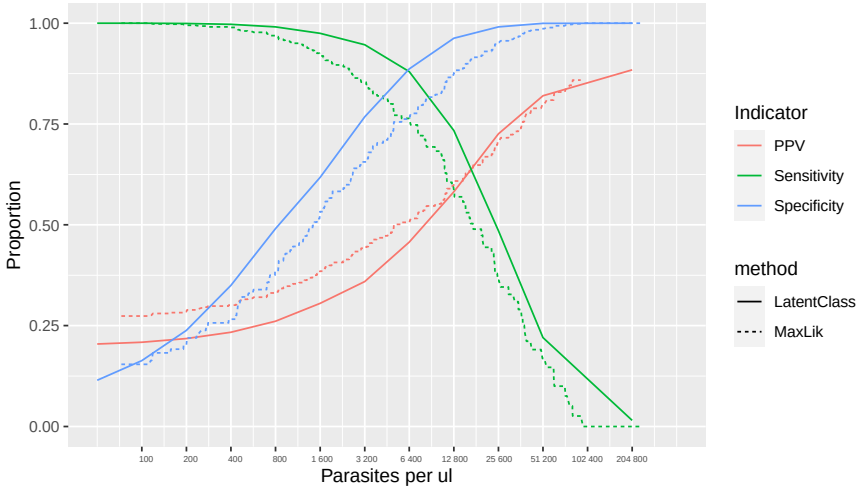
$$\Pr(k \geq C \mid \text{malaria episode}) = \sum_{k=C}^{K} \theta_m(k) \qquad (3)$$

$$\Pr(\text{malaria episode} \mid k \geq C) = \frac{\sum_{k=C}^{K} \lambda(k)\theta_s(k)}{\sum_{k=C}^{K} \theta_s(k)} \qquad (4)$$

$$\Pr(k < C \mid \text{non-malaria illness} = \Psi(C) = \frac{\sum_{k=0}^{C-1} (1-\lambda(k))\theta_s(k)}{\sum_{k=0}^{K} (1-\lambda(k))\theta_s(k)}. \qquad (5)$$

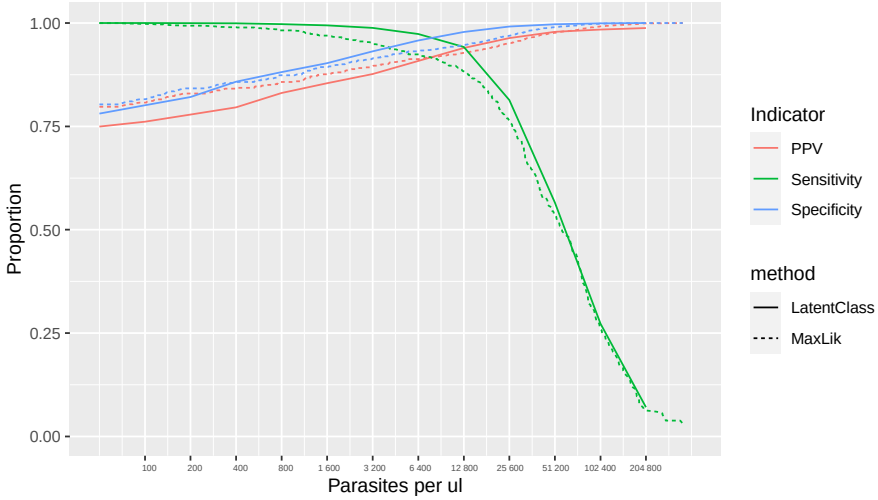
Müller, I., Genton, B., Rare et al., 2009. Three different Plasmodium species show similar patterns of clinical tolerance of malaria infection. Malar J 8, 158. https://doi.org/10.1186/1475-2875-8-158

Comparison of the two methods



Kilombero test dataset

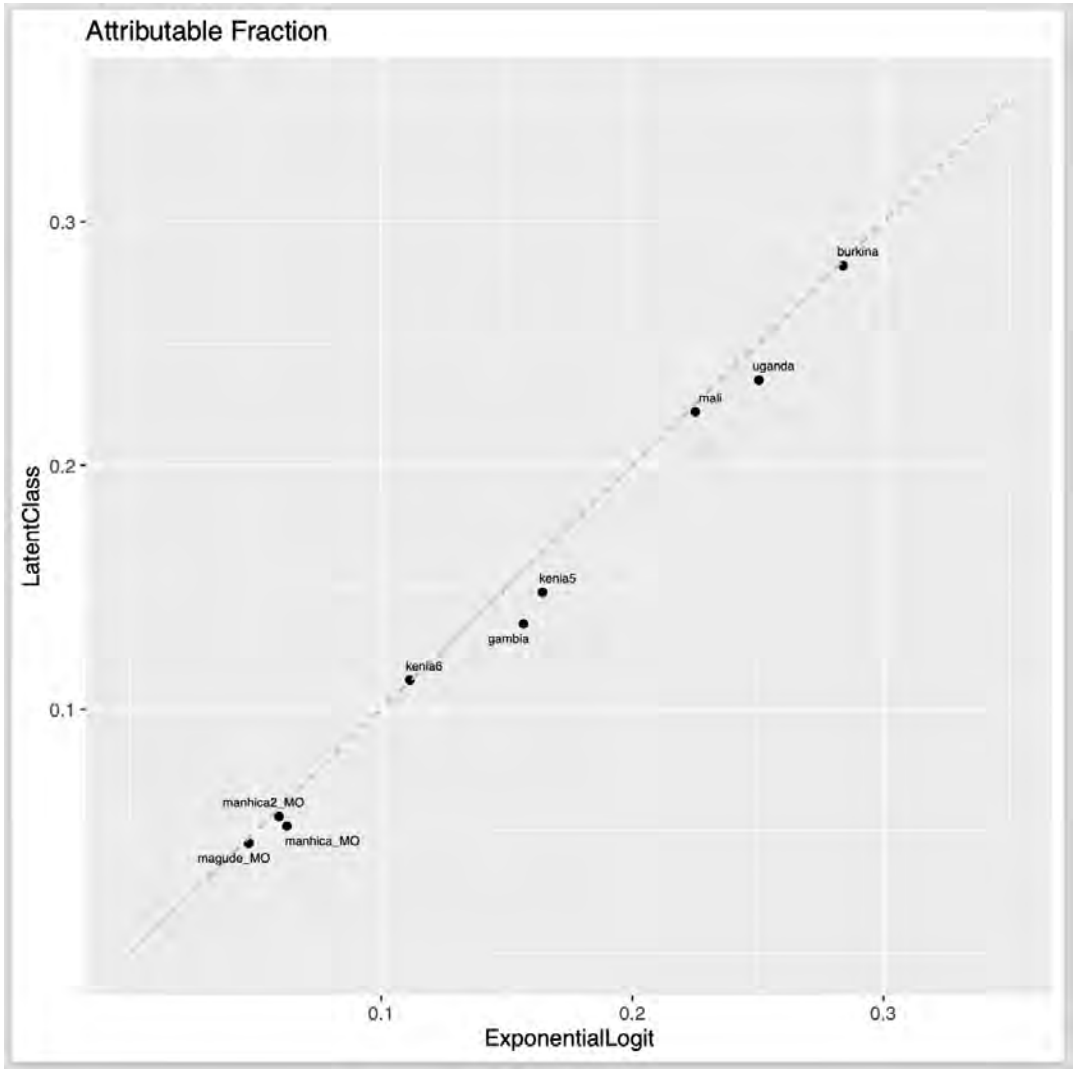
AF: 24.2% Logistic
19.0% Bayesian



Manhiça test dataset

AF: 43.7 % Logistic
41.2 % Bayesian

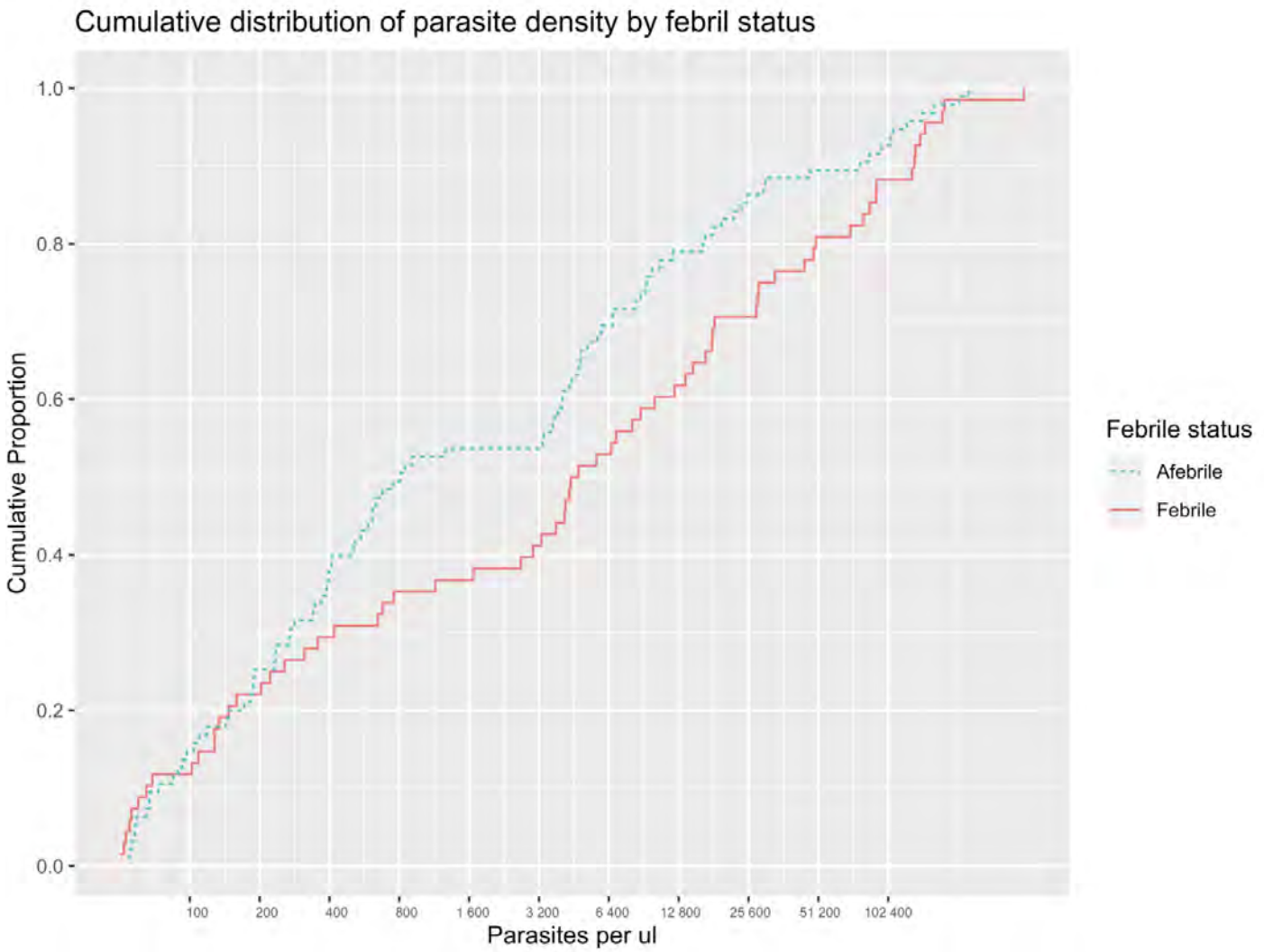
Both methods produce comparable AF estimates



Datasets available to evaluate AF using OM

Report name	Country	Population	Source	timeset
burkina	Burkina Faso	3-59 months	Chandramohan et al NEJM 2019	2014-2016
gambia	Gambia	6m to 14y	MRC Gambia	2016-2020
kenia5	Kenia	All population	Kemri	2010-2014
kenia6	Kenia	All population	Kemri	2016-2020
magude_MO	Mozambique	All ages	CISM	2015-2018
mali	Mali	3-59 months	Chandramohan et al NEJM 2019	2014-2016
manhica_MO	Mozambique	All ages	CISM	2010-2014
manhica2_MO	Mozambique	All population	CISM	2015-2018
uganda	Uganda	All population	Public data	2015-2018

Mozambique: Manhiça – all ages – 2015-2018



Mozambique: Manhiça

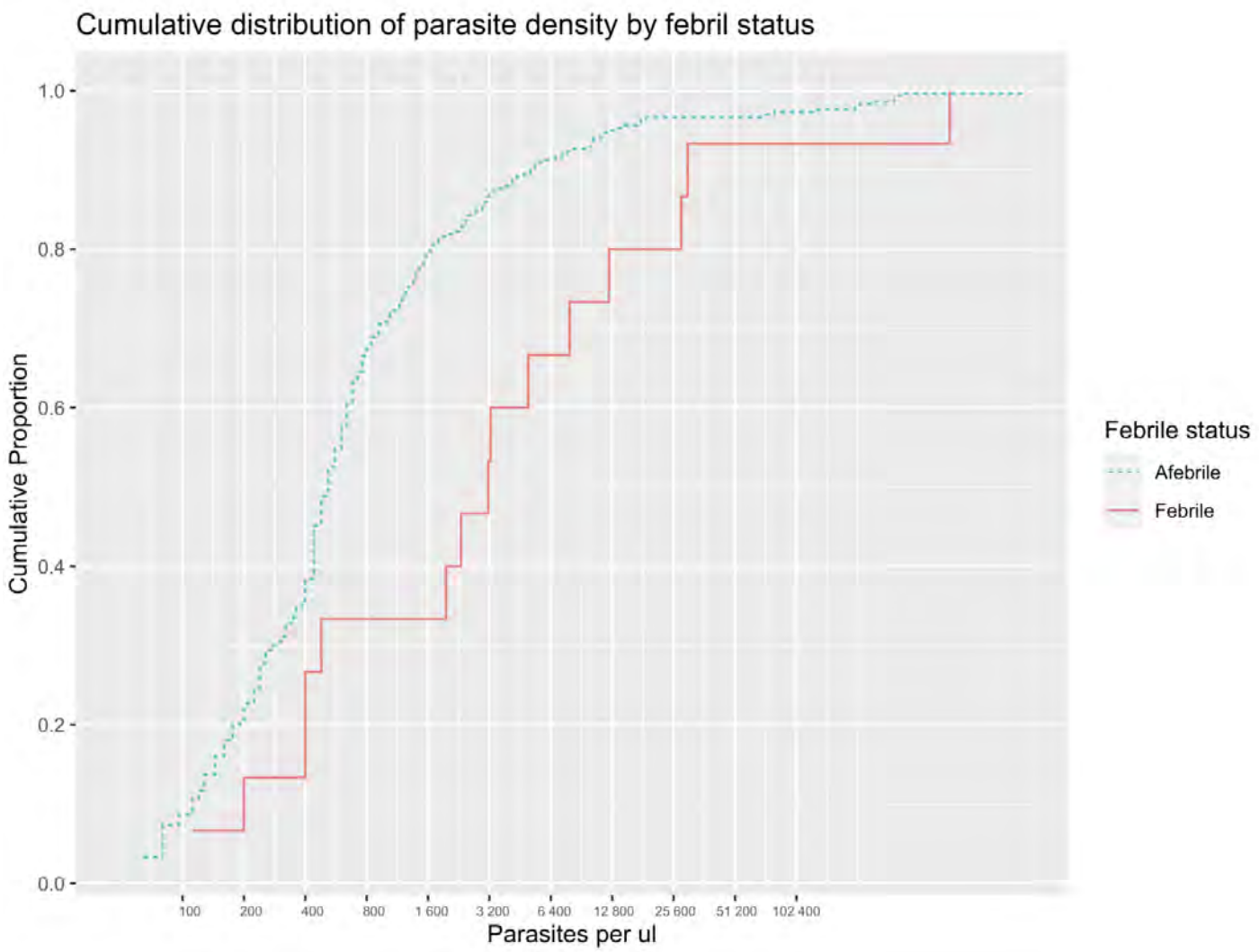
Latent Class Bayesian model

Diagnostic characteristics using Bayesian Latent Class model

cutoff	sensitivity	sens_lci	sens_uci	specificity	spec_lci	spec_uci	ppv	ppv_lci	ppv_uci	npv	npv_lci	npv_uci
1	1.000	1.000	1.000	0.978	0.972	0.982	0.635	0.488	0.750	1.000	1.000	1.000
100	0.971	0.899	1.000	0.981	0.976	0.985	0.696	0.559	0.796	0.998	0.993	1.000
200	0.950	0.847	1.000	0.985	0.980	0.988	0.726	0.596	0.819	0.997	0.989	1.000
400	0.921	0.785	0.999	0.988	0.984	0.991	0.758	0.638	0.842	0.995	0.985	1.000
800	0.885	0.720	0.998	0.988	0.985	0.992	0.788	0.678	0.864	0.993	0.980	1.000
1600	0.868	0.696	0.996	0.989	0.985	0.992	0.798	0.691	0.871	0.992	0.978	1.000
3200	0.853	0.679	0.990	0.993	0.990	0.995	0.802	0.698	0.875	0.991	0.977	1.000
6400	0.713	0.529	0.908	0.995	0.993	0.997	0.838	0.747	0.902	0.984	0.967	0.996
12800	0.597	0.415	0.810	0.997	0.995	0.998	0.863	0.777	0.922	0.978	0.959	0.993
25600	0.469	0.303	0.678	0.998	0.997	0.999	0.884	0.803	0.940	0.971	0.951	0.988
51200	0.347	0.207	0.532	0.999	0.998	0.999	0.897	0.819	0.951	0.965	0.943	0.984
102400+	0.236	0.125	0.391	1.000	1.000	1.000	0.906	0.828	0.959	0.960	0.937	0.979

Attributable fraction: 0.052 95%CI(0.031, 0.076)

Kenya: Kilifi (Junjo) – all ages – 2010-2014



Kenya: Kilifi (Junjo)

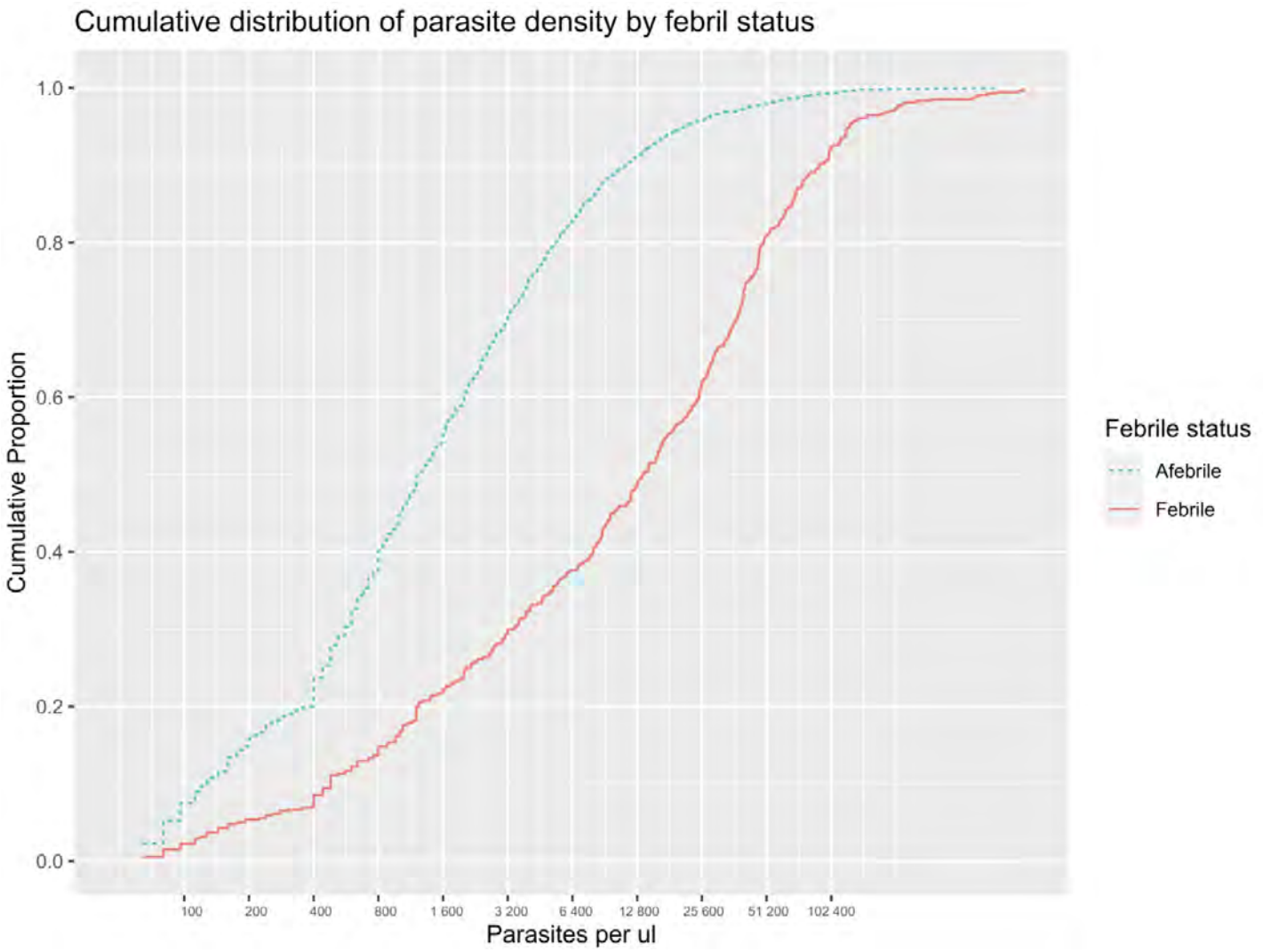
Latent Class Bayesian model

Diagnostic characteristics using Bayesian Latent Class model

cutoff	sensitivity	sens_lci	sens_uci	specificity	spec_lci	spec_uci	ppv	ppv_lci	ppv_uci	npv	npv_lci	npv_uci
1	1.000	1.000	1.000	0.851	0.834	0.867	0.480	0.200	0.696	1.000	1.000	1.000
100	0.997	0.980	1.000	0.870	0.854	0.885	0.514	0.225	0.727	0.999	0.995	1.000
200	0.992	0.953	1.000	0.894	0.879	0.908	0.545	0.248	0.752	0.998	0.988	1.000
400	0.979	0.900	1.000	0.945	0.934	0.955	0.589	0.286	0.785	0.995	0.975	1.000
800	0.922	0.717	0.999	0.965	0.956	0.973	0.714	0.422	0.870	0.984	0.929	1.000
1600	0.874	0.607	0.998	0.978	0.971	0.984	0.784	0.519	0.910	0.976	0.904	1.000
3200	0.783	0.495	0.990	0.986	0.980	0.991	0.834	0.609	0.935	0.959	0.872	0.999
6400	0.679	0.386	0.968	0.992	0.988	0.996	0.871	0.685	0.953	0.942	0.843	0.998
12800	0.545	0.262	0.909	0.995	0.991	0.997	0.907	0.762	0.969	0.922	0.810	0.994
25600	0.470	0.201	0.857	0.996	0.992	0.998	0.923	0.795	0.977	0.911	0.794	0.991
51200	0.422	0.169	0.799	0.997	0.993	0.999	0.928	0.807	0.979	0.904	0.785	0.988
102400+	0.361	0.133	0.719	1.000	1.000	1.000	0.931	0.814	0.981	0.897	0.775	0.984

Attributable fraction: 0.148 95%CI(0.042, 0.281)

Uganda (ICMER) – all ages – 2015-2018



Uganda (ICMER)

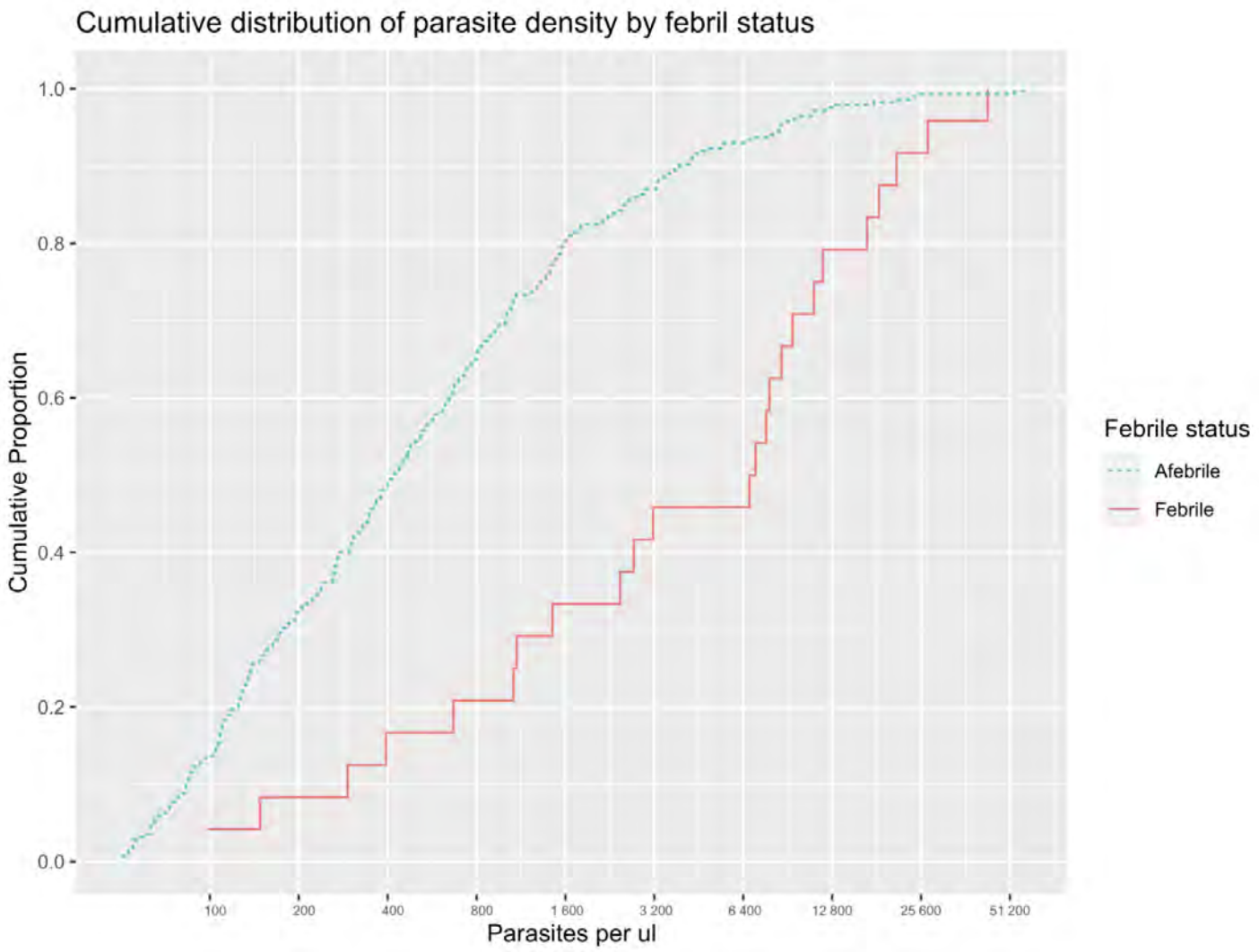
Latent Class Bayesian model

Diagnostic characteristics using Bayesian Latent Class model

cutoff	sensitivity	sens_lci	sens_uci	specificity	spec_lci	spec_uci	ppv	ppv_lci	ppv_uci	npv	npv_lci	npv_uci
1	1.000	1.000	1.000	0.901	0.897	0.904	0.722	0.690	0.750	1.000	1.000	1.000
100	0.996	0.983	1.000	0.909	0.905	0.912	0.754	0.725	0.780	0.998	0.994	1.000
200	0.991	0.973	1.000	0.914	0.911	0.918	0.769	0.741	0.794	0.997	0.991	1.000
400	0.987	0.964	0.999	0.933	0.930	0.936	0.779	0.753	0.803	0.996	0.988	1.000
800	0.958	0.922	0.990	0.951	0.948	0.954	0.814	0.791	0.835	0.987	0.974	0.997
1600	0.914	0.869	0.957	0.968	0.966	0.970	0.851	0.832	0.868	0.973	0.957	0.988
3200	0.859	0.804	0.914	0.981	0.980	0.983	0.891	0.875	0.904	0.958	0.938	0.975
6400	0.790	0.728	0.851	0.990	0.989	0.992	0.928	0.917	0.939	0.939	0.917	0.959
12800	0.670	0.608	0.733	0.995	0.995	0.996	0.955	0.947	0.963	0.908	0.884	0.930
25600	0.509	0.451	0.568	0.998	0.997	0.998	0.971	0.964	0.977	0.869	0.844	0.892
51200	0.262	0.221	0.307	0.999	0.999	1.000	0.975	0.968	0.981	0.815	0.790	0.839
102400+	0.106	0.080	0.137	1.000	1.000	1.000	0.979	0.970	0.987	0.785	0.760	0.810

Attributable fraction: 0.235 95%CI(0.21, 0.26)

Gambia – 6mo-14yrs – 2016-2020



Gambia

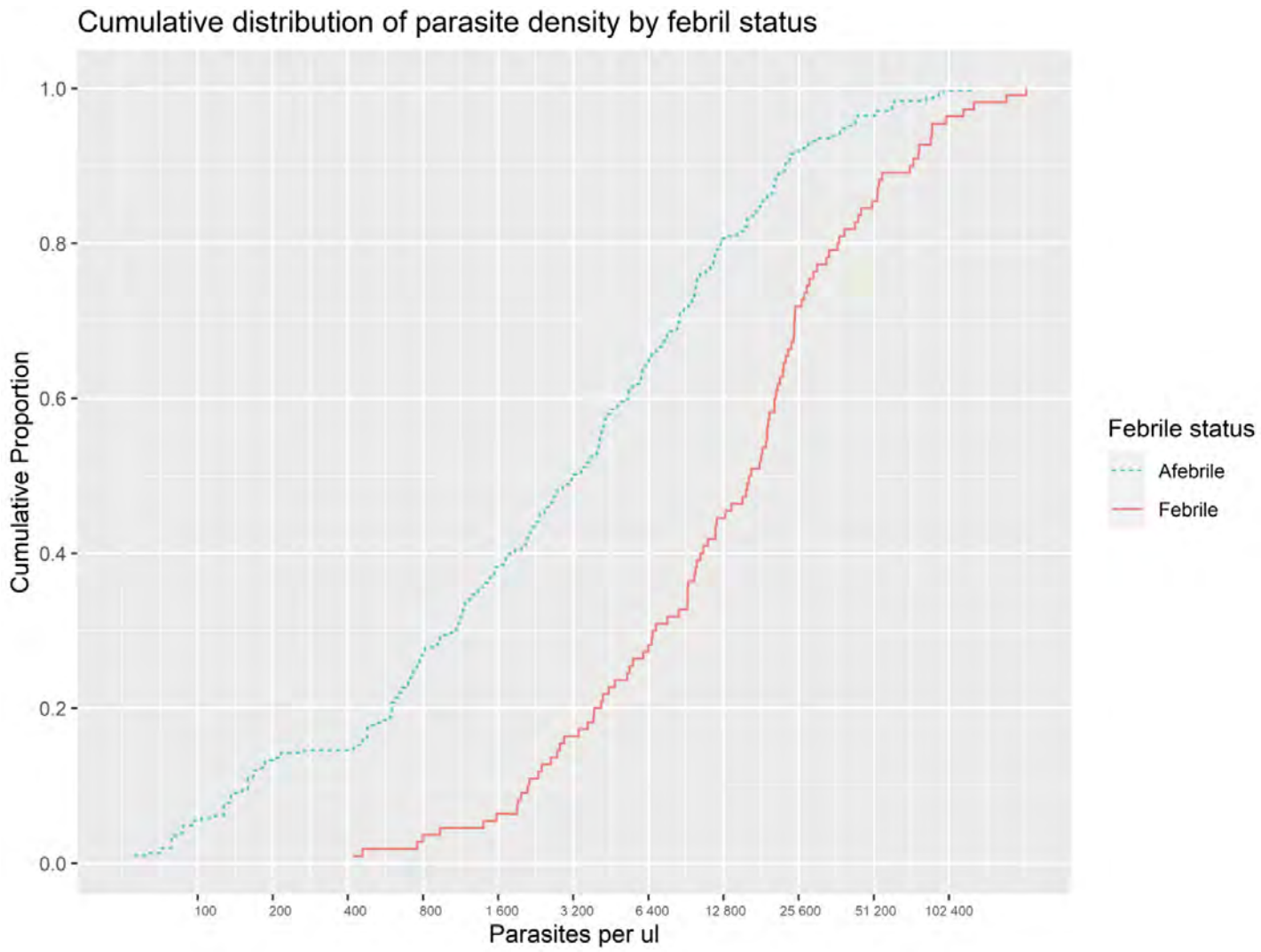
Latent Class Bayesian model

Diagnostic characteristics using Bayesian Latent Class model

cutoff	sensitivity	sens_lci	sens_uci	specificity	spec_lci	spec_uci	ppv	ppv_lci	ppv_uci	npv	npv_lci	npv_uci
1	1.000	1.000	1.000	0.939	0.931	0.946	0.496	0.333	0.648	1.000	1.000	1.000
100	0.963	0.812	1.000	0.952	0.945	0.958	0.699	0.548	0.810	0.993	0.958	1.000
200	0.953	0.776	1.000	0.964	0.957	0.969	0.744	0.605	0.843	0.991	0.951	1.000
400	0.937	0.733	1.000	0.975	0.970	0.980	0.789	0.663	0.874	0.988	0.942	1.000
800	0.906	0.668	0.999	0.986	0.982	0.989	0.840	0.736	0.908	0.984	0.930	1.000
1600	0.840	0.571	0.995	0.991	0.988	0.994	0.895	0.818	0.942	0.973	0.909	0.999
3200	0.771	0.487	0.985	0.995	0.992	0.997	0.924	0.864	0.961	0.963	0.892	0.998
6400	0.687	0.389	0.962	0.998	0.997	0.999	0.948	0.900	0.976	0.951	0.874	0.996
12800	0.328	0.150	0.560	1.000	0.999	1.000	0.966	0.926	0.988	0.904	0.824	0.959
25600	0.139	0.035	0.317	1.000	0.999	1.000	0.978	0.944	0.996	0.881	0.800	0.942
51200	0.075	0.009	0.214	1.000	1.000	1.000	0.982	0.950	0.997	0.873	0.792	0.936
102400+	0.000	0.000	0.000	1.000	1.000	1.000	0.991	0.967	1.000	0.865	0.783	0.929

Attributable fraction: 0.135 95%CI(0.071, 0.217)

Burkina Faso



Burkina Faso

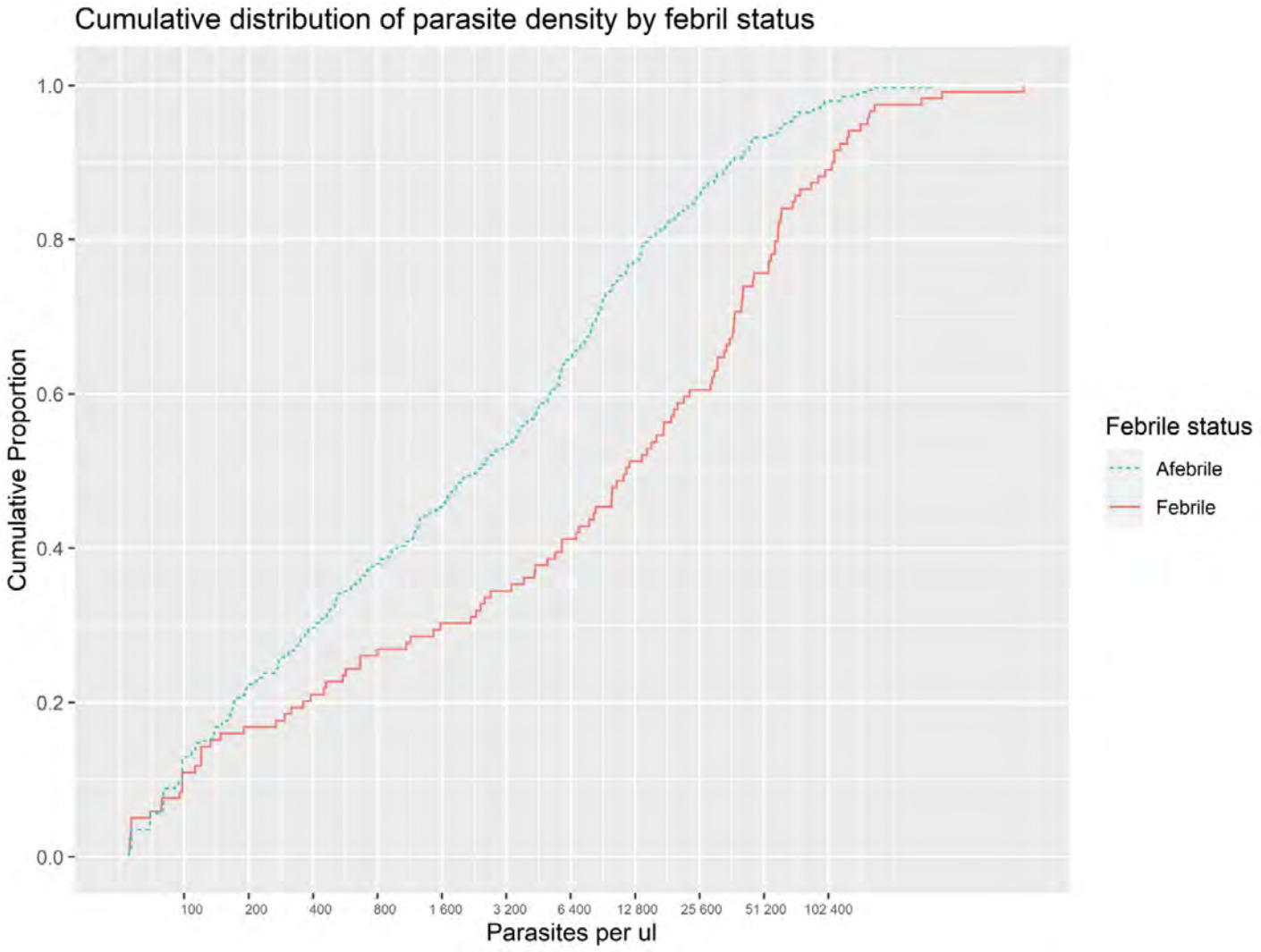
Latent Class Bayesian model

Diagnostic characteristics using Bayesian Latent Class model

cutoff	sensitivity	sens_lci	sens_uci	specificity	spec_lci	spec_uci	ppv	ppv_lci	ppv_uci	npv	npv_lci	npv_uci
1	1.000	1.000	1.000	0.948	0.942	0.954	0.876	0.842	0.904	1.000	1.000	1.000
100	0.999	0.997	1.000	0.952	0.947	0.958	0.882	0.849	0.910	1.000	0.999	1.000
200	0.998	0.990	1.000	0.953	0.947	0.958	0.891	0.860	0.916	0.999	0.996	1.000
400	0.998	0.989	1.000	0.960	0.955	0.965	0.892	0.861	0.917	0.999	0.995	1.000
800	0.984	0.954	1.000	0.966	0.961	0.970	0.905	0.877	0.928	0.993	0.981	1.000
1600	0.961	0.909	0.996	0.973	0.968	0.977	0.916	0.891	0.937	0.984	0.963	0.999
3200	0.888	0.822	0.944	0.981	0.977	0.984	0.927	0.904	0.946	0.957	0.928	0.980
6400	0.770	0.685	0.852	0.989	0.986	0.992	0.939	0.919	0.956	0.916	0.877	0.950
12800	0.591	0.488	0.694	0.996	0.994	0.997	0.955	0.935	0.969	0.861	0.813	0.904
25600	0.317	0.236	0.408	0.998	0.997	0.999	0.966	0.948	0.980	0.788	0.737	0.835
51200	0.170	0.109	0.244	1.000	0.999	1.000	0.973	0.955	0.986	0.754	0.701	0.803
102400+	0.038	0.012	0.081	1.000	1.000	1.000	0.984	0.964	0.998	0.726	0.673	0.776

Attributable fraction: 0.282 95%CI(0.232, 0.336)

Mali


Cumulative distribution of parasite density by febril status
Cumulative Proportion
1.0
0.8
0.6
0.4
0.2
0.0
100
200
400
800
1 600
3 200
6 400
12 800
25 600
51 200
102 400
Parasites per ul
Febrile status
Afebrile
Febrile

Latent Class Bayesian model

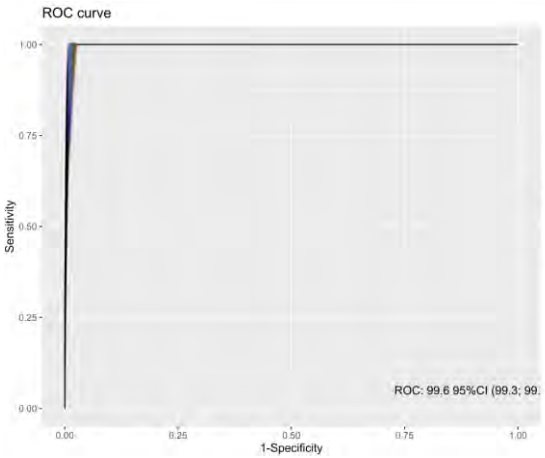
Diagnostic characteristics using Bayesian Latent Class model

cutoff	sensitivity	sens_lci	sens_uci	specificity	spec_lci	spec_uci	ppv	ppv_lci	ppv_uci	npv	npv_lci	npv_uci
1	1.000	1.000	1.000	0.947	0.941	0.953	0.815	0.764	0.856	1.000	1.000	1.000
100	0.951	0.917	0.982	0.953	0.948	0.959	0.836	0.790	0.873	0.985	0.973	0.995
200	0.918	0.869	0.965	0.958	0.952	0.963	0.847	0.804	0.882	0.976	0.958	0.991
400	0.888	0.830	0.946	0.963	0.958	0.968	0.856	0.815	0.889	0.968	0.946	0.986
800	0.848	0.779	0.918	0.968	0.963	0.972	0.867	0.828	0.898	0.957	0.931	0.979
1600	0.810	0.732	0.888	0.972	0.968	0.976	0.876	0.839	0.906	0.947	0.918	0.973
3200	0.762	0.677	0.851	0.979	0.975	0.982	0.886	0.850	0.914	0.935	0.902	0.964
6400	0.682	0.587	0.782	0.986	0.983	0.989	0.901	0.868	0.927	0.915	0.878	0.949
12800	0.566	0.464	0.676	0.991	0.989	0.993	0.920	0.890	0.944	0.888	0.847	0.927
25600	0.457	0.355	0.567	0.996	0.994	0.997	0.936	0.907	0.958	0.865	0.820	0.906
51200	0.290	0.206	0.388	0.999	0.998	0.999	0.951	0.923	0.972	0.831	0.784	0.874
102400+	0.130	0.075	0.202	1.000	1.000	1.000	0.964	0.935	0.986	0.801	0.754	0.846

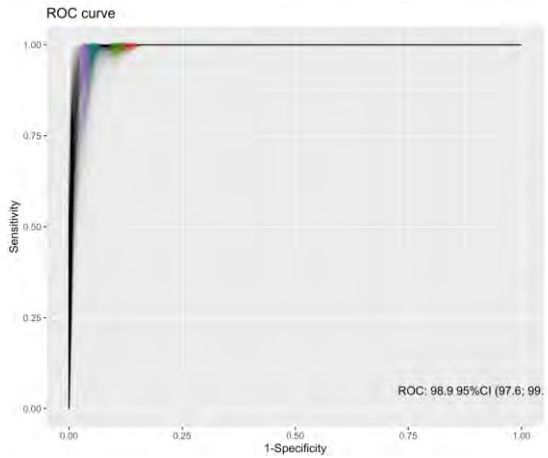
Attributable fraction: 0.222 95%CI(0.177, 0.27)

ROC curve

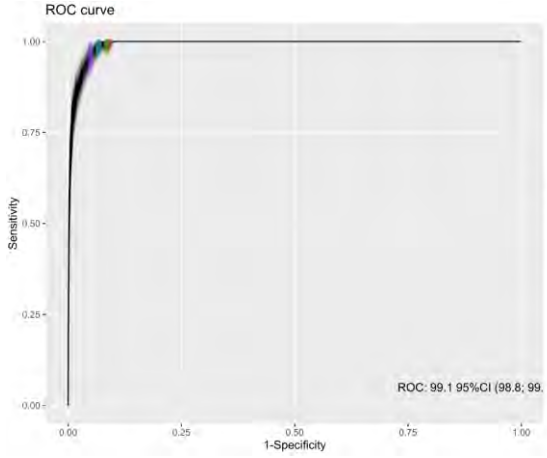
Mozambique (99.6)



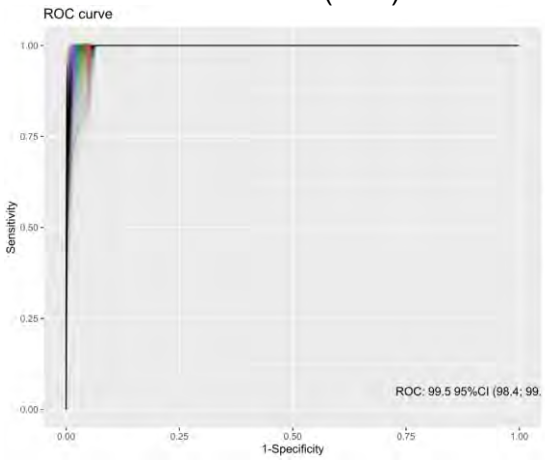
Kenya (97.6)



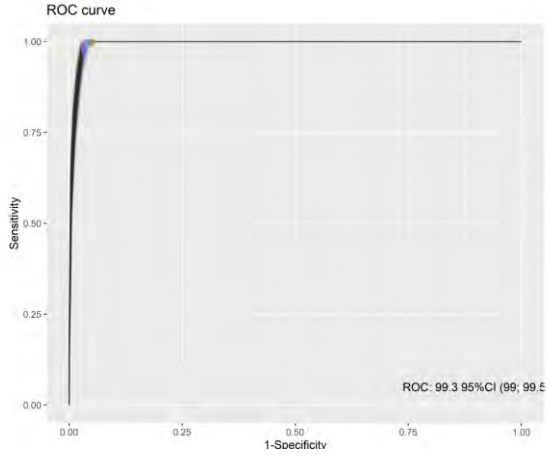
Uganda (99.1)



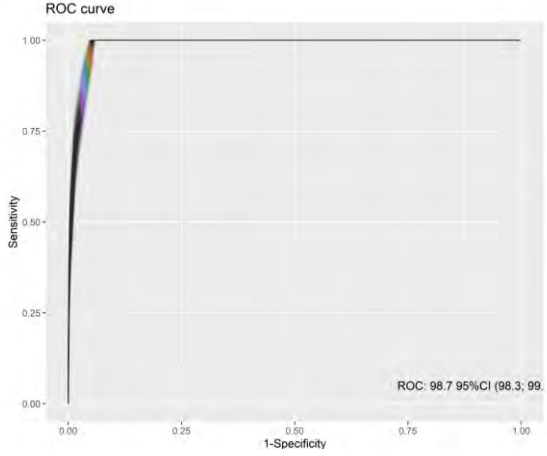
Gambia (98.4)



Burkina Faso (99.3)



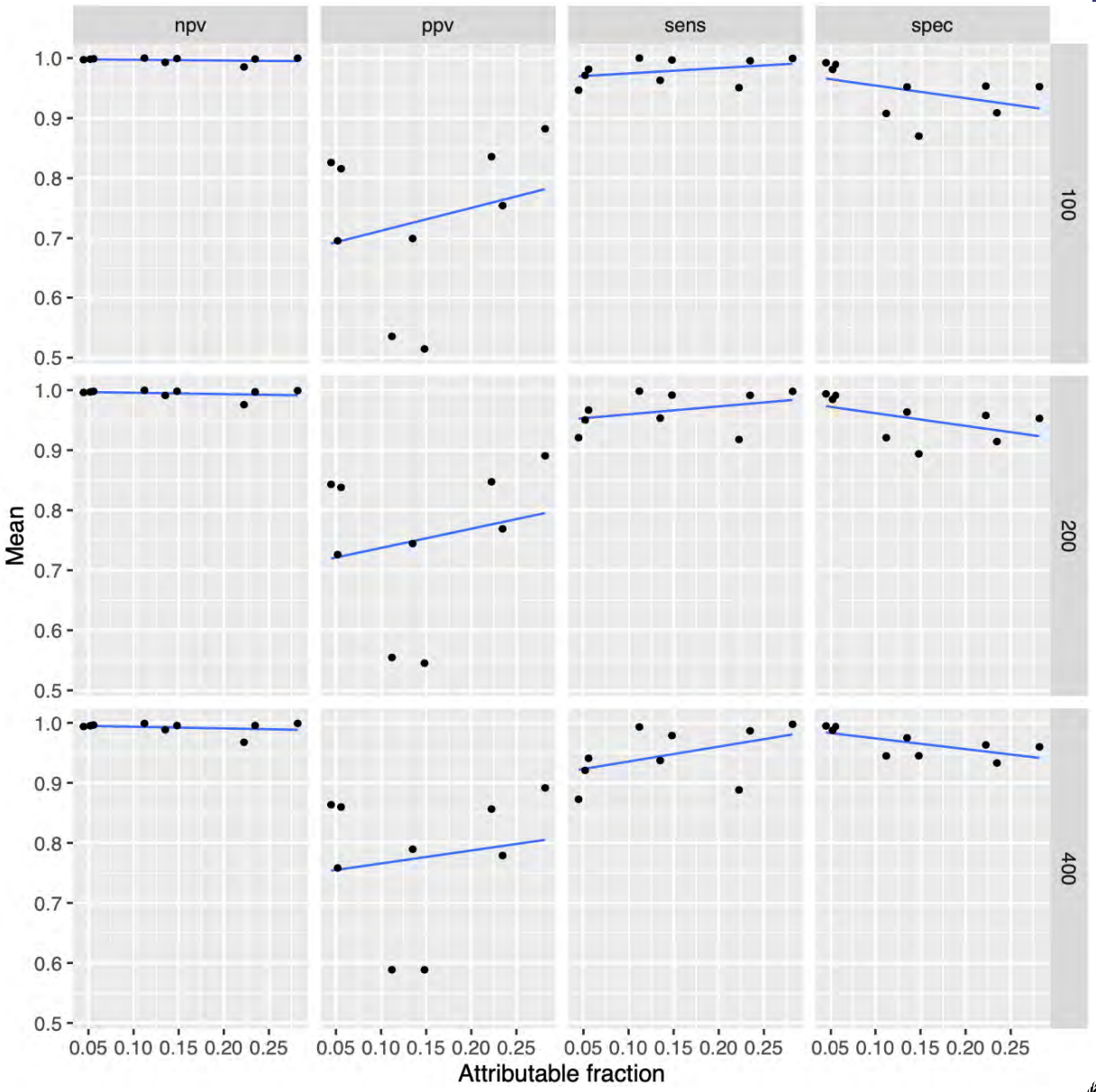
Mali (98.7)



Summary of diagnosis performance according to 100 and 200p/uL cut-offs/LOD

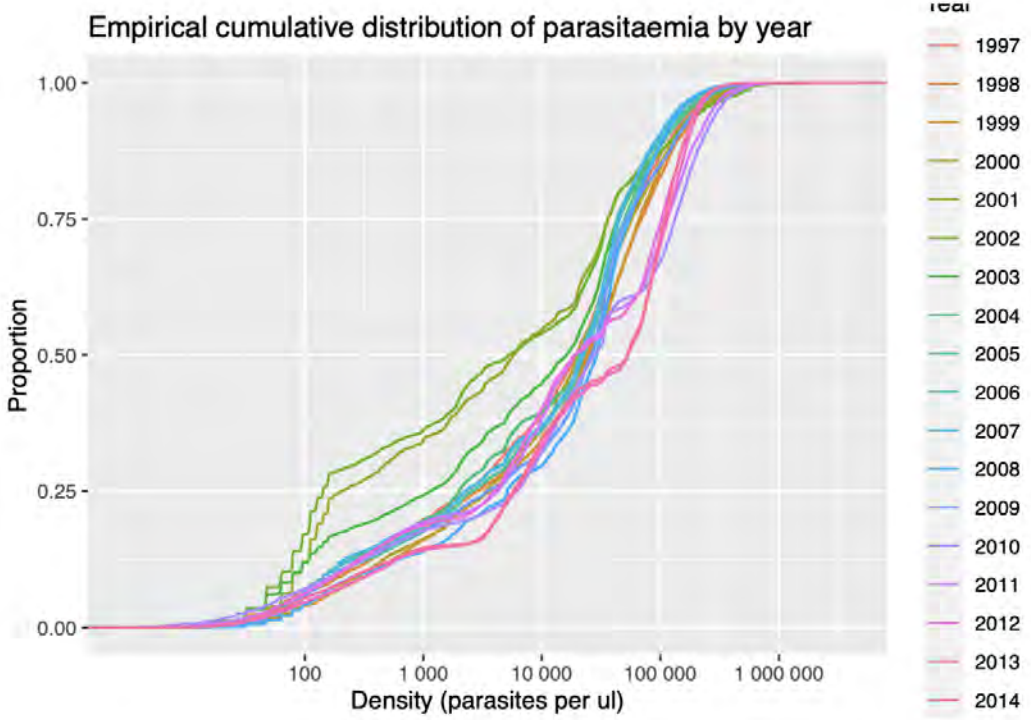
report	At 100 parasites / ul				At 200 parasites /ul			
	sens	spec	ppv	npv	sens	spec	ppv	npv
burkina	99.9	95.2	88.2	100.0	99.8	95.3	89.1	99.9
gambia	96.3	95.2	69.9	99.3	95.3	96.4	74.4	99.1
kenia5	99.7	87.0	51.4	99.9	99.2	89.4	54.5	99.8
kenia6	100.0	90.8	53.5	100.0	99.8	92.1	55.5	100.0
magude_MO	94.7	99.2	82.6	99.7	92.1	99.4	84.3	99.6
mali	95.1	95.3	83.6	98.5	91.8	95.8	84.7	97.6
manhica_MO	97.1	98.1	69.6	99.8	95.0	98.5	72.6	99.7
manhica2_MO	98.1	98.9	81.6	99.9	96.7	99.1	83.8	99.8
uganda	99.6	90.9	75.4	99.8	99.1	91.4	76.9	99.7

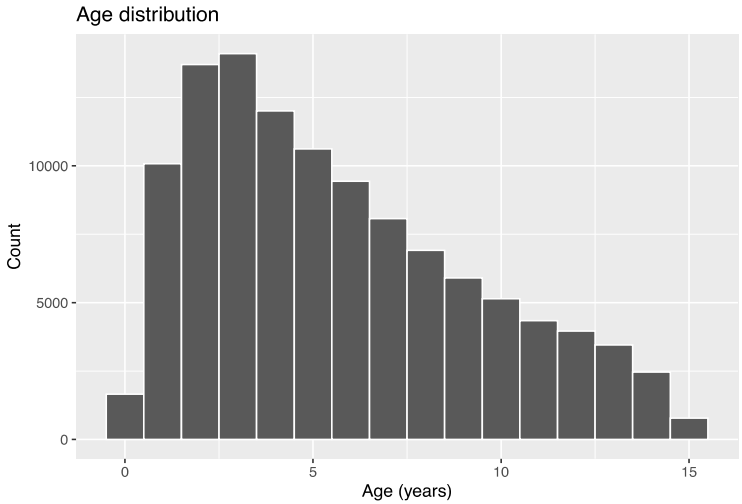
Performance as function of the AF and cutoff

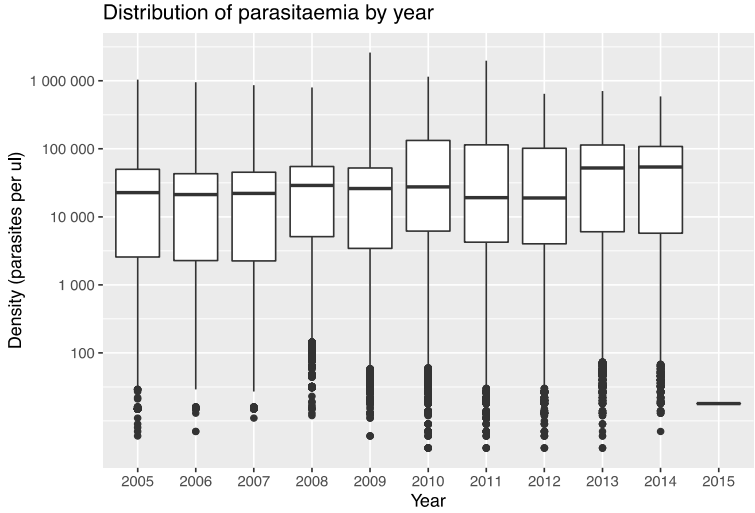


Outpatient data

Manhiça 2005-2014







Distribution of positives (Manhica)

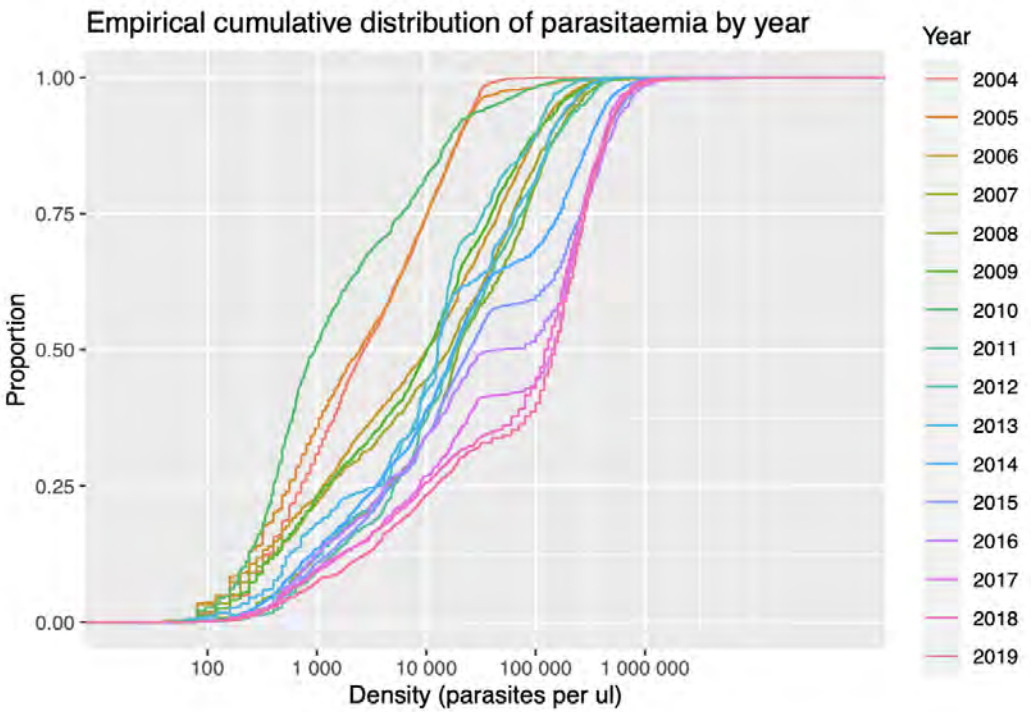
Table 1: Parasitaemia density distribution

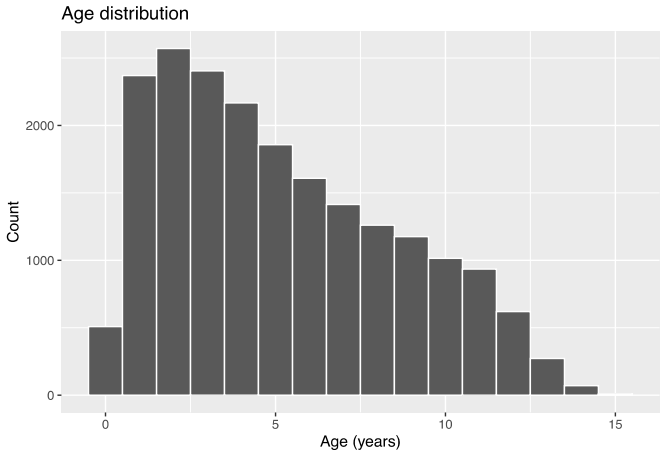
Density	2005	2006	2007	2008	2009
1>	895 (5.9)	900 (6.3)	615 (6.5)	446 (4.2)	533 (5.8)
100>	685 (4.5)	737 (5.1)	509 (5.4)	433 (4)	430 (4.7)
200>	391 (2.6)	384 (2.7)	279 (3)	277 (2.6)	271 (3)
400>	509 (3.4)	476 (3.3)	297 (3.2)	273 (2.5)	314 (3.4)
800>	599 (4)	547 (3.8)	312 (3.3)	306 (2.9)	280 (3.1)
1600>	903 (6)	872 (6.1)	551 (5.9)	538 (5)	401 (4.4)
3200>	980 (6.5)	943 (6.6)	656 (7)	679 (6.3)	451 (4.9)
6400>	985 (6.5)	862 (6)	556 (5.9)	590 (5.5)	565 (6.2)
12300>	2027 (13.4)	2135 (14.9)	1262 (13.4)	1494 (13.9)	1294 (14.1)
25600>	3414 (22.6)	3432 (24)	2336 (24.9)	2826 (26.3)	2302 (25.1)
51200>	2025 (13.4)	1628 (11.4)	1181 (12.6)	1693 (15.8)	962 (10.5)
102400>	1200 (8)	1055 (7.4)	638 (6.8)	945 (8.8)	914 (10)
204800>	461 (3.1)	358 (2.5)	205 (2.2)	235 (2.2)	443 (4.8)

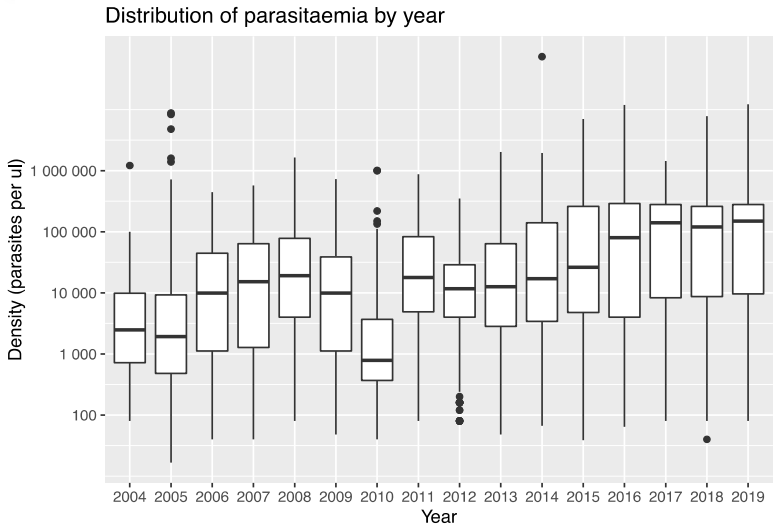
Density	2010	2011	2012	2013	2014
1>	449 (7.2)	827 (6.4)	975 (6.3)	655 (4.6)	282 (5.4)
100>	183 (2.9)	464 (3.6)	560 (3.6)	354 (2.5)	136 (2.6)
200>	218 (3.5)	568 (4.4)	688 (4.5)	443 (3.1)	161 (3.1)
400>	202 (3.2)	529 (4.1)	557 (3.6)	466 (3.3)	149 (2.9)
800>	126 (2)	271 (2.1)	256 (1.7)	170 (1.2)	61 (1.2)
1600>	120 (1.9)	287 (2.2)	465 (3)	288 (2)	63 (1.2)
3200>	286 (4.6)	1001 (7.7)	1437 (9.3)	1265 (9)	530 (10.2)
6400>	685 (11)	1759 (13.6)	1997 (13)	1640 (11.6)	588 (11.3)
12300>	773 (12.4)	1153 (8.9)	1129 (7.3)	973 (6.9)	376 (7.2)
25600>	727 (11.7)	806 (6.2)	865 (5.6)	698 (4.9)	227 (4.4)
51200>	443 (7.1)	1641 (12.7)	2635 (17.1)	3088 (21.8)	1235 (23.7)
102400>	1232 (19.8)	2320 (17.9)	3138 (20.4)	3282 (23.2)	1177 (22.6)
204800>	787 (12.6)	1332 (10.3)	689 (4.5)	812 (5.7)	228 (4.4)

Density	Total
1>	13649 (7.6)
100>	9575 (5.3)
200>	5803 (3.2)
400>	6326 (3.5)
800>	5615 (3.1)
1600>	9017 (5)
3200>	12645 (7)
6400>	14192 (7.9)
12300>	20204 (11.2)
25600>	29790 (16.6)
51200>	23105 (12.9)
102400>	21158 (11.8)
204800>	8685 (4.8)

Kilifi (Pingilikani) 2004-2020







Distribution of positives (Kilifi)

Table 1: Parasitaemia density distribution

Density	2004	2005	2006	2007	2008	2009
1>	37 (1.6)	59 (3.6)	57 (3.2)	25 (1.9)	4 (0.3)	19 (1.2)
100>	74 (3.2)	86 (5.3)	101 (5.8)	70 (5.4)	18 (1.2)	65 (4.1)
200>	252 (10.9)	184 (11.3)	102 (5.8)	69 (5.3)	57 (3.8)	112 (7)
400>	268 (11.6)	203 (12.4)	120 (6.8)	98 (7.6)	74 (5)	127 (8)
800>	304 (13.1)	191 (11.7)	135 (7.7)	86 (6.7)	93 (6.2)	121 (7.6)
1600>	288 (12.5)	156 (9.6)	137 (7.8)	67 (5.2)	80 (5.4)	103 (6.5)
3200>	287 (12.4)	194 (11.9)	128 (7.3)	93 (7.2)	98 (6.6)	119 (7.5)
6400>	331 (14.3)	231 (14.2)	151 (8.6)	102 (7.9)	140 (9.4)	197 (12.4)
12300>	328 (14.2)	218 (13.4)	212 (12.1)	146 (11.3)	257 (17.3)	224 (14.1)
25600>	135 (5.8)	69 (4.2)	215 (12.2)	151 (11.7)	150 (10.1)	184 (11.6)
51200>	8 (0.3)	14 (0.9)	210 (12)	183 (14.2)	232 (15.6)	158 (9.9)
102400>	NA	18 (1.1)	135 (7.7)	129 (10)	188 (12.6)	103 (6.5)
204800>	1 (0)	9 (0.6)	53 (3)	71 (5.5)	98 (6.6)	57 (3.6)

Density	2010	2011	2012	2013	2014
1>	57 (2.7)	2 (0.1)	6 (0.5)	20 (1)	5 (0.2)
100>	145 (6.9)	10 (0.5)	15 (1.1)	46 (2.3)	37 (1.3)
200>	332 (15.9)	32 (1.6)	39 (2.9)	85 (4.3)	103 (3.7)
400>	450 (21.5)	101 (5.2)	80 (6)	173 (8.7)	185 (6.6)
800>	262 (12.5)	118 (6.1)	83 (6.2)	111 (5.6)	163 (5.8)
1600>	190 (9.1)	92 (4.7)	62 (4.7)	53 (2.7)	181 (6.5)
3200>	151 (7.2)	182 (9.4)	163 (12.2)	174 (8.8)	202 (7.2)
6400>	189 (9)	265 (13.6)	247 (18.5)	359 (18.1)	332 (11.9)
12300>	171 (8.2)	286 (14.7)	256 (19.2)	215 (10.9)	397 (14.2)
25600>	53 (2.5)	244 (12.6)	144 (10.8)	168 (8.5)	216 (7.7)
51200>	57 (2.7)	255 (13.1)	100 (7.5)	203 (10.3)	106 (3.8)
102400>	25 (1.2)	214 (11)	118 (8.9)	270 (13.7)	302 (10.8)
204800>	11 (0.5)	142 (7.3)	19 (1.4)	101 (5.1)	571 (20.4)

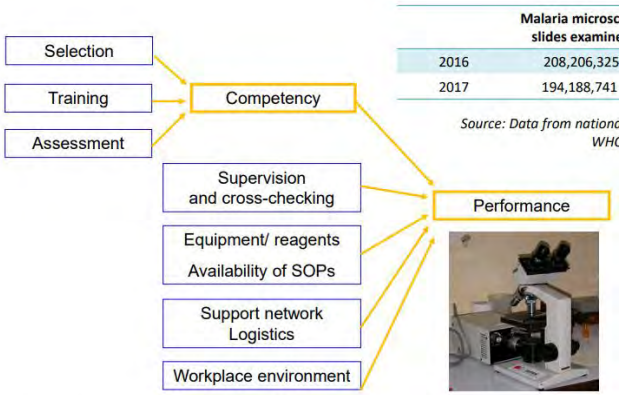
Density	2015	2016	2017	2018	2019
1>	8 (0.3)	2 (0.1)	2 (0.2)	5 (0.2)	1 (0.1)
100>	19 (0.7)	19 (1.1)	15 (1.3)	24 (0.9)	8 (0.8)
200>	67 (2.3)	42 (2.5)	24 (2.1)	58 (2.2)	22 (2.1)
400>	159 (5.5)	115 (6.9)	50 (4.3)	123 (4.6)	35 (3.3)
800>	167 (5.8)	90 (5.4)	53 (4.6)	109 (4.1)	33 (3.1)
1600>	164 (5.7)	96 (5.7)	48 (4.1)	114 (4.3)	43 (4)
3200>	209 (7.3)	110 (6.6)	64 (5.5)	130 (4.9)	65 (6.1)
6400>	314 (10.9)	141 (8.4)	80 (6.9)	173 (6.5)	66 (6.2)
12300>	343 (11.9)	176 (10.5)	111 (9.6)	122 (4.6)	62 (5.8)
25600>	222 (7.7)	50 (3)	38 (3.3)	99 (3.7)	30 (2.8)
51200>	51 (1.8)	42 (2.5)	32 (2.8)	237 (8.9)	63 (5.9)
102400>	296 (10.3)	229 (13.6)	229 (19.7)	574 (21.6)	234 (22)
204800>	855 (29.7)	566 (33.7)	415 (35.7)	884 (33.3)	403 (37.8)

Density	Total
1>	309 (1)
100>	752 (2.5)
200>	1580 (5.3)
400>	2361 (8)
800>	2119 (7.1)
1600>	1874 (6.3)
3200>	2369 (8)
6400>	3318 (11.2)
12300>	3524 (11.9)
25600>	2168 (7.3)
51200>	1951 (6.6)
102400>	3064 (10.3)
204800>	4256 (14.4)

Note:
Number of samples within the category (Column Percentage)

Circling back to current requirements for diagnostic tests

- Microscopy



WHO competence levels and criteria

Competence Level	Parasite detection (%)	Species identification (%)	Parasite count within 25% of true count (%)
1	90-100	90-100	50-100
2	80-89	80-89	40-49
3	70-79	70-79	30-39
4	0-69	0-69	0-29

Challenge for microscopists – parasite detection: 80-200p/µL – Level 3 – 70-79% grade on these samples

- RDTs
  - Antigen concentrations found in samples with 200p/µL – Panel detection score > 75%

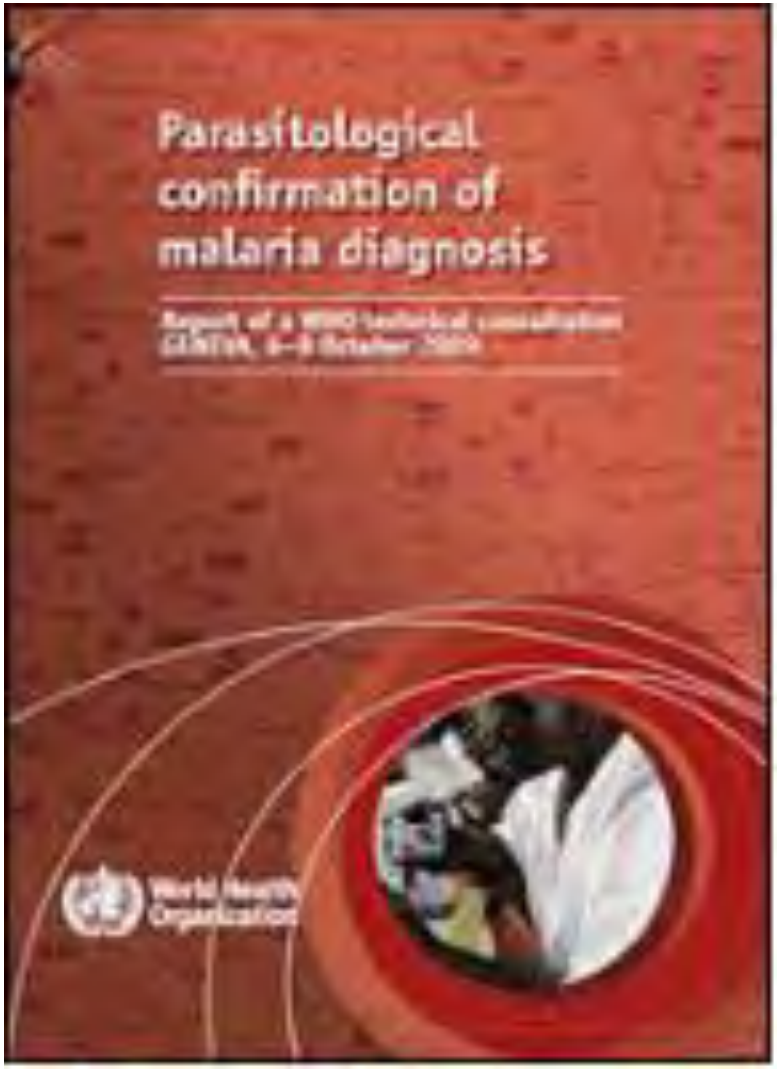
- PCR
  - 2 p/µL

With these requirements/ limits of detection and based on datasets presented – test should detect the majority of clinical Pf malaria cases in Africa.

Conclusions

- Quality datasets from range of transmission setting in Africa and over different time periods
- Based on the model using cut-offs of 100 or 200p/µL
  - Does not significantly affect ability to detect clinical malaria ; improvements in sensitivity are coupled with reductions in specificity and poorer PPV; NPV very high
  - Implications for burden of disease estimates - may overestimate true burden with more sensitive tests
- More sensitive tests/lower cut-offs may well detect more malaria infections but not malaria 'disease'
- Diagnosis of clinical malaria should always include assessment for other non-malaria causes of fever – PPV not good when using cut-offs < 400p/µL

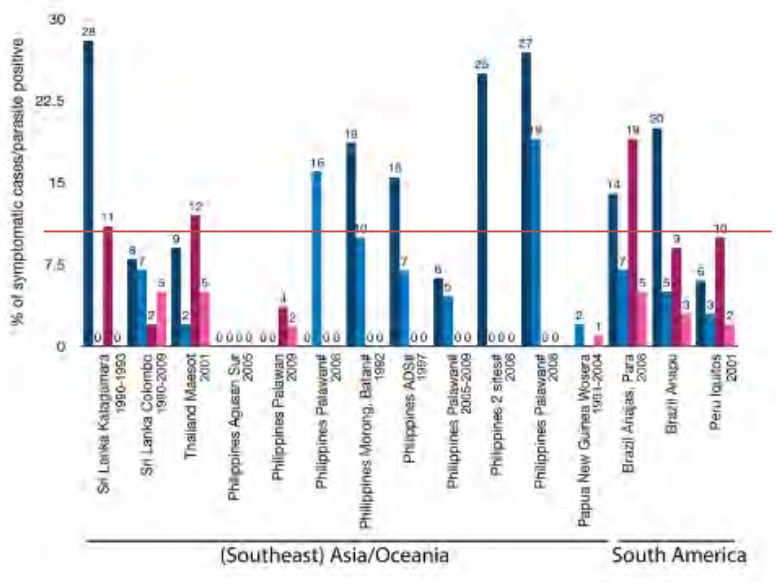
Background



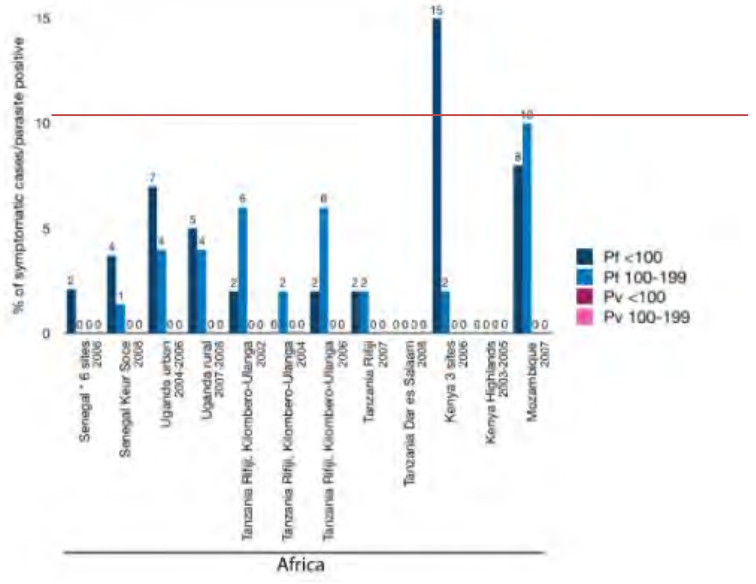
- In 2009 WHO set minimum specifications for RDTs - consistently detect 200p/µL, < 5% FP rate; based on data from health facilities or symptomatic sub-populations from cross-sectional surveys.

Datasets reviewed in 2009

SEA, Oceania, S America



Africa



Conclusions in 2009: RDTs with LOD ≈ 200 p/µL will capture the majority of patients with clinical malaria/disease in Africa but may miss some clinically relevant malaria infections in SEA, PNG and South America – both Pf and Pv

Background

- Over past 7 years ++ interest in low density infections and role of more sensitive diagnostics – ? relevance for case management, surveillance, screening, transmission, elimination..
- WHO consultations – 2013, 2017
  - upheld use of microscopy and RDTs for clinical case management

- World Malaria Day – April 2017- Alere released its high/ultra sensitive RDTs - LOD ≈10p/ µL; 10 fold greater LOD; 2021 – price drop to coRDT

- 2020 – strengthen past efforts to answer whether or not we are missing clinical malaria cases ?

https://www.who.int/malaria/mpac/mpac_mar2014_diagnosis_low_transmission_settings_report.pdf
https://www.who.int/malaria/mpac/mpac-oct2017-erg-malaria-low-density-infections-session2.pdf?ua=1
https://apps.who.int/iris/bitstream/handle/10665/44323/9789241599412_eng.pdf;jsessionid=5CED33AD244825D7D6B29D5A689BF7D0?sequence=1

Questions for MPAG

- Do you agree yield of more sensitive tests for detection of clinical malaria would be negligible or do we need more sensitive POC Pf tests for case management in Africa ?

- What are the weaknesses of this approach/model?

- How best to respond to country demands for selection of high sensitive tests over 'conventional' RDTs for case management of *P.falciparum* ?
    - Yes, they detect more cases of malaria infection but …….

- Is this same analysis needed outside Africa for *P.falciparum* and *P.vivax*?
    - more sensitive pLDH tests are on the horizon – will we recommend them over conventional RDTs ?

Acknowledgements

- **WHO/GMP**
  - Abdisalan Noor
  - Beatriz Galatas Andrade
  - Pedro Alonso
- Data analysis and modelling
  - Matthew Cairns – LSHTM
  - Tom Smith
  - Orvalho Augusto

Institutes who provided data sets:

- London School Hygiene and Tropical Diseases (LSHTM)
- Kenya Medical Research Institute
- Medical Research Council, Gambia
- CISM - Manhiça Health Research Centre
- ICMER

Summary of the situation of antimalarial drug efficacy and artemisinin resistance in Africa

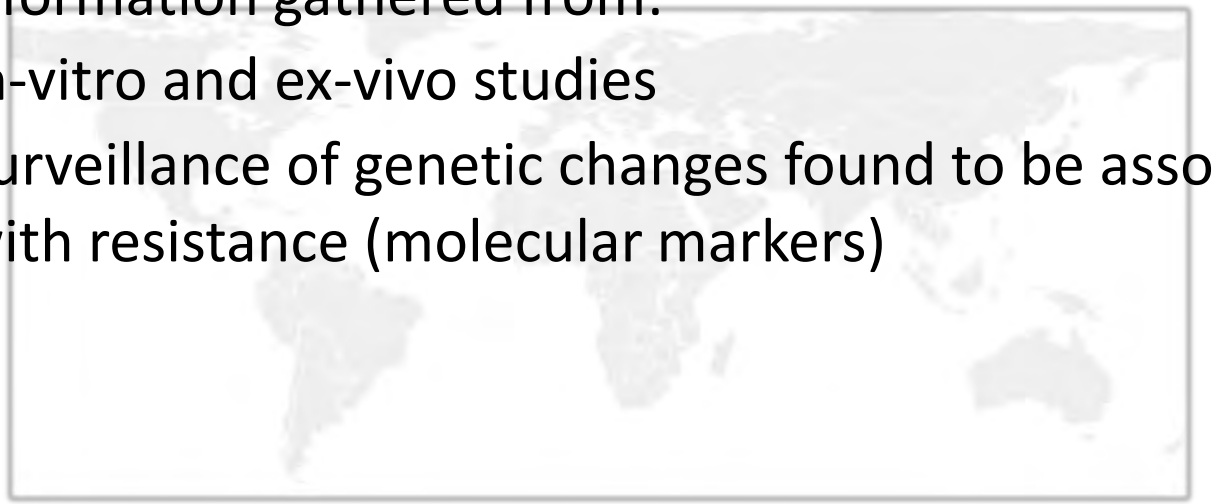
P. Ringwald
C. Rasmussen
A. Barrette

Global Malaria Programme

World Health Organization

Introduction: Antimalarial drug efficacy and resistance

- To respond to malaria drug resistance, we need systems that:
  1) Can detect changes in how well the recommended treatment is working
  2) That can implement changes in policy when needed
- Therapeutic efficacy studies (TES) are the gold standard for monitoring drug efficacy to inform treatment policy
- In countries implementing malaria elimination activities, efficacy can be monitored by integrated Drug Efficacy Surveillance (iDES)
- Additional information gathered from:
  - In-vitro and ex-vivo studies
  - Surveillance of genetic changes found to be associated with resistance (molecular markers)



Molecular markers of drug resistance

- Once the genetic changes associated with resistance are identified, drug resistance can be confirmed and monitored with molecular techniques.

Chemical family	Drug	Molecular Marker
4-aminoquinolines	Chloroquine	*Pfcrt* mutations *Pfmdr1* mutations (in combination with *Pfcrt* mutations only)
	Amodiaquine	Yet to be validated
	Piperaquine	*Pfpm2-3* increased copy number *Pfcrt* mutations
Antifolates	Pyrimethamine	*Pfdhfr* mutations
	Sulfadoxine	*Pfdhps* mutations
	Proguanil	*Pfdhfr* mutations
Amino-alcohols	Lumefantrine	Yet to be validated
	Mefloquine	*Pfmdr1 increased copy number*
	Quinine	*Yet to be validated*
Mannich base	Pyronaridine	*Yet to be validated*
Sesquiterpene lactones	Artemisinin and its derivatives	*PfK13 mutations*

Artemisinin-based combinations therapy (ACTs)

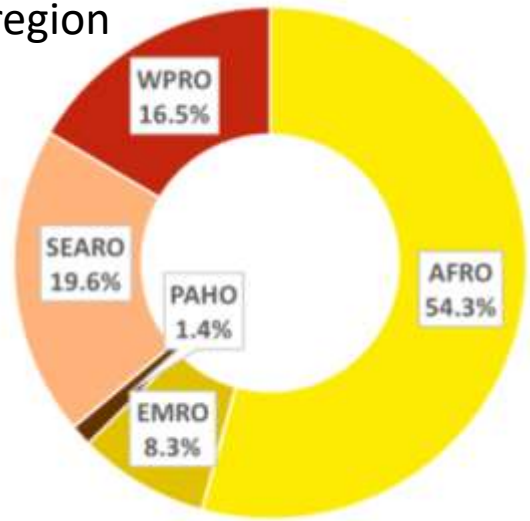
- Resistance in *P. falciparum* has posed the greatest challenge
- ACTs recommended treatment of uncomplicated *P. falciparum* malaria:
  - Artemether-lumefantrine (AL)
  - Artesunate-amodiaquine (AS-AQ)
  - Artesunate-mefloquine (AS-MQ)
  - Artesunate-SP (AS-SP)
  - Dihydroartemisinin-piperaquine (DHA-PPQ)
  - Artesunate-pyronaridine (AS-PY)

- In most of the world, these antimalarial drugs are highly efficacious
- However, *P. falciparum* resistance in the Greater Mekong Sub-region does pose a challenge.

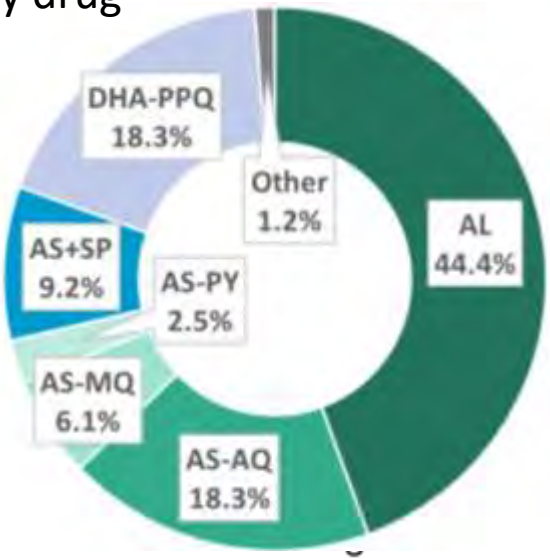
TES *P. falciparum* studies 2010-2020

TES studies for *P. falciparum*: 1103

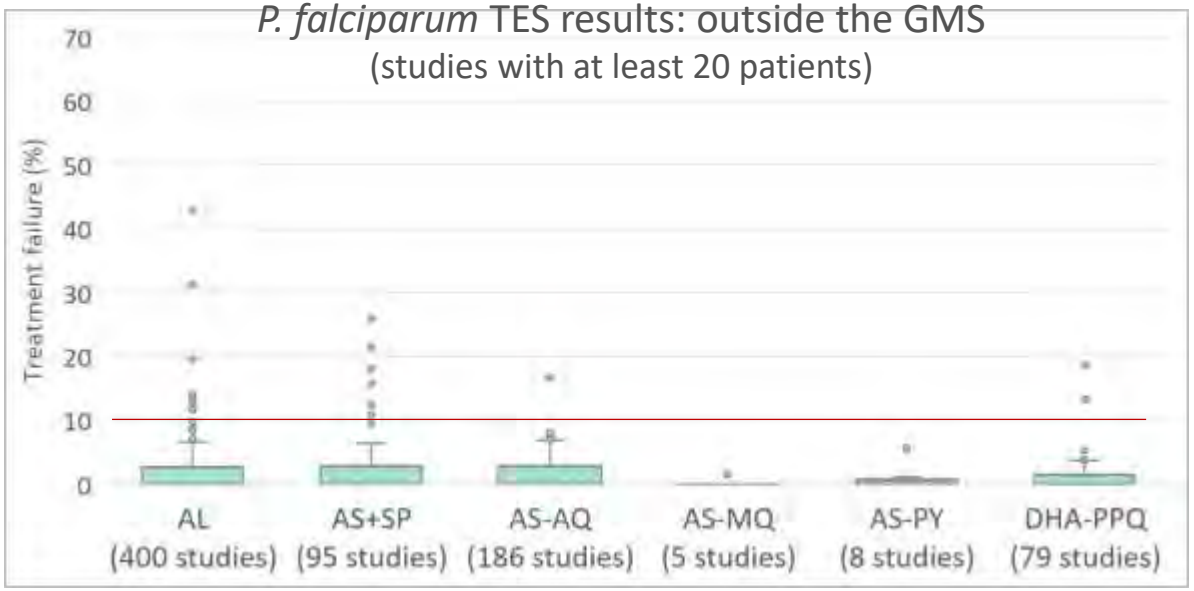
By region



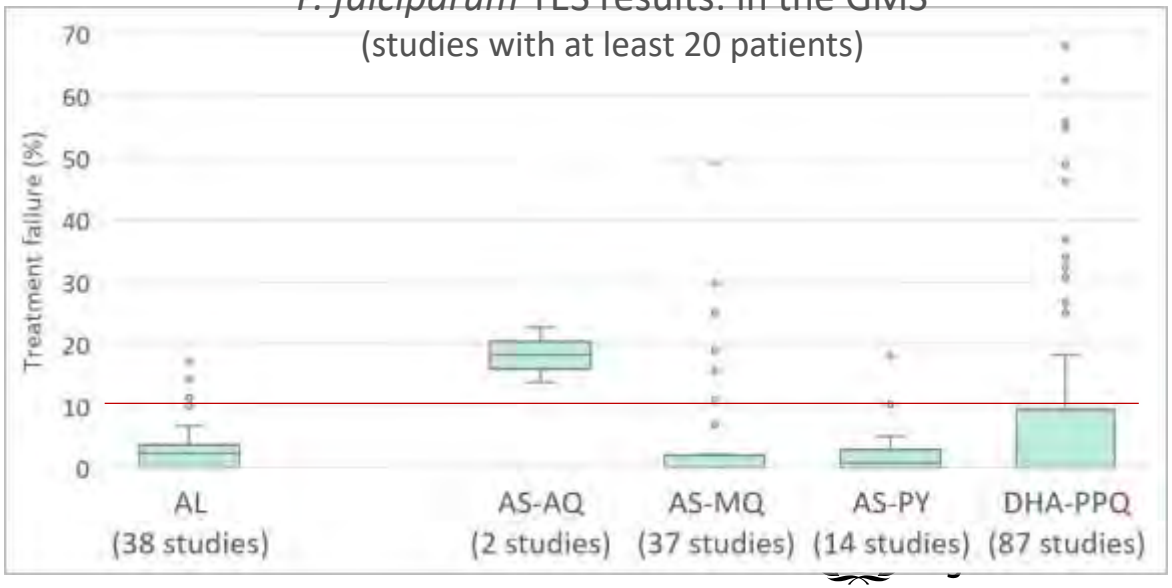
By drug



P. falciparum TES results: outside the GMS
(studies with at least 20 patients)



P. falciparum TES results: in the GMS
(studies with at least 20 patients)



Criteria for classification of K13 markers of resistance

Candidate or associated K13 markers of artemisinin partial resistance

- A statistically significant association ($p<0.05$) between a K13 mutation and clearance half-life >5 hours or day 3 parasitaemia via a chi-squared test or appropriate multivariable regression model on a sample of at least 20 clinical cases.

or

- Survival of >1% using the $RSA_{0–3h}$ in at least five individual isolates with a given mutation or a statistically significant difference ($p<0.05$) in the $RSA_{0–3h}$ assay between culture-adapted recombinant isogenic parasite lines, produced using transfection and gene editing techniques, which express a variant allele of K13 as compared with the wild-type allele.

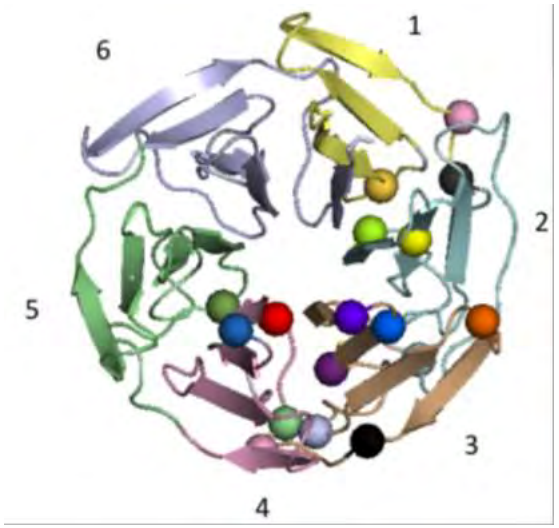
Validated K13 markers of artemisinin partial resistance

- Both requirements 1 and 2 are met.

K13: marker of artemisinin (partial) resistance

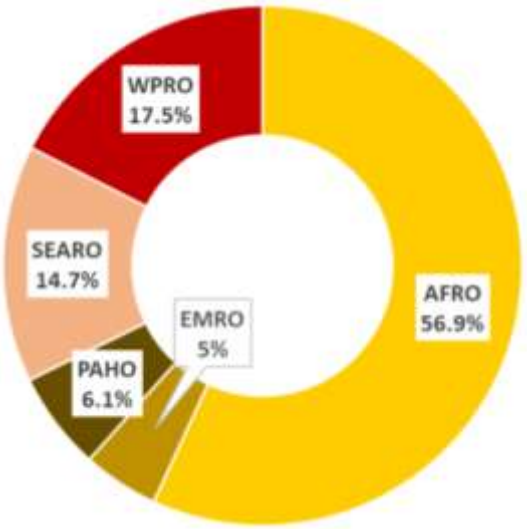
- Found to be associated with delayed parasite clearance;
- In TES, seen as increases in the patients with day-3 parasitemia;
- Found to be associated with several *PfKelch13* (K13) mutations:

Candidates/associated		Validated	
P441L	N537I/D	**F446I**	**I543T**
G449A	G538V	**N458Y**	**P553L**
C469F/Y	V568G	**M476I**	**R561H**
A481V	R622I	**Y493H**	**P574L**
R515K	A675V	**R539T**	**C580Y**
P527H			

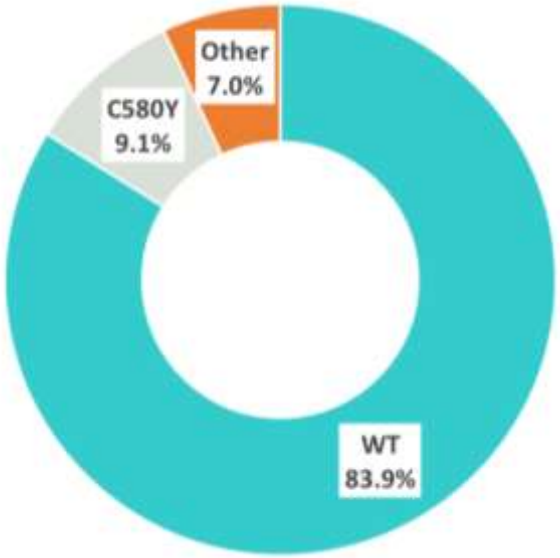


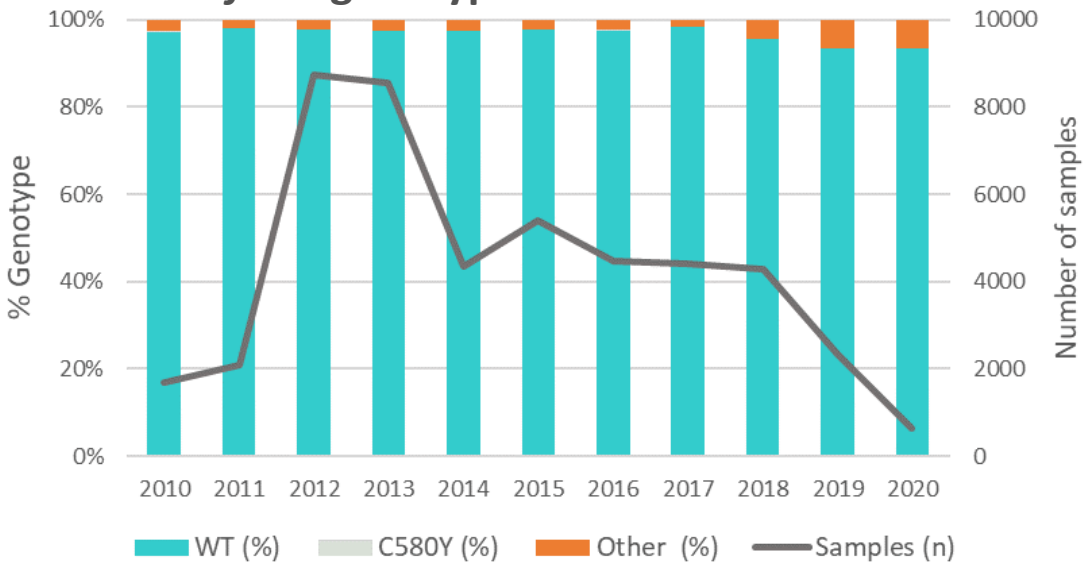
K13 genotyped samples (2010-2020)

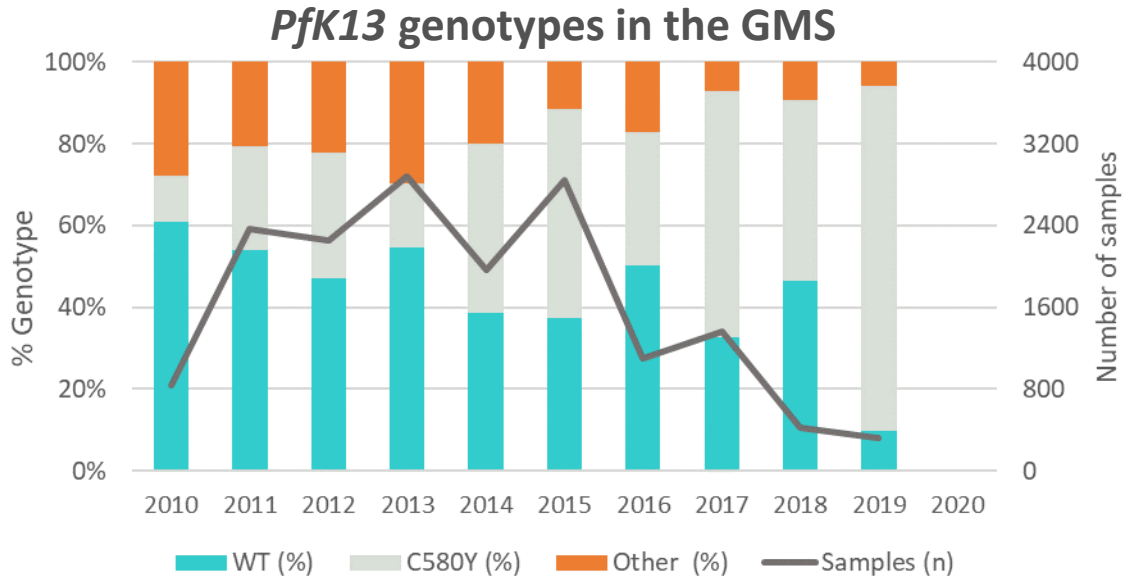
By region (n = 63,298)



By genotype





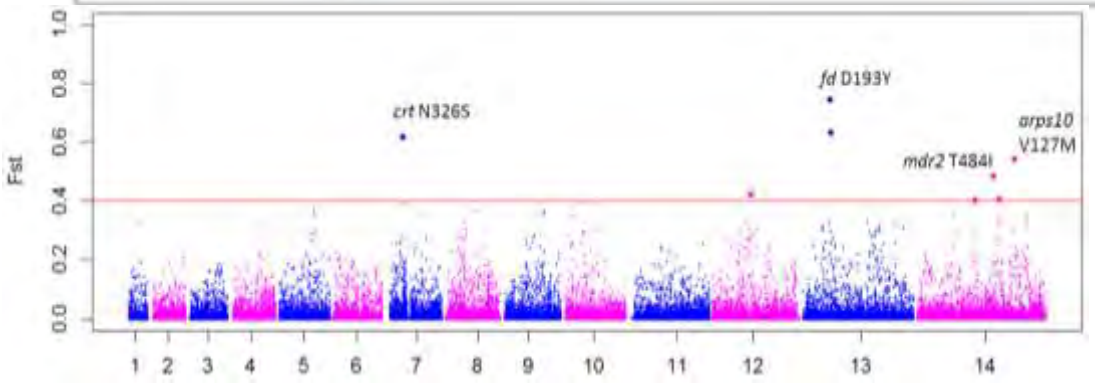


K13 validated molecular markers outside the GMS

- Validated molecular markers of artemisinin (partial) resistance have been detected outside the GMS.
- Of special concern is C580Y and detection of high numbers of R561H

★ C580Y detected

★ High frequency of R561H



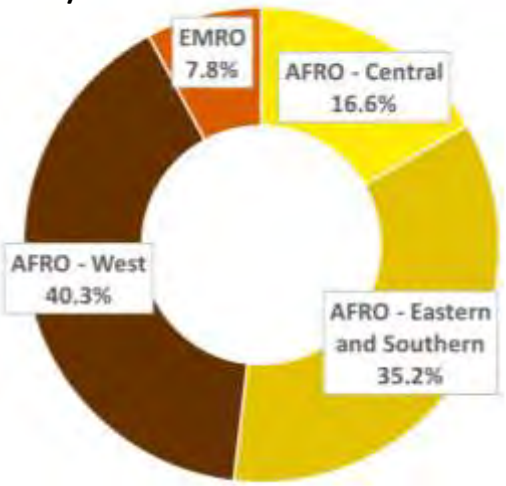
Possible “permissive” or compensatory background mutations

Miotto *et al.*, *Nature Genetics.* 2015

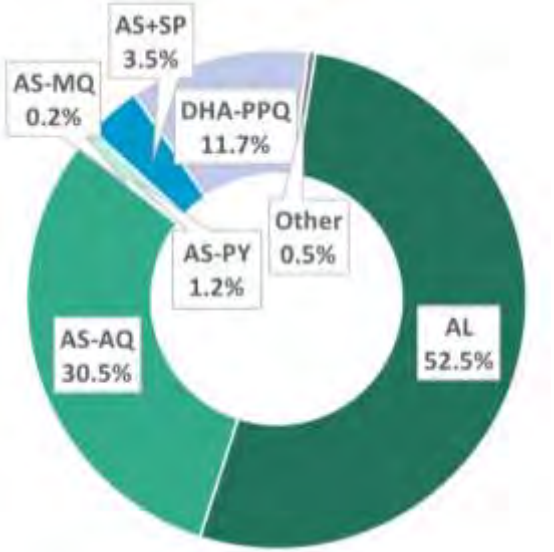
TES *P. falciparum* studies in Africa 2010-2020

TES studies for *P. falciparum*: 650

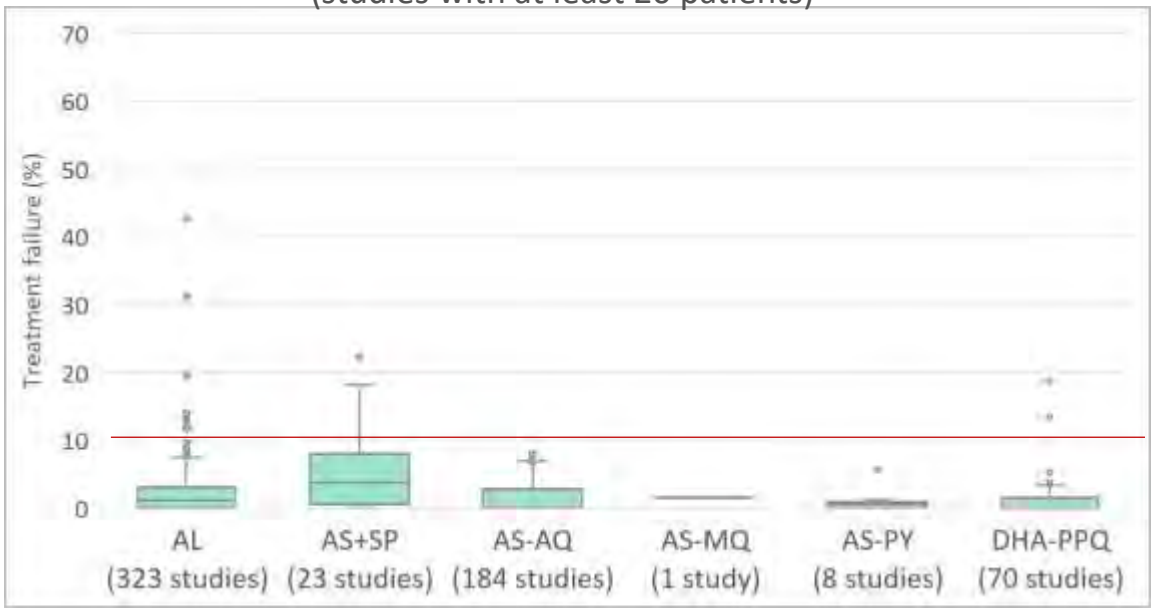
By region /IST



By drug



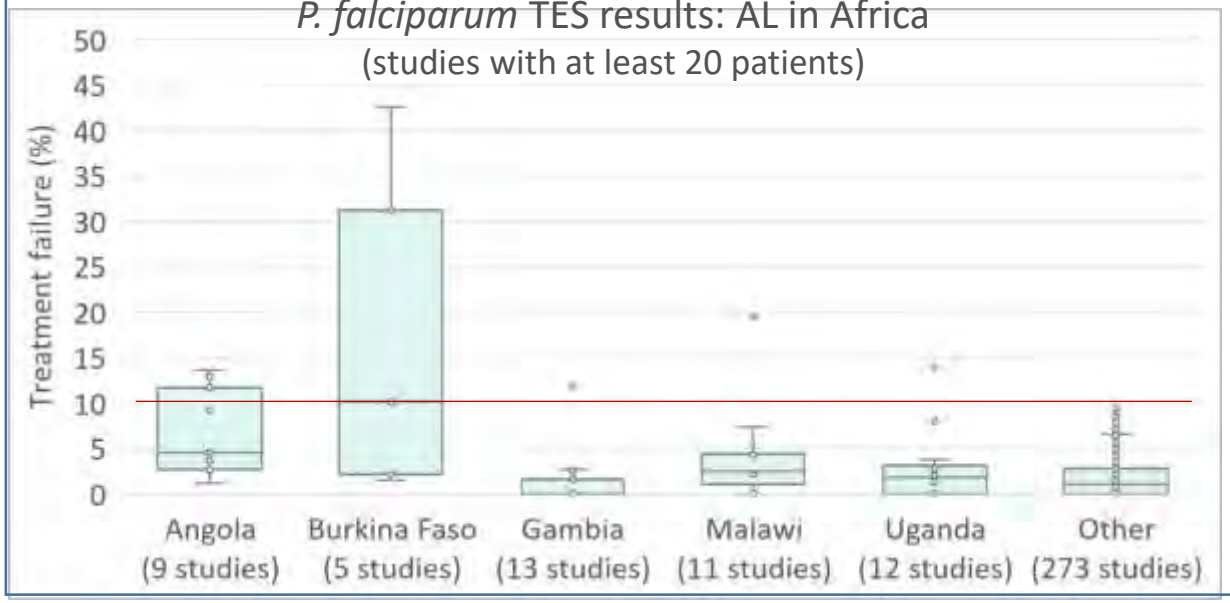
P. falciparum TES results: In Africa
(studies with at least 20 patients)



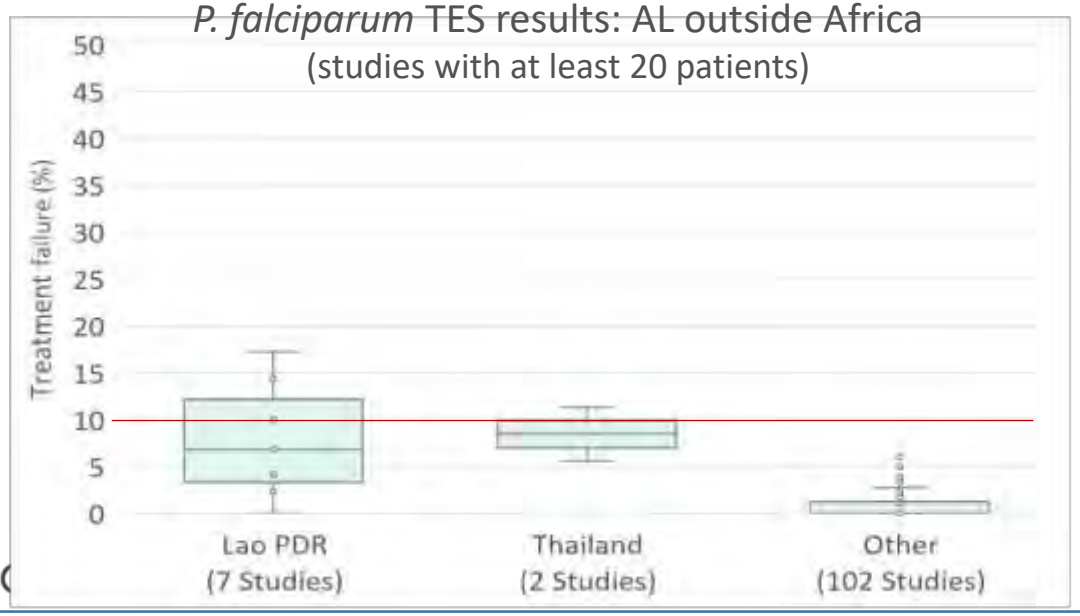
Source: WHO database

World Health Organization

Artemether-Lumefantrine studies 2010 - 2020



- 9 of 323 AL studies done in Africa found >10% failure rate
- For the 5 older studies, later studies in the same area have shown <10% failure rate.
- For 4 recent studies in Angola (1 study) and Burkina Faso (3 studies) confirmation of results are needed.



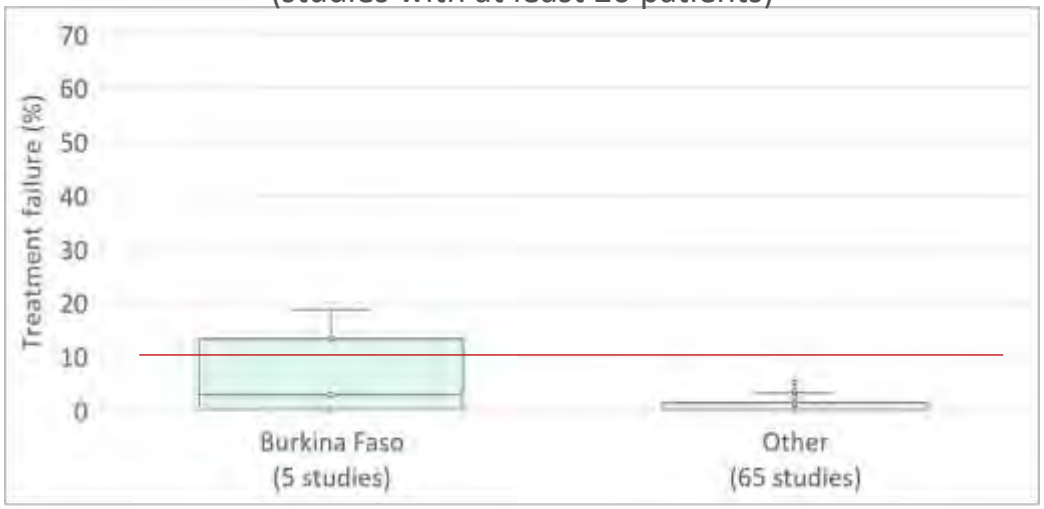
- 3 of 111 AL studies done outside Africa found >10% failure rate
- Studies ongoing in Lao PDR have not found <10% failure rate

Source: WHO database

World Health Organization

Dihydroartemisinin-Piperaquine studies 2010 - 2020

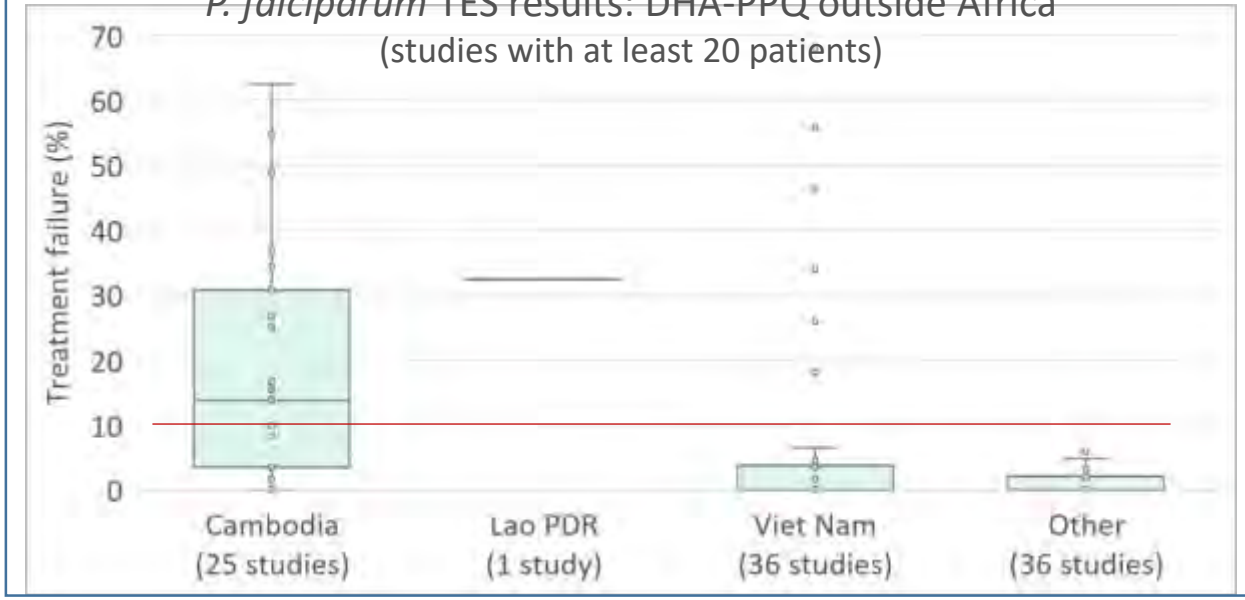
P. falciparum TES results: DHA-PPQ in Africa
(studies with at least 20 patients)



- 2 of 70 DHA-PPQ studies done in Africa found >10% failure rate
- Both studies are recent studies in Burkina Faso (same studies that recently reported high AL failure rates)

Source: WHO database

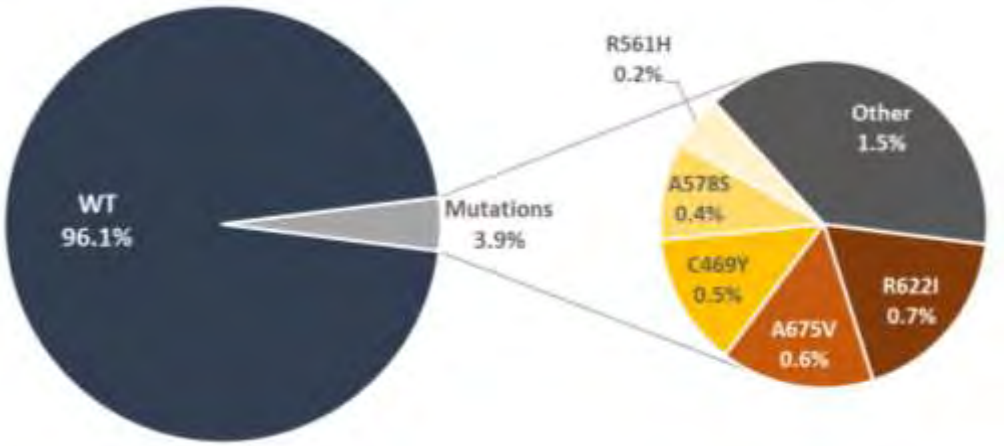
P. falciparum TES results: DHA-PPQ outside Africa
(studies with at least 20 patients)

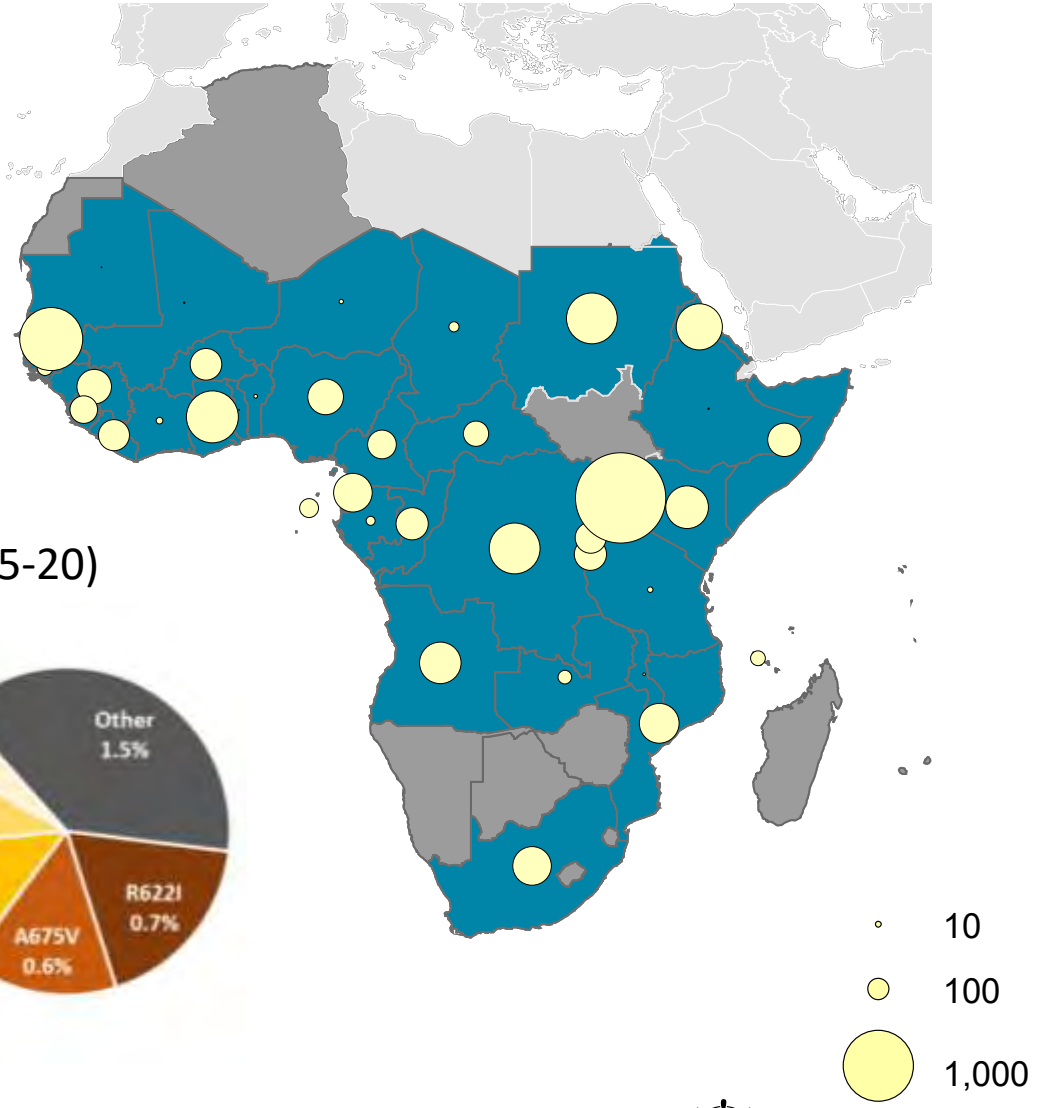


- TES have found high failure rates caused by piperaquine resistance in Cambodia, Lao PDR and Viet Nam
- iDES with fever than 20 patients have also found high failure rates in eastern Thailand

World Health Organization

K13 genotype in Africa countries (2015-2020)



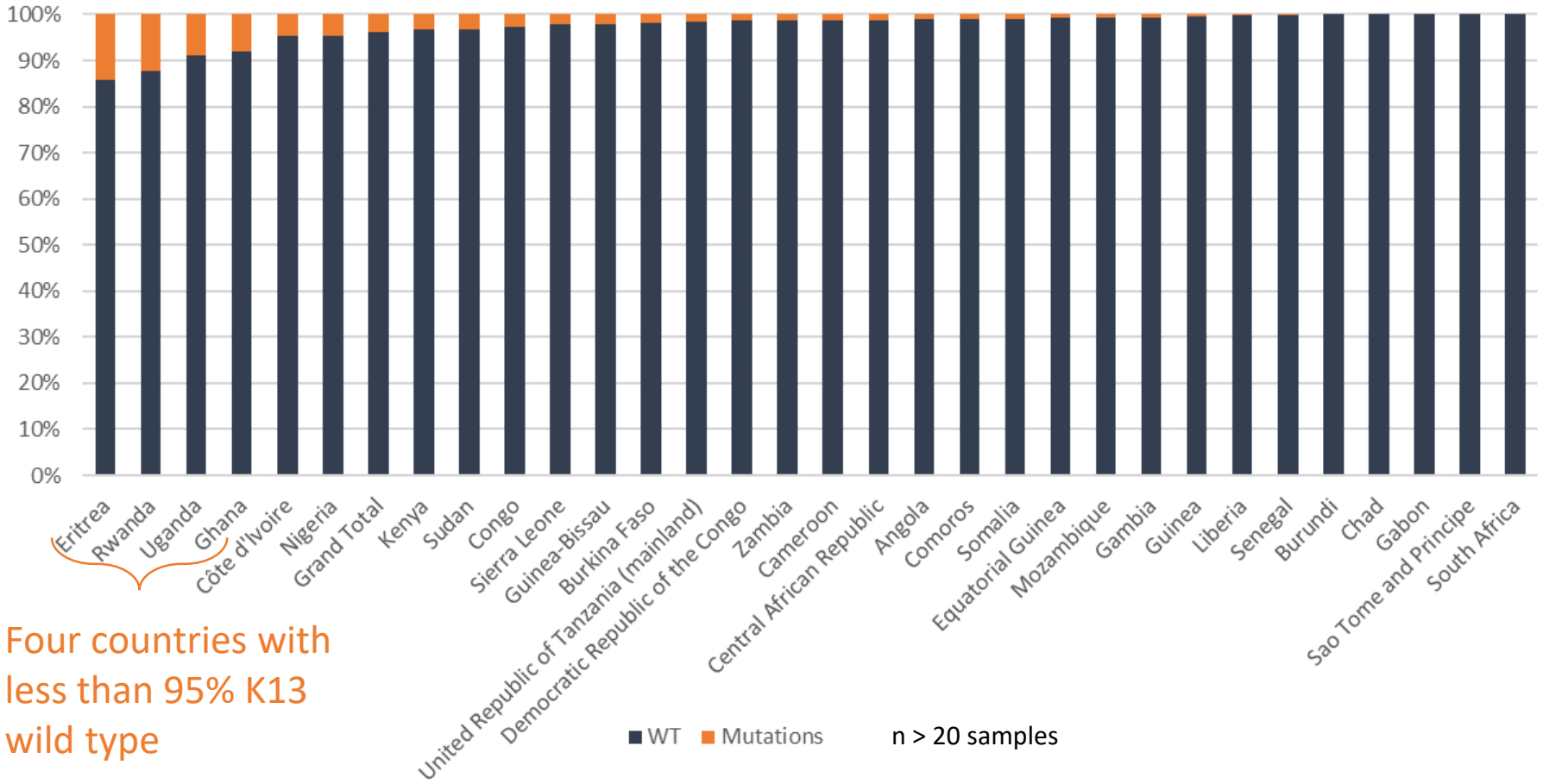


Global **Malaria** Programme

Source: WHO database

World Health Organization

K13 genotype in Africa countries (2015-2020)



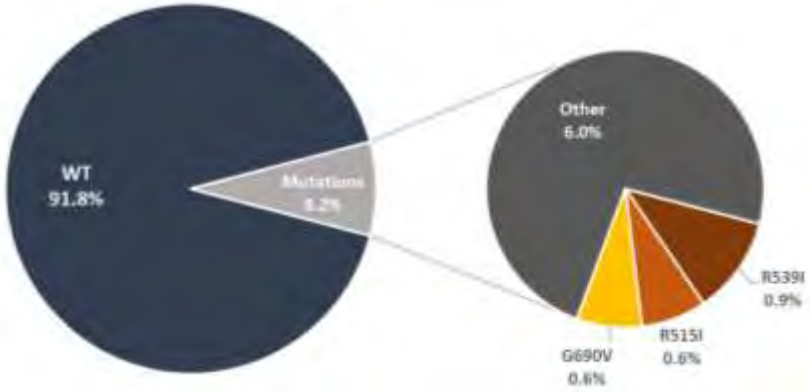
Four countries with less than 95% K13 wild type

Source: WHO database

K13 genotype in 4 Africa countries (2015-2020)

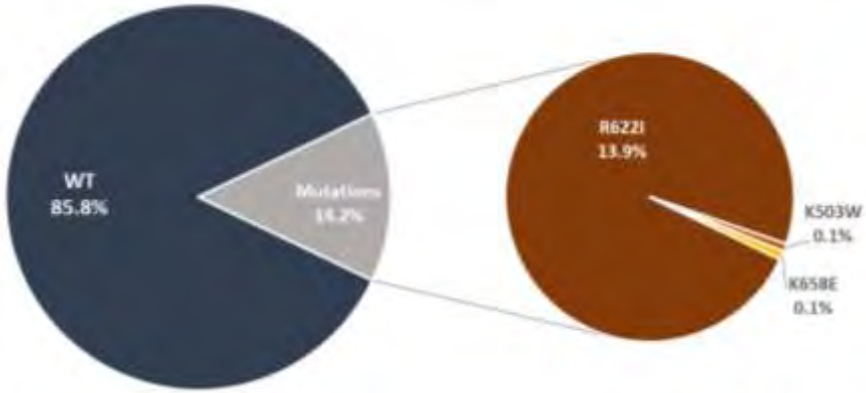
Ghana (n=968)

36 different K13 mutations detected



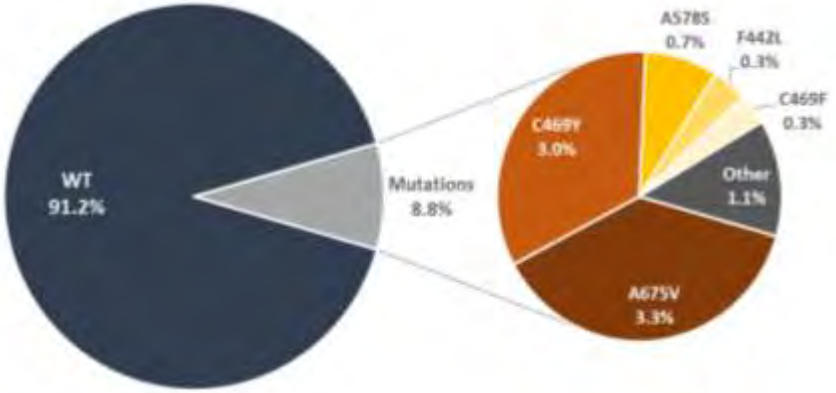
Eritrea (n=769)

3 different K13 mutations detected



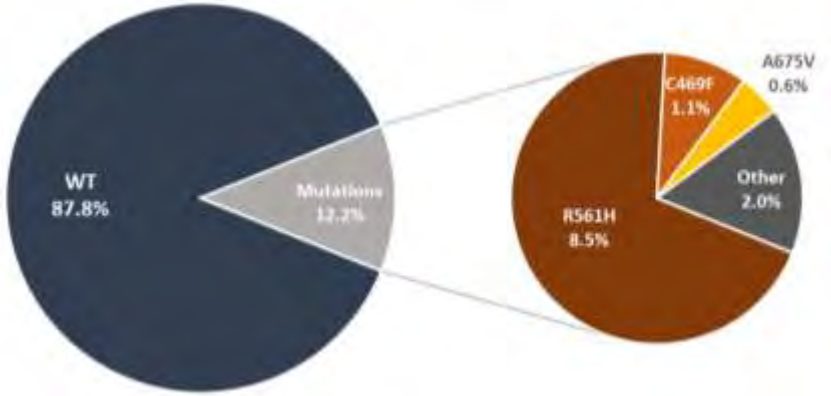
Uganda (n=2872)

21 different K13 mutations detected



Rwanda (n=352)

10 different K13 mutations detected



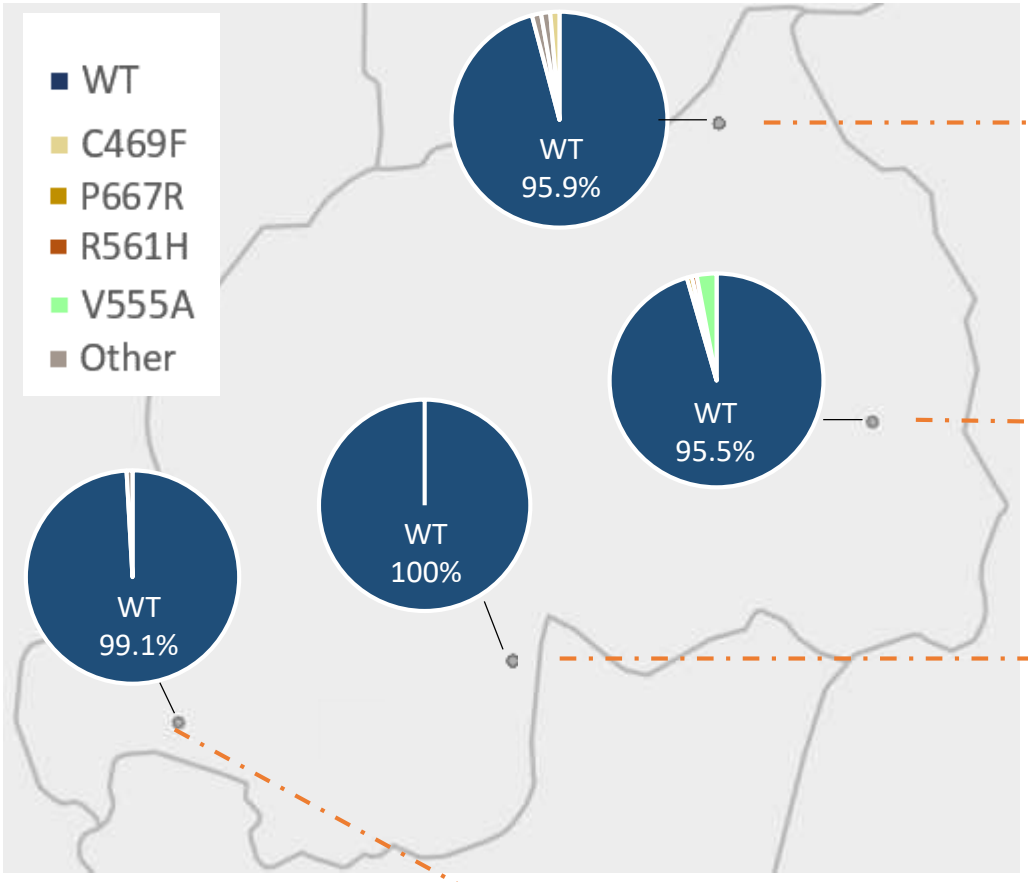
Global **Malaria** Programme

Source: WHO database

World Health Organization

TES and K13 prevalence in Rwanda (1)

Studies 2012-2015



Nyarurema, Province de l'Est	n	D3	TF
Artemether-lumefantrine	63	1.3	3.2

Rukara, Province de l'Est	n	D3	TF
Artemether-lumefantrine	120	0	5.8

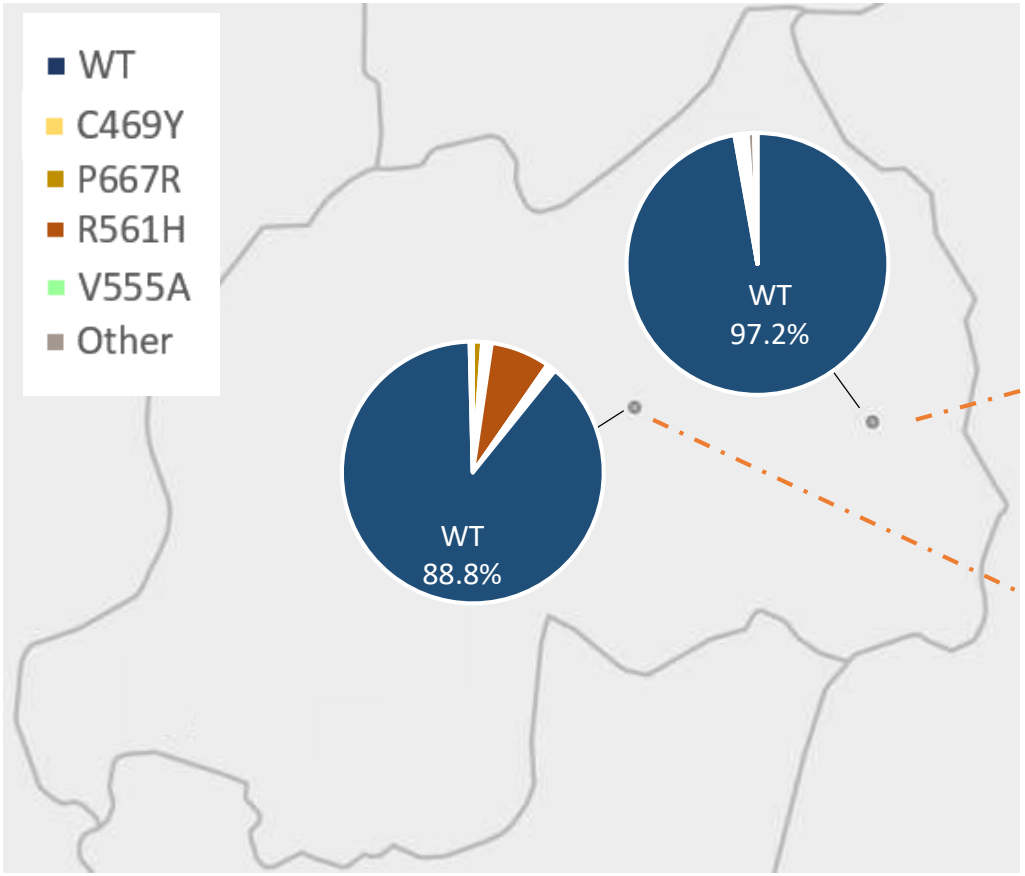
Kibirizi, Province du Sud	n	D3	TF
Artemether-lumefantrine	88	0	2.3

Muganza (Bugarama), Province de l'Ouest	n	D3	TF
Artemether-lumefantrine	101	0.9	3.9

Uwimana A et al. *Nat Med.* 2020

TES and K13 prevalence in Rwanda (2)

Studies 2013-2015



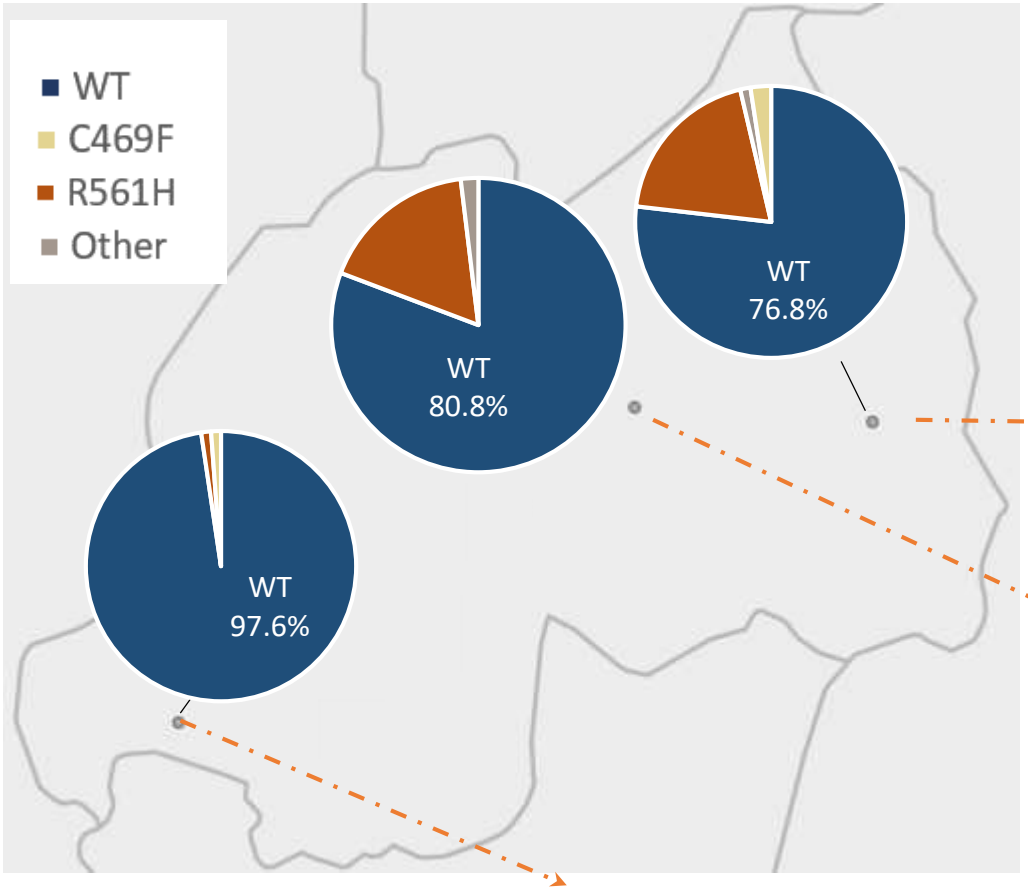
Rukara, Province de l'Est	n	D3	TF
Artemether-lumefantrine	124	0.7	0.8
Dihydroartemisinin-piperaquine	129	0.8	0.8

Masaka, Province de Kigali	n	D3	TF
Artemether-lumefantrine	111	0.8	2.7
Dihydroartemisinin-piperaquine	121	0.7	3.3

Uwimana A et al. *Nat Med.* 2020

TES and K13 prevalence in Rwanda (3)

Studies 2018



Emergence of K13 R561H appears to have an effect on clearance rate but efficacy remains high

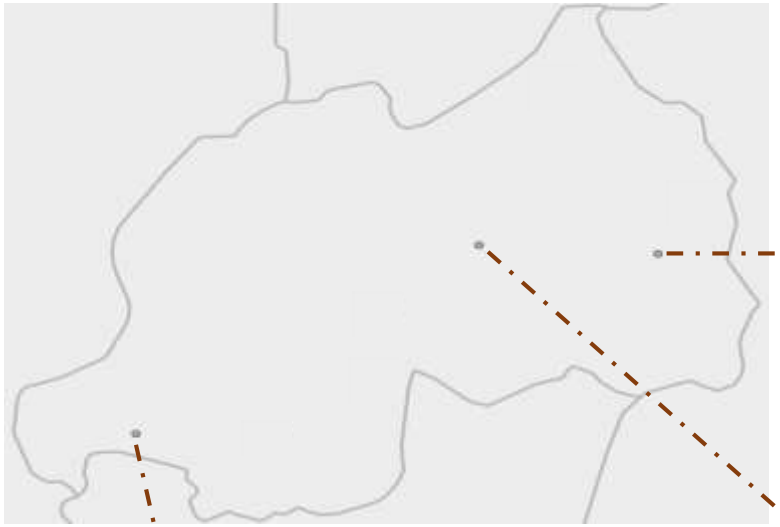
Rukara, Province de l'Est	n	D3	TF
Artemether-lumefantrine	69	13.6	5.8

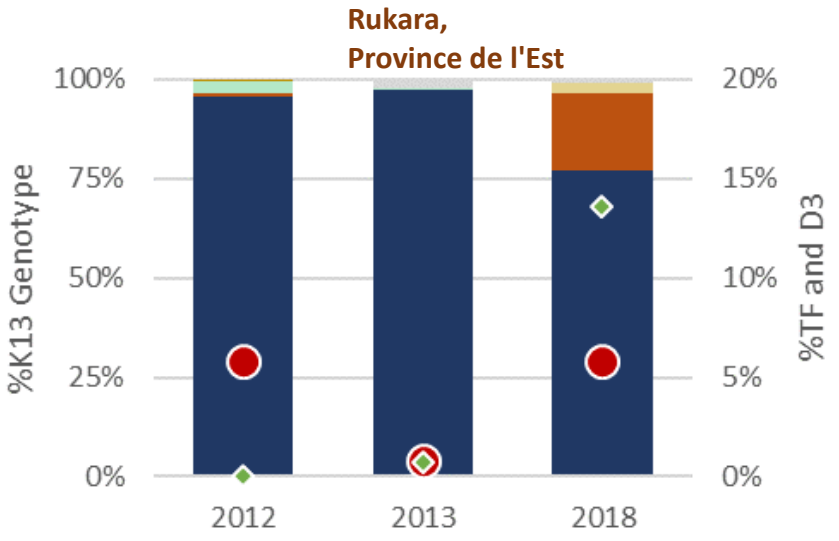
Masaka, Province de Kigali	n	D3	TF
Artemether-lumefantrine	50	15.4	4.0

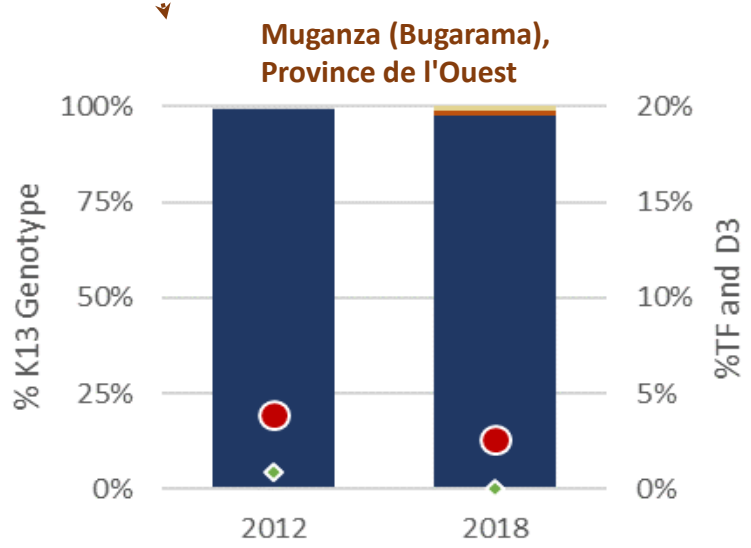
Source: CDC Atlanta

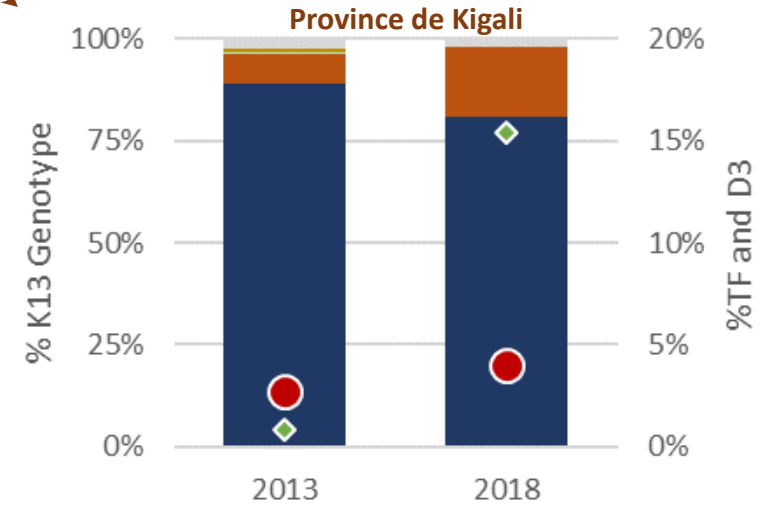
Muganza (Bugarama), Province de l'Ouest	n	D3	TF
Artemether-lumefantrine	76	0	2.6

Trend of AL efficacy and K13 prevalence in Rwanda









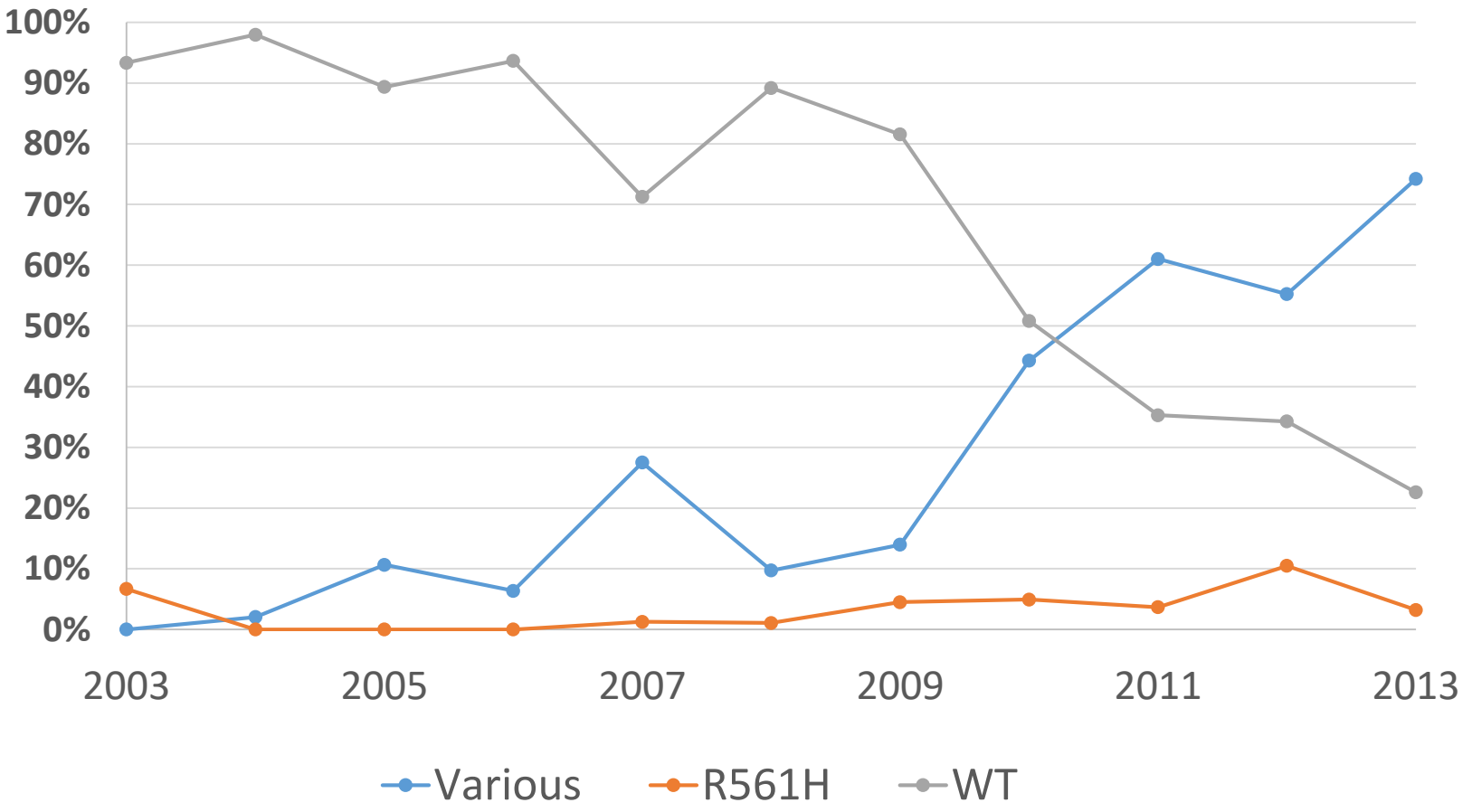
WT R561H V555A C469F P667R Other D3 TF

Relation between partner drug efficacy and K13 mutations (2)

Year	Site	ACT	N	Efficacy 28/42 days (%)	K13 mutant (%)
2016	Kampong Speu, Kratie	Artesunate-mefloquine	69	100	95.6% (C580Y)
2017	Kampong Speu, Pursat, Stungtreng	Artesunate-mefloquine	170	99.5	78.2% (C580Y, R539T, Y493H)
2017	Ratanakiri, Mondulkiri	Artesunate-pyronaridine	123	97.6	72.4 (C580Y)
2017	Kachin, N. Shan	Artemether-lumefantrine	71	97.2	43.7 (F446I, R561H)

Source: WHO database from studies and surveys

Trend of R561H in Tak province, Thailand

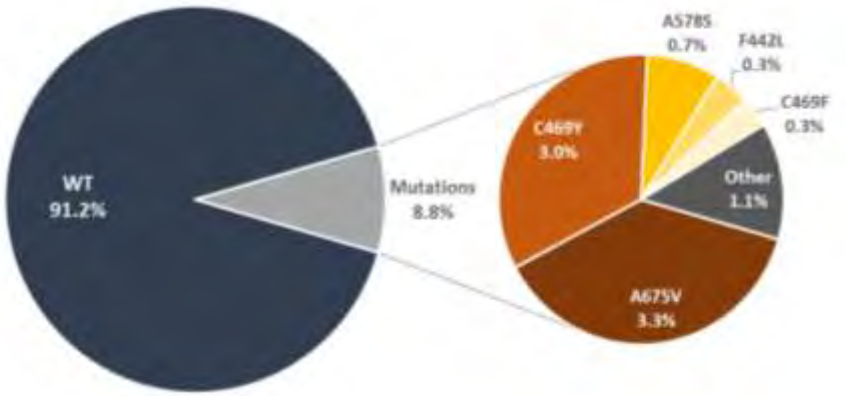


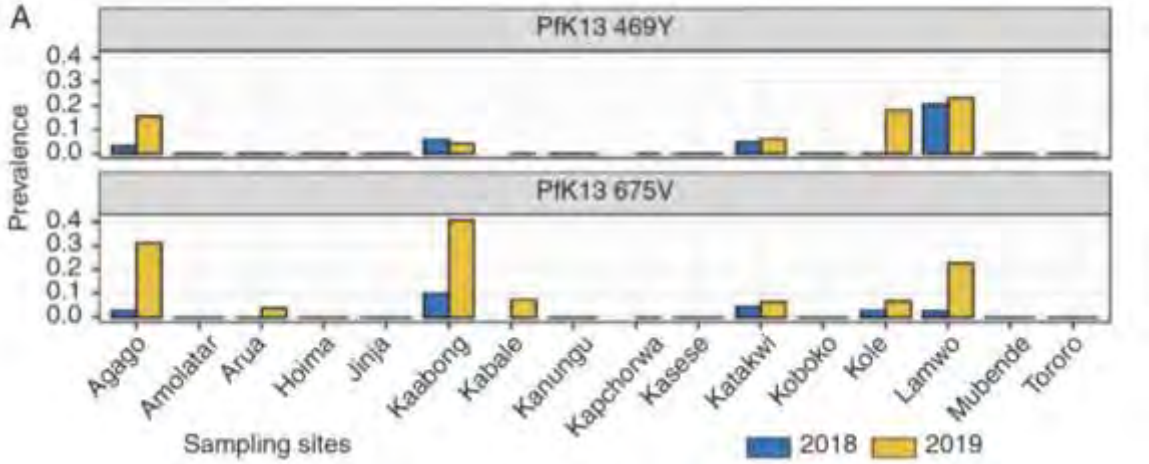
Global Malaria Programme

Source: WHO database from studies and surveys

World Health Organization

K13 genotypes in Uganda 2015-19 (n = 2872)





Prevalence of *Plasmodium falciparum* kelch protein (PfK13) 469Y and 675V mutations

Global **Malaria** Programme

Source: WHO database from studies and surveys
Asua V et al. *J Infect Dis.* 2020

World Health Organization

Activities to minimize risk of emergence and spread of resistance

Preventing

Preventing resistance

- Reduce the incidence of malaria through a combination of interventions including vector control
- Improve access to quality diagnosis and treatment
- Optimize case management
- Lower the risk that a recrudescent case transmits malaria

Monitoring

Monitoring drug efficacy & resistance

- Therapeutic efficacy surveillance to inform treatment policies
- Track resistance through molecular markers

Responding

Responding to drug resistance deemed to be a potential threat to public health

- Accelerate efforts to reduce malaria transmission in areas of resistance
- Monitor patterns of morbidity and mortality
- Minimise the risk of spread of resistance

Delivering

Delivering quality services and targeting of activities

- Accelerate uptake and implementation of policies preventing and mitigating resistance
- Promote access to services for high-risk and hard-to-reach groups
- Strengthen surveillance and monitor quality and coverage of the service delivery

Developing

Developing the tools, knowledge and evidence base

- Accelerate the development of new tools and treatments
- Develop understanding of causes of resistance
- Improve ability to detect and track resistance
- Strengthen knowledge on delivery of services preventing and mitigating resistance

Updating methodologies to distinguish reinfection from recrudescence in high malaria transmission areas

- WHO published guidance in 2008 on genotyping to identify parasite populations for clinical trials;
- The guidance published were reviewed in a 2017 meeting of the Technical Expert Group on Drug Efficacy and Response (TEG DER);
- Recent publications in 2019 and 2020 triggered the need to review the new suggested methodologies through an informal consultation;
- The aim of this consultation is to collate comparative data, and for the experts to provide advice on any changes needed in the currently recommended methodologies as well as provide direction to the development of tools and methods for use in the future.

Objectives of the informal consultation

The objectives of the consultation are to:

- Review data and assess advantages and disadvantages of:
  - Changes in the markers used to differentiate recrudescence from reinfections (msp/glurp vs microsatellites);
  - Changes in the algorithms used to classify recrudescence and reinfections (match-counting vs 2/3 vs Bayesian algorithm);
- Assess in which transmission settings a change in the current methodology could improve the precision of the classification of recurrent *P. falciparum* as recrudescence or reinfection.
- Discuss potential alternative tools for use in the future and suggest research needed to validate these tools.

Conclusions

- There is a critical need for surveillance outside the GMS to detect potential de novo resistance or the potential introduction of resistant parasites;
- Where surveillance signals a potential threat to leading ACTs, effective alternative ACTs should be identified and implemented before resistance reaches critical levels.
- All putative *Pfkelch13* mutants potentially conferring artemisinin resistance be independently verified as being associated with resistance both in gene editing, in vitro and clinical studies, ideally before publication claiming such association.
- Worldwide situation of artemisinin resistance is evolving constantly, and messaging should should take into account these changes.

Antimalarial drug efficacy and drug resistance WHO website

WHO website
https://www.who.int/teams/global-malaria-programme

Malaria threats maps

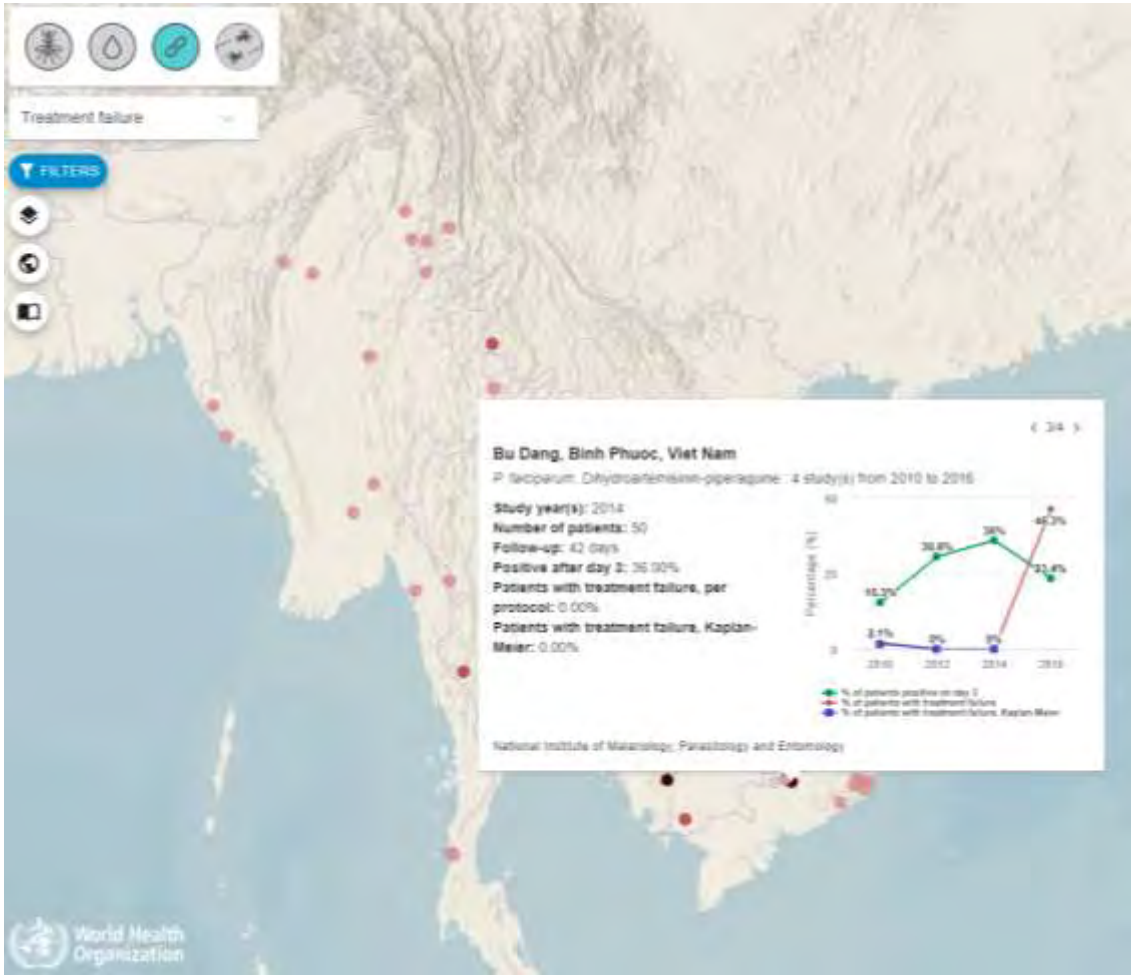
https://www.who.int/news/item/24-10-2020-malaria-threats-map-making-data-available-for-download

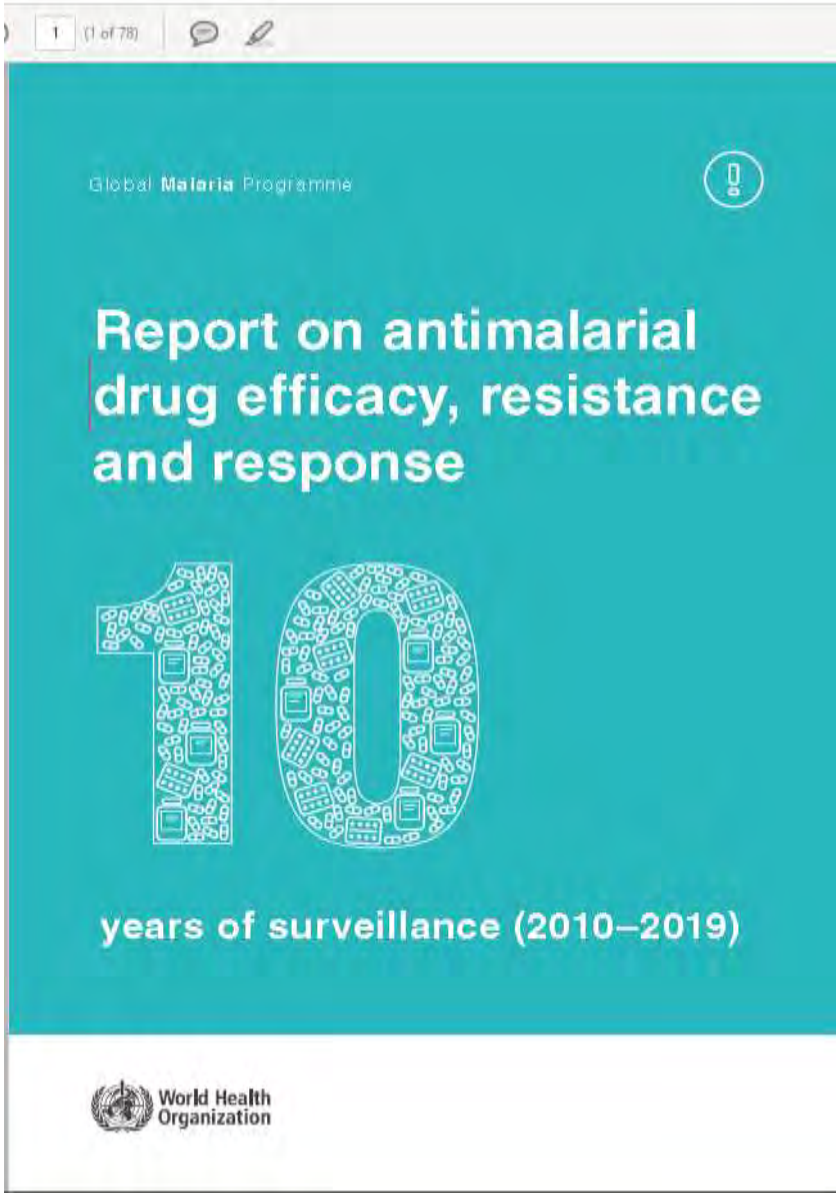
Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010-2019)
https://www.who.int/publications/m/item/WHO-UCN-GMP-2020.07

Threats map

Malaria threat maps

https://www.who.int/news/item/24-10-2020-malaria-threats-map-making-data-available-for-download




1 (1 of 78)
Global Malaria Programme
Report on antimalarial drug efficacy, resistance and response
10
years of surveillance (2010–2019)
World Health Organization

Thank you for your attention

Malaria Policy Advisory Group Meeting

13—15 April 2021, Geneva, Switzerland
Background document for Session 2

World Health Organization

WHO technical consultation to stage *P. knowlesi* along the continuum between animal and human pathogens

Drs K. A. Lindblade, A. Tiffany, Li X-H, P. Ringwald, J. Kolaczinski and A. Noor

Technical consultation proposed for the 4th Quarter 2021, Geneva, Switzerland

Background

Plasmodium knowlesi is a zoonotic malaria parasite species, transmitted between non-human primate hosts by *Anopheles* mosquitoes of the Leucosphyrus group. The parasite frequently spills over into the human population in areas where the parasite, vector, primate host and humans converge. *P. knowlesi* in humans largely affects people who live in or travel to forests and forest fringes where macaques, the main primate reservoir host, and vectors are commonly found. The parasite was first isolated and studied in India in the early 1930s *(1)*, but naturally acquired human cases were thought to be rare until a cluster of human infections was found in Sarawak, Malaysian Borneo in 2004 *(2)*. Since that time, most countries in South-East Asia have reported *P. knowlesi* infections in humans *(3–10)*.

Malaysia, where *P. knowlesi* infections in humans were first reported, has now eliminated all other malaria species that infect humans and has maintained its status for more than three years. However, since 2009, when the country began retesting all samples identified as *P. malariae* using PCR, the country has reported between 300 and 4000 cases of *P. knowlesi* each year (J Jelip, personal communication).

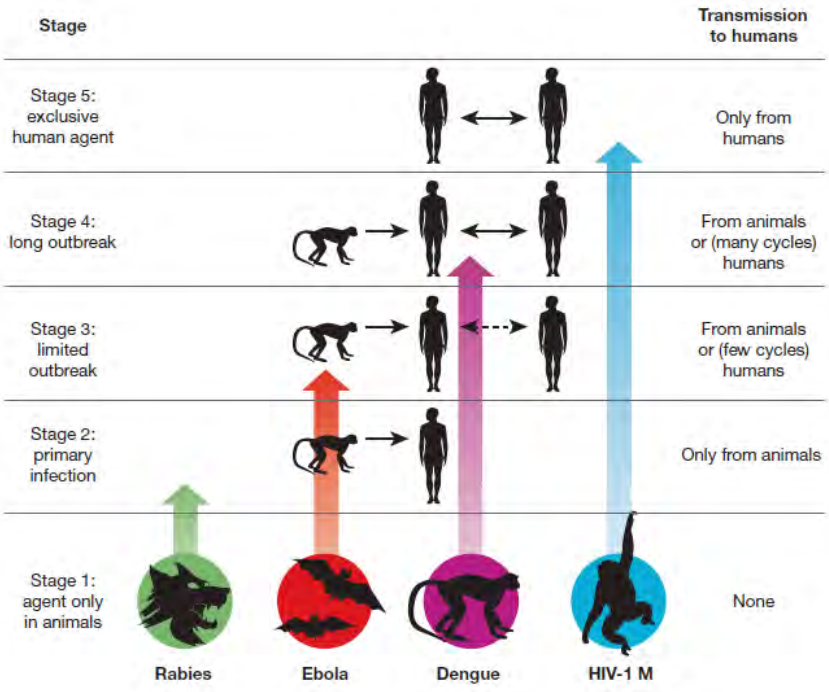
Despite being referred to as the 'fifth human malaria species *(11)*, it is not yet clear where *P. knowlesi* lies on the continuum between animal and human pathogens. Emerging zoonotic infections are classified into five stages based on epidemiological dynamics in the incidental host (Fig. 1) *(12)*. Stage 3 is characterized by stuttering chains of human cases because the pathogen is weakly transmissible between humans (R_0<<1). Stage 3 pathogens are exemplified by SARS-CoV and MERS-CoV, which have caused outbreaks and limited human-to-human transmission that ultimately dies out or is controlled. Stage 4 pathogens are those that have long sequences of transmission between humans without involvement of animal hosts (R_0>1). This stage has been further divided based on the relative importance of transmission within the reservoir or incidental host:

- Stage 4a: The sylvatic cycle is much more important than direct human-to-human spread, e.g., Chagas disease and yellow fever.
- Stage 4b: Both the sylvatic cycle and human-to-human transmission are important, e.g., dengue fever in some forested areas of West Africa and South-East Asia.
- Stage 4c: Transmission between humans is more important, e.g., influenza A, cholera, typhus, SARS-CoV2.

WHO/UCN/GMP/MPAG/2021.02

Pathogens in Stages 1–2 are not considered to be human infections, but those that pass on to Stage 4 are considered to be human pathogens, with critical implications for elimination and eradication. For example, yellow fever, a Stage 4a pathogen, was considered for eradication during the first decades of the 20th century. However, when the importance of the sylvatic reservoir was identified, it was determined that eradication was not feasible. The implications for eradication of the classification of zoonotic pathogens as Stage 3 are less clear.

FIG. 1.
Illustration of the five stages through which pathogens of animals evolve to cause diseases confined to humans *(12)*



A 2017 WHO Evidence Review Group (ERG) examined the available evidence on *P. knowlesi* to consider whether sustained human–mosquito–human transmission of *P. knowlesi* was occurring.[1] The ERG concluded that *P. knowlesi* infection remained primarily a zoonotic infection (i.e., Stage 2), but stressed the need to further investigate the possibility of human–mosquito–human transmission.

The staging of *P. knowlesi* along the continuum between animal and human pathogens is important for determining the feasibility of the ultimate goal of eradicating human malaria, as well as the near-term goal of eliminating malaria from countries in South-East Asia. If there is evidence that *P. knowlesi* is a Stage 3 or 4 pathogen, the criteria for the certification of malaria elimination may need to be revisited.

Objectives of the technical consultation

1. To review the evidence from a systematic review of the literature on *P. knowlesi* to determine whether human–mosquito–human transmission is occurring and whether sustained transmission is possible.

[1] The report of the ERG can be found at: https://apps.who.int/iris/rest/bitstreams/1096713/retrieve.

2. To review the results of spatiotemporal analysis of *P. knowlesi* case data from Malaysia that attempt to identify clusters of cases that could have arisen from human–mosquito–human transmission.

3. To recommend to WHO a current staging of *P. knowlesi* on the zoonotic continuum based on the evidence reviewed.

4. To outline a research and surveillance plan to monitor for emergent changes in the human transmission potential of *P. knowlesi*.

Process

The Elimination Unit has prepared Terms of Reference for a literature review and contracted Drs Chris Drakeley and Kim Fornace of the London School of Hygiene and Tropical Medicine (LSHTM) to undertake the review. The literature review is not limited to any specific country and will cover epidemiological, entomological, ecological and laboratory evidence for actual or potential sustained transmission between humans of *P. knowlesi* (Annex 1), based on recommendations from the 2017 ERG on *P. knowlesi* regarding the categories of data that would be needed to provide evidence for current or potential sustained human–vector–human transmission. More than 7229 records were identified after duplicates were removed, and 456 are being assessed for eligibility. The review should be concluded by May 2021.

In addition to the literature review, the University of Malaysia Sabah, together with Drs Chris Drakeley and Kim Fornace from LSHTM and Dr Azra Ghani from Imperial College, will undertake a spatiotemporal analysis of the *P. knowlesi* case data, which have included geographic coordinates for households with detected infections since 2015. A protocol for the analysis has been drafted and is currently under review by authorities in Malaysia (Annex 2). The analysis is expected to take 4–5 months and will identify space–time clusters of cases that could represent sustained chains of human–mosquito–human transmission.

Three GMP units – Elimination, Entomology and Vector Control (EVC), and Surveillance – will collaborate on the technical preparations for the meeting. The Elimination Unit will provide administrative support and manage the contracts for the literature review and analysis.

WHO/GMP will convene a group of 12 independent experts in zoonoses, parasitology, entomology, surveillance and elimination from leading technical agencies to address the objectives of the meeting. Among these experts will be researchers with specific expertise in *P. knowlesi*. The LSHTM will be asked to present the findings of the systematic review. Based on an advanced review of the most important findings of the systematic review, experts in the domains identified as key to understanding the current and potential future status of *P. knowlesi* transmission among humans will be invited to give presentations on their research findings. Experts on other emerging zoonoses, such as MERS-CoV, avian influenza and SARS-CoV, will be invited to present on the processes used to understand the ecology and epidemiology of these pathogens and their classifications. The University of Malaysia Sabah will present the findings from the spatiotemporal analysis of *P. knowlesi* cases in Malaysia. Experts from the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) will be invited to present on frameworks for addressing and classifying emerging zoonoses.

The technical consultation will involve up to 30 participants and will require three days. Following the advice and recommendations of the Malaria Policy Advisory Group (MPAG) in April 2018, the tentative dates proposed for the meeting are 8–10 December 2021. It is expected that the outcome of this meeting will be presented to MPAG for debate and discussion during the April 2022 meeting.

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Malaria Policy Advisory Group Meeting

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Background document for Session 2

WHO technical consultation to stage *P. knowlesi* along the continuum between animal and human pathogens – Annexes

Drs K. A. Lindblade, A. Tiffany, Li X-H, P. Ringwald, J. Kolaczinski and A. Noor
Technical consultation proposed for the 4th Quarter 2021, Geneva, Switzerland

1. What evidence is there of sustained human-mosquito-human transmission of *Plasmodium knowlesi*? A systematic review protocol (Authors: Pablo Ruiz Cuenca, Kimberly Fornace, Chris Drakeley)
2. Study Protocol: An observational spatiotemporal analysis of routinely collected malaria surveillance reports to assess probability of nonzoonotic *Plasmodium knowlesi* transmission

What evidence is there of sustained human-mosquito-human transmission of *Plasmodium knowlesi*? A systematic review protocol

Authors: Pablo Ruiz Cuenca, Kimberly Fornace, Chris Drakeley
Support: Funds provided by WHO

Introduction

Rationale

Zoonotic malaria, caused by the parasite *Plasmodium knowlesi*, has increasingly become a public health concern in South East Asia. It is maintained in the wild by non-human primates (including macaques) and is transmitted by mosquitoes from the Leucosphyrus group of *Anopheles*. The geographic distribution of the disease is limited to areas where both primate hosts and vectors are present, mostly affecting individuals who live in or travel through forests and forest fringes. [1] The incidence has been increasing in Malaysia, with cases caused by *P. knowlesi* being the predominant cause of indigenous malaria in the country since 2017. [2]

As an emerging infectious disease, zoonotic malaria may be classified into 5 stages based on its epidemiological dynamics (Figure 1). [3] Stages 1 and 2 represent pathogens which are found in animals and have either not been found to naturally infect humans or have not been found to cause secondary human infections. Stage 3 pathogens are those that can cause secondary human infections but can only undergo a limited number of cycles. Stage 4 represents those pathogens that have a natural cycle of infecting humans from the primary animal host and can produce long sequences of secondary human cases. These are divided further into subgroups according to the importance of the animal and human cycles. Stage 5 pathogens are those that originated in animals but are now exclusively found in humans.

Stages 1 and 2 are not considered human pathogens, whilst stages 4 and 5 do fall into this category with important implications for control measures and, ultimately, elimination and eradication. The implications of pathogens which are classified within stage 3 are less clear, requiring further clarifications to inform control policies.

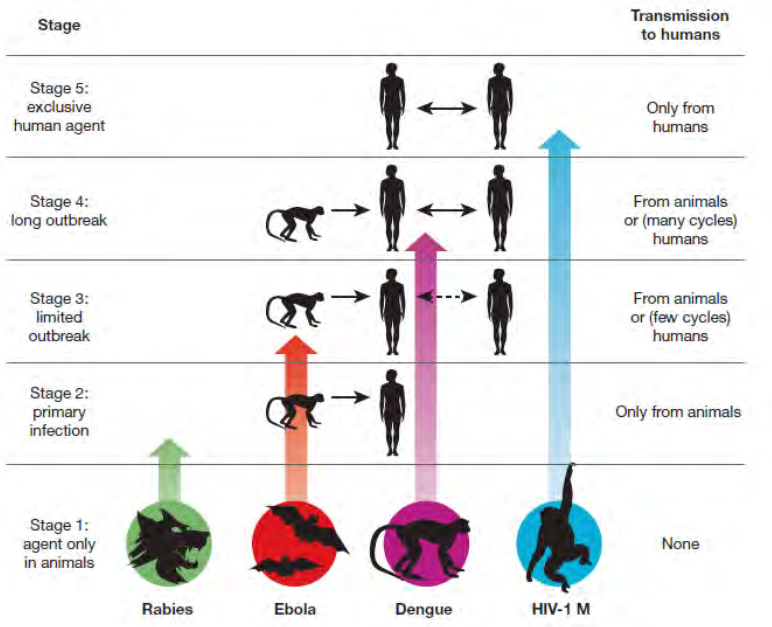


Figure 1 *– Depiction of suggested stages of an animal pathogen evolving to cause disease in humans. From Wolfe et al, Nature 2007.* [3]

In 2017, a WHO evidence review group (ERG) examined the available evidence on zoonotic malaria to determine which stage it could be classified as. It concluded that, given there was limited evidence of sustained human transmission following an initial spillover, it should be considered primarily a zoonotic infection and classified within stage 2. However, if this were to change to stage 3 or above, criteria for malaria elimination certification would have to be revisited. The ERG also concluded that further research was necessary, as significant knowledge gaps were identified, and categorised the necessary evidence that would prove or refute sustained human transmission.

Objectives

The purpose of this systematic review is to examine current evidence to determine if any human-mosquito-human transmission of *Plasmodium knowlesi* occurs and if sustained transmission, defined as multiple generations of human cases with no spillover from macaques, is possible. This will inform WHO's decision to classify zoonotic malaria and help determine appropriate elimination and eradication strategies and targets.

Furthermore, to help inform transmission models distinguishing zoonotic from non-zoonotic transmission, we will assemble data on the distribution and abundance of specific simian host and vector species. However, as this is not the main focus of the review, this will become the secondary objective.

Methods

We will adapt the CoCoPop framework (Condition, Context, Population) suggested for prevalence and incidence questions to create our question and guide our work. [4] Given the broad scope of the question, the evidence required was categorised by the WHO evidence review group.

Evidence suggestive of sustained human transmission chains was defined as:

- Epidemiological evidence
    - Evidence of R_0 >= 1in human population
    - Identification of space-time clusters consistent with human-mosquito-human transmission, including human case reports
- Laboratory evidence
    - Mixed (human-zoonotic) infections in mosquitoes
    - *P. knowlesi* infected mosquitoes with human blood only
    - Human infections successfully infecting malaria vectors (focus on Leucosphyrus group)
    - Demonstrating distinct haplotypes between parasites infecting humans and macaques
- Environmental/ecological evidence
    - Simian host distribution and abundance consistent with no possible spillover events
    - Vector distribution and density, specifically Leucosphyrus group, proving no possibility of spillover from simian hosts
- Other suggestive evidence, for example:
    - *Plasmodium cynomolgi* research
    - Other regional zoonotic malarias

Additionally, evidence that refuted the possibility of sustained human transmission included:

- Laboratory evidence
    - Identifying molecular barriers to successful invasion of human red blood cells
- Epidemiological evidence
    - Prevalence of ligands associated with molecular barriers to parasitic invasion of human red blood cells

Eligibility criteria

Types of studies

We will only exclude literature reviews from our search. Otherwise, there will be no exclusions on the types of studies reviewed. These will include, but are not limited to, epidemiological observational and interventional studies, laboratory studies and modelling studies. We will also perform a search of the grey literature for any suitable evidence, including human case reports and vector and simian host distributions.

Language

We will conduct the search in English but will be aiming to include studies published in any language. We are able to directly consider sources in English, Spanish, French and Malay. For those in languages we are not familiar with, support with screening and/or translating will be sought from our professional networks.

Condition

The search will focus on *P. knowlesi* research but will also include relevant work carried out with regional *Plasmodium* species which could be suggestive of sustained human transmission chains. We will limit diagnoses to molecular confirmation methods. We will consider genomic sequencing to be the strongest evidence of a positive diagnostic followed by PCR.

Context

In light of the epidemiology of zoonotic malaria, our interest is focused on understanding if there are biological reasons that support or hinder sustained human transmission, and if this is occurring naturally already. Given the complexity of studying this phenomenon, we will be using studies carried out in both controlled laboratory conditions and in the field, and will not be restricting based on location. We will also be looking for any suggestive evidence produced by modelling studies.

Population

No exclusion criteria will be applied to populations included in the review.

Information sources

The following databases will be used to search for published research:

- Medline
- EMBASE
- Web of Science

We will also include searches for grey literature, including reports and data sets on human and simian hosts and vectors from the following organisations:

- ProMed
- IUCN
- PREDICTS project
- Cambridge Conservation Initiative
- Zoological Society of London
- Zenodo

Search strategy

As there is limited published research on *P. knowlesi*, we will be using a wide search strategy in hopes of capturing all necessary evidence. The search strategy has been peer reviews by an Information Scientist from the London School of Hygiene and Tropical Medicine library, using PRESS peer review standard and guidelines.[5]

An example of the search strategy we will use in Medline is detailed below:

1 Plasmodium knowlesi/
2 plasmodium knowles*.mp.
3 1 or 2

4 Zoonoses/
5 catarrhini/ or cercopithecidae/ or cercopithecinae/ or exp macaca/
6 (monkey* or simian* or zoono* or macaca or macaque*).mp.
7 4 or 5 or 6

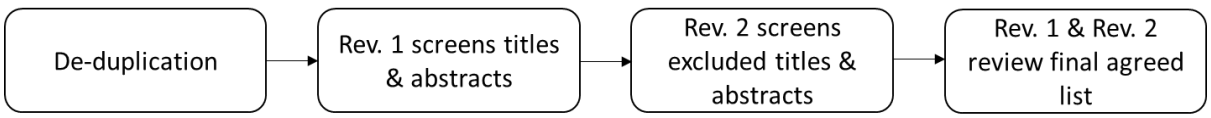
8 (malaria* or plasmodium).mp.
9 Malaria/
10 8 or 9

11 7 and 10
12 3 or 11

Study records

Selection process

All published literature will be imported into a reference manager, which will be used to de-duplicate any papers. Titles and abstracts will then be screened and removed as necessary. We will utilise the Rayyan tool for screening titles and abstracts. [6] One reviewer will screen all titles and abstracts, whilst the second reviewer will assess those that have been excluded. Once the final list of texts has been agreed, both reviewers will review all texts concurrently.



Data extraction and management

A standardised form will be used to extract necessary data from published literature. Given the varied evidence we will be collecting, the synthesis without meta-analysis (SWiM) framework will be used, applying vote counting to combine evidence. [7] Using the categories of evidence outlined above, the evidence suggestive of sustained human transmission will be considered a positive vote (▲) whilst the evidence refuting sustained transmission will be considered a negative vote (▼).

The data extraction form will include year of publication, population, setting (including georeferenced data if possible), diagnostic used (if applicable), category and direction of evidence (vote counting). A separate form will be used to extract necessary data from grey literature, including host and vector data to improve model parameters. Sample extraction forms are included in Appendix II.

Outcomes and prioritization

Our main outcomes will be guided by the evidence categories defined above. We will prioritise definitive evidence of sustained human transmission, but will also seek suggestive evidence, such as modelling work performed with host and vector data.

Guided by our secondary objectives of finding parameters to improve transmission models, our secondary outcomes will be identifying data sets and evidence to improve parameterisation of models. These parameters are detailed in Appendix I.

Risk of bias in individual studies

As we are expecting a broad range of types of studies, the risk of bias will be assessed according to each category of evidence. We will assess what types of evidence need to be assessed before applying specific tools to assess risk of bias. Examples are included in Table 1.

Table 1 *– Possible tools assessing risk of bias*

Tool	Type of study
Cochrane RoB 2	Experimental epidemiological study
Newcastle - Ottowa Scale (NOS)	Case-control studies
QUADAS-2	Diagnostic studies

Data Synthesis

Data will be synthesised using the SWiM framework, combining evidence by vote counting. This will be done narratively, graphically (eg harvest plots[8]) and in tabular form to help interpret results. Given the comprehensive nature of the evidence we will be searching, we will not be assessing meta-biases of the studies included.

Confidence in cumulative evidence

Given the range of evidence we will be searching, we will be using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach applicable to each type of study (epidemiological, diagnostic and modelling). [9,10]

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Appendix I – Model Parameters

Data and evidence will be sought to help improve and inform the following model parameters:

- Human host-parasite interactions
    - Parasite virulence
    - Parasite binding to human red blood cells
    - Duration of infection/infectivity
- Human host-vector interactions
    - Human-mosquito infectiousness
    - Human host biting rate
    - Biting preference
    - Mosquito:humans ratio
- Vector-parasite interactions
    - Mosquito lifespan
    - Reproductive ability of parasite inside mosquito
    - Duration of development in mosquito
- Simian host-vector interactions
    - Simian ecology
    - Biting rate
    - Biting preference
    - Duration of infection/infectivity

Appendix II – Sample extraction forms

Evidence synthesis:

ID	Title	Abstract	Authors	Year	Journal	Evidence category (epi/lab/eco)	Sub-category	Risk of bias	Certainty of evidence	Direction of evidence (vote counting: ↑, - , ↓)

Vector/host data extraction:

ID	Vector/Host	Species	Presence/abundace	Number	Date	Country	Location	Latitude	Longitude	Source_ID	Source

Study Protocol

An observational spatiotemporal analysis of routinely collected malaria surveillance reports to assess probability of nonzoonotic *Plasmodium knowlesi* transmission

Protocol number, version number and date:
Version 1.0, 3 March 2021

Name and Institution of Principal investigator:
Prof. Kamruddin Ahmed, Borneo Medical and Health Research Centre, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia

Name and Institution of Co-Investigators:
Prof. Chris Drakeley, London School of Hygiene and Tropical Medicine, London, United Kingdom
Dr. Kimberly Fornace, London School of Hygiene and Tropical Medicine, London, United Kingdom
Prof. Azra Ghani, Imperial College London, London, United Kingdom

List of Abbreviations

GIS	Geographical Information System
GPS	Global Positioning System
LSHTM	London School of Hygiene and Tropical Medicine
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
UMS	Universiti Malaysia Sabah
WHO	World Health Organization

Research Synopsis

Study title	An observational spatiotemporal analysis of routinely collected malaria surveillance reports to assess probability of nonzoonotic *Plasmodium knowlesi* transmission
Study Population	All confirmed malaria cases reported to the Malaysian Ministry of Health from 2000 - 2021
Study Design	A retrospective longitudinal study. Surveillance reports for the period 2000 - 2021 will be reviewed and study data extracted.
General Objective	To examine evidence of human-to-human transmission of *Plasmodium knowlesi* in Malaysia
Specific Objectives	a. To investigate existing modelling approaches to characterize zoonotic and non-zoonotic transmission; b. To clean and validate reported data on cases and household locations; c. To describe the spatiotemporal distribution of reported malaria cases and assemble potential environmental covariates influencing transmission pathways; d. To identify clusters of cases which could have arisen from human-to-human transmission; and e. To estimate spillover rates and reproductive rates for *Plasmodium knowlesi*
Study endpoints/outcomes	Reported malaria case confirmed by PCR, microscopy or RDT
Sample Size	Not applicable, all reported malaria cases
Study Duration	2000 - 2021

1.Background and Significance

Disease emergence is a complex process in which pathogens overcome evolutionary and ecological dynamics to establish in human populations [1]. Pathogens have been classified into different stages of emergence, from entirely driven by spillover from animal populations (e.g. rabies) to widespread transmission maintained by human populations (e.g. HIV). Many emerging pathogens have both zoonotic and nonzoonotic transmission pathways in which an initial spillover event may lead to stuttering chains of human-to-human transmission or larger outbreaks [2].

Plasmodium knowlesi is an emerging disease carried by simian reservoirs and transmitted to people through bites of infected *Anopheles* mosquitoes. Since the first report of a large cluster of human *P. knowlesi* cases in Malaysian Borneo, reported *P. knowlesi* incidence has markedly increased and *P. knowlesi* is now the main cause of human malaria in Malaysia [3-6]. These increases in incidence are strongly associated with deforestation, suggesting landscape change may be increasing contact between people, mosquitoes and macaque reservoirs [7, 8]. Genetic evidence suggest that transmission remains primarily zoonotic from wild non-human primate populations [9]. However, the possibility of human-mosquito-human transmission of *P. knowlesi* has been demonstrated in laboratory studies [10]. Further evidence is needed to evaluate whether nonzoonotic transmission of *P. knowlesi* is occurring naturally.

As countries move towards malaria elimination certification, understanding the transmission pathways of *P. knowlesi* was identified as a priority by the World Health Organization (WHO). The London School of Hygiene and Tropical Medicine (LSHTM) is currently leading a systematic literature review to examine existing evidence of whether nonzoonotic *P. knowlesi* transmission occurs. However, in addition to examining previous studies, analysis of reported malaria case records can be used to assess whether spatiotemporal patterns are consistent with different transmission mechanisms.

Quantifying relative contributions of zoonotic and nonzoonotic transmission is essential for designing effective surveillance and control strategies. These transmission pathways are represented by two epidemiological parameters: the spillover rate (λ), the rate at which a pathogen is transmitted from an animal reservoir to a human, and the reproductive rate (R), the number of human cases resulting from an infectious individual. Stuttering chains of transmission occur when pathogens are transmissible between humans but not capable of sustained transmission ($0<R<1$). While routinely collected surveillance data is frequently the only available source of information, estimating these parameters is challenging when distributions and movements of wildlife hosts, vectors and human populations are unknown and a human case could result from multiple

sources of infection. These data may be further limited by biases in health-seeking behaviour and health facility coverage.

Model-based inference methods provide a powerful tool for disentangling relative contributions of zoonotic and nonzoonotic transmission mechanisms, using times of infection, spatial locations of reported cases and estimates of heterogenous mixing to infer transmission trees and predict the source of infection (e.g. [11-13]). We aim to adapt these approaches to examine evidence for human-to-human transmission of *P. knowlesi* in Malaysia. By evaluating spatiotemporal patterns of reported malaria cases, we will identify possible malaria clusters which could have occurred through nonzoonotic transmission and quantify estimates and uncertainty around spillover rates and reproductive rates of *P. knowlesi*. This will enable identification of priority areas for surveillance and control measures.

2. Objective

To examine evidence of human-to-human transmission of *Plasmodium knowlesi* in Malaysia, with specific objectives to:

a. To investigate existing modelling approaches to characterize zoonotic and non-zoonotic transmission;
b. To clean and validate reported data on cases and household locations;
c. To describe the spatiotemporal distribution of reported malaria cases and assemble potential environmental covariates influencing transmission pathways;
d. To identify clusters of cases which could have arisen from human-to-human transmission; and
e. To estimate spillover rates and reproductive rates for *Plasmodium knowlesi*.

3.Methodology

3.1 Study Type and Design

This will be an observational, retrospective longitudinal study using routinely reported malaria surveillance data in Malaysia from 2000 – 2021.

We will assemble historical records of malaria reports, including data on date of malaria confirmation, date of symptom onset, patient age, patient gender, patient residence (locality and GPS coordinates if available), malaria parasite species diagnosed, diagnostic method and reported travel history or location of infection. Data will be checked for completeness and all spatial data will be mapped in GIS software (e.g. Quantum GIS), with inaccurate data or data which cannot be geolocated excluded from further analysis.

We will additionally assemble open source satellite-derived data on key environmental and spatial factors throughout this time period, including forest cover [14], elevation [15], population density [16] and climate variables [17]. We will extract key variables for locations of malaria reports. Data will be incorporated into modelling frameworks to assess the probability of human to human transmission and to identify locations for further surveillance efforts.

3.2 Study Population
We will include all malaria patients reported to the Malaysian national surveillance system from 2000 – 2021.

3.3 Inclusion Criteria
All patients with a confirmed malaria diagnosis will be included in this study.

3.4 Exclusion Criteria
We will exclude patients without either the date of the malaria report or date of malaria onset. We will additionally exclude any patients who cannot be geolocated to at least a district-level.

3.5 Withdrawal Criteria
Subjects will be withdrawn if consent for record use is withdrawn by the Ministry of Health. All records will be anonymized and other personal data will not be used.

3.6 Sample Size
As this is a retrospective study of reported cases, no sample size is applicable.

3.7 Study Duration and Timeline
This study will be conducted from May 2021 – April 2022.

Activity	5/21	6/21	7/21	8/21	9/21	10/21	11/21	12/21	1/22	2/22	3/22	4/22
Assemble spatial and environmental data	■	■										
Review and map surveillance records	■	■	■									
Initial model development and parameterization	■	■	■									
Identification of spatiotemporal clusters			■	■								
Model development to estimate R and λ				■	■	■						
Present intermediate results to MoH and WHO							■					
Finalise and refine model								■	■	■	■	
Publication and report											■	■

3.8 Study Visits and Procedures
No study visits will be conducted. Data will updated if new records become available from the Ministry of Health.

3.9 Statistical Analysis Plan
We will use these records to fit Bayesian mathematical models to assess the probability of nonzoonotic *P. knowlesi* transmission. We will first use results from the systematic literature review to estimate likely timing and geographic spread of human-to-human *P. knowlesi* transmission. We will incorporate estimates of parasite prevalence in humans and macaques, incubation and parasite development durations, spatial mixing within the same village or travel to neighbouring areas and other parameters to identify spatiotemporal windows in which two human cases could be part of the same transmission chain, conducting sensitivity analysis on key model parameters. While this cannot be used to conclude human-to-human transmission occurred, this approach allows identification of clusters where human-to-human transmission is highly unlikely to have occurred. We will compare clusters of nonzoonotic malaria transmission (e.g. historical *P. falciparum* transmission) with potential clusters of *P. knowlesi* to cross-validate this approach.

Next, we will model the spatiotemporal distributions of *P. knowlesi* cases using Bayesian latent process methods to estimate the latent (unobserved) sources of infection and true numbers of infections adjusted for detection probabilities (e.g. diagnostic method, health coverage). We assume the total numbers of infections in a time and location are a product of the likelihood of zoonotic spillover and human-to-human transmission, with new infections resulting from either spillover events or contacts with infectious humans (through mosquitoes). We will model the distribution of zoonotic infections as a random variable dependent on forest cover or other remotely sensed metrics of macaque and mosquito habitat while cases arising from human-to-human transmission will be modelled as a random variable dependent on the reproductive rate in humans, generational time and proximity and connectivity of a location to infectious individuals. We will apply Bayesian data augmentation approaches to infer unobserved parameters [13]. Models will be used to explore probabilities of zoonotic and nonzoonotic infection sources and identify future priorities for surveillance.

3.10 Risk and benefit to study participants
As this study will analyze anonymized secondary data only, we do not anticipate any significant risks to study participants. Data will be managed to ensure confidentiality and security.

This study does not present any direct benefit to the participants. However, the study will provide a better understanding of *P. knowlesi* transmission and improve targeting of surveillance and control activities within Malaysia. This will also support malaria elimination efforts in Malaysia, a critical national priority.

3.11 Risk Benefit Assessment
As stated above, there is minimal risk from the study procedures. Study findings shall potentially greatly improve surveillance and control efforsts. The expected benefit outweighs the minimal risk to subjects and thus this study should be supported.

3.12 Ethics of Study
This study will be conducted in compliance with ethical principles outlined in the Declaration of Helsinki and Malaysian national guidelines. Ethical approvals will be obtained from the Malaysian Research Ethics Committee and the Institutional Review Boards at the London School of Hygiene and Tropical Medicine and Universiti Malaysia Sabah.

3.13 Informed Consent/Assent Process
This study will only use anonymized secondary records. As such, no additional consent will be obtained.

3.14 Privacy and Confidentiality
All records will be anonymized and no personal data will be collected. However, as GPS coordinates on household locations are available for recent surveillance records, we will treat this as identifiable information. Electronic data access will be restricted to authorized study personnel only and all data will be password protected. Data will be backed up externally on secure encrypted cloud storage and external hard drives accessible only to study personnel. Study codes will be used to identify all records and no patient identifiable information (including GPS data) will be published or available to people outside the study team.

Data will be archived according to the Research Data Management Policies at Universiti Malaysia Sabah and London School of Hygiene and Tropical Medicine. After the study, research data will be archived and destroyed according to the records and disposal strategy.

3.15 Conflict of Interest
The investigators declare they have no conflict of interest.

3.16 Publication Policy
No personal information will be disclosed and subjects will not be identified when the findings of the survey are published. The Ministry of Health will be provided with initial data as soon as it is available and updated on the results from subsequent analyses. Results will be published as soon as this study is complete. Guidelines for authorship of international peer-reviewed journals will be used to establish authorship. All results will be published in open access journals and presented to the Ministry of Health and World Health Organization.

3.17 Termination of Study
The Ministry of Health may decide to terminate the study at any time.

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Proposed WHO technical consultation to stage *P. knowlesi* along the continuum between animal and human pathogen

Dr Kim Lindblade, Elimination Unit Head

Global Malaria Programme

Global **Malaria** Programme

World Health Organization

Reasons for technical consultation

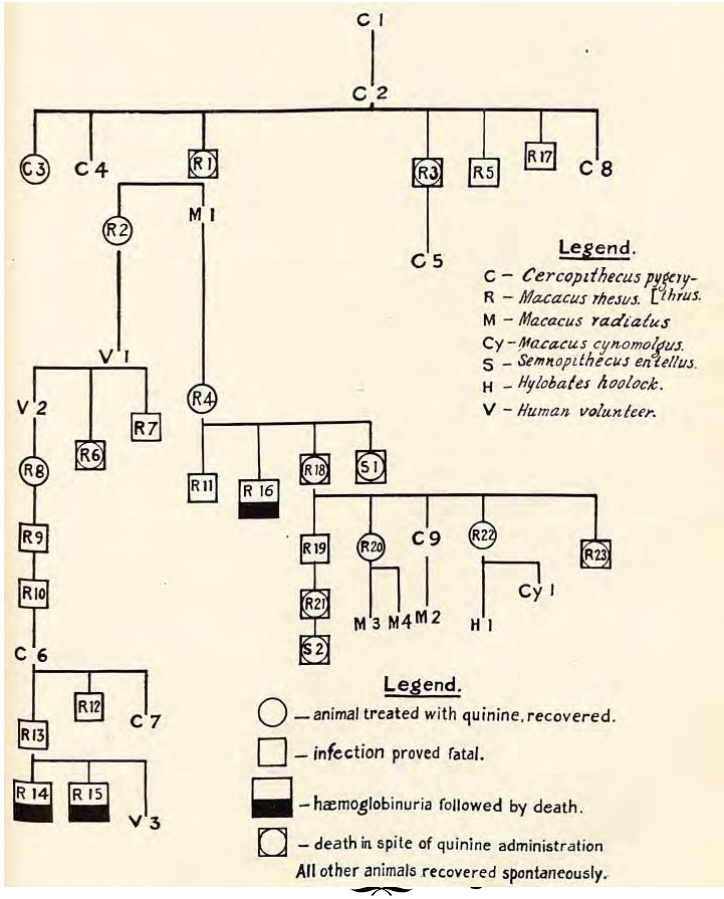
- Malaysia has eliminated human malaria* and is eligible to request WHO certification
- *P. knowlesi* has been increasing over the past decade
  - Referred to by some as 'fifth human malaria parasite'
- An ERG convened in 2017 concluded:

  'P. knowlesi infection remains primarily a zoonotic infection but . . . Important to further investigate the possibility of human-mosquito-human transmission.'
- Need for more precise designation of status of Pk to inform recommendations

*As currently defined

Plasmodium knowlesi

- First identified in non-human primates in 1931 in India (monkey from Singapore)
- Experimental infections between non-human primates and human volunteers demonstrated possibility of human infection
- First cluster of cases identified in Sarawak, Malaysian Borneo, 2004



First human cluster detected

- Difference in species distribution noted within Sarawak
- Kapit Division, *P. malariae* cases identified by microscopy- 20% cases

	Microscopy				
PCR	**PF**	**Pm**	**Pv**	**Po**	**Total**
Pf	16	15	1	0	32
Pm	0	0	0	0	0
Pv	2	9	37	0	48
Po	0	1	1	0	2
Pk	3	101	2	0	106
Pv+Pk	0	7	1	0	8
Pv+Pf	3	3	0	0	6
Pf+Pk	0	5	0	0	5
Pf+Pf+Pk	1	0	0	0	1
Total	25	141	42	0	208

12% of Pf cases and 5% of Pv found to be Pk

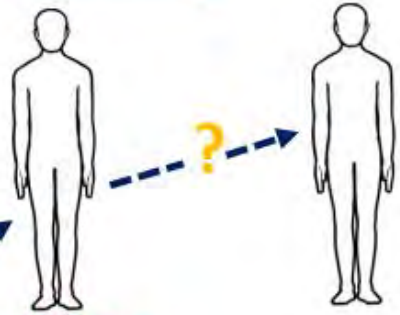
80% of Pm cases found to be Pk

Transmission of *P. knowlesi*

HOST

Long-tailed macaque *(M. fascicularis)*
Pig-tailed macaque *(M. nemestrina)*
Banded leaf monkey *(P. melalophus)*

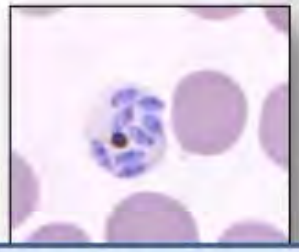
SOCIAL
Employment
Migration
Others



Humans:
Zoonotic infections

VECTOR

An. leucosphyrus mosquitoes:
An. latens (Sarawak)
An. balabacencis (Sabah)
An. cracens (Peninsular Malaysia)
An. dirus (Viet Nam)



MALARIA

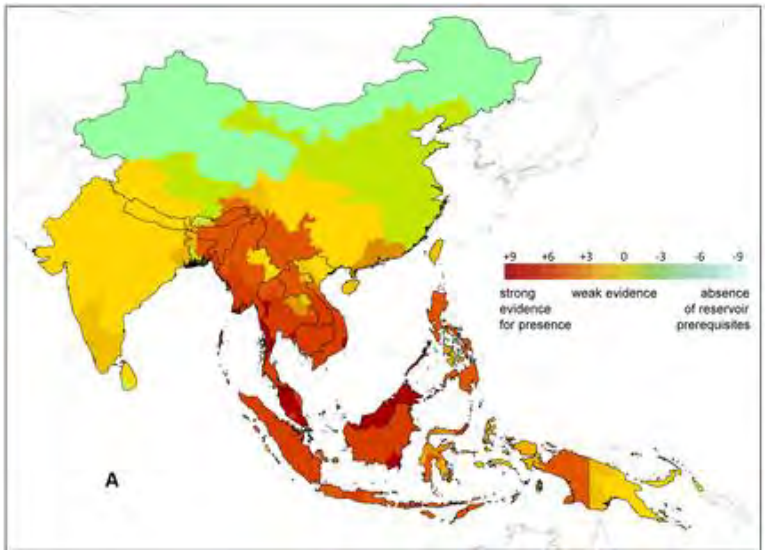
Plasmodium knowlesi

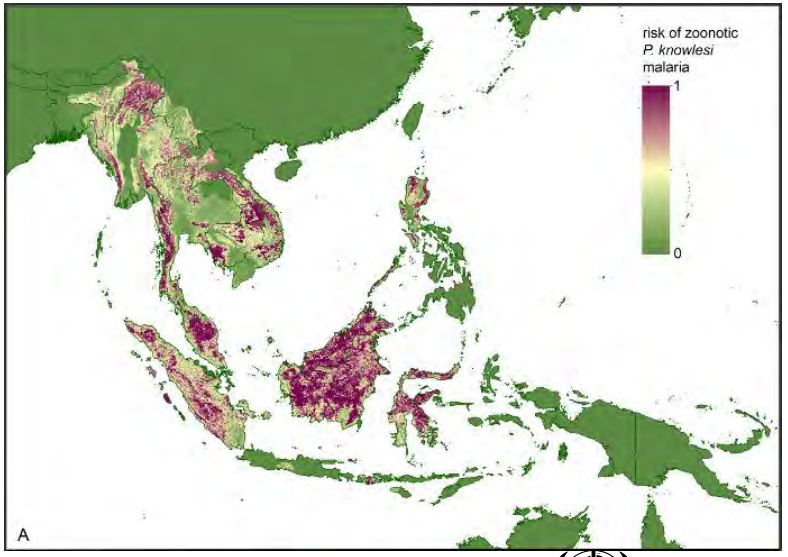
ENVIRONMENT

Dense jungle and forest fringe areas

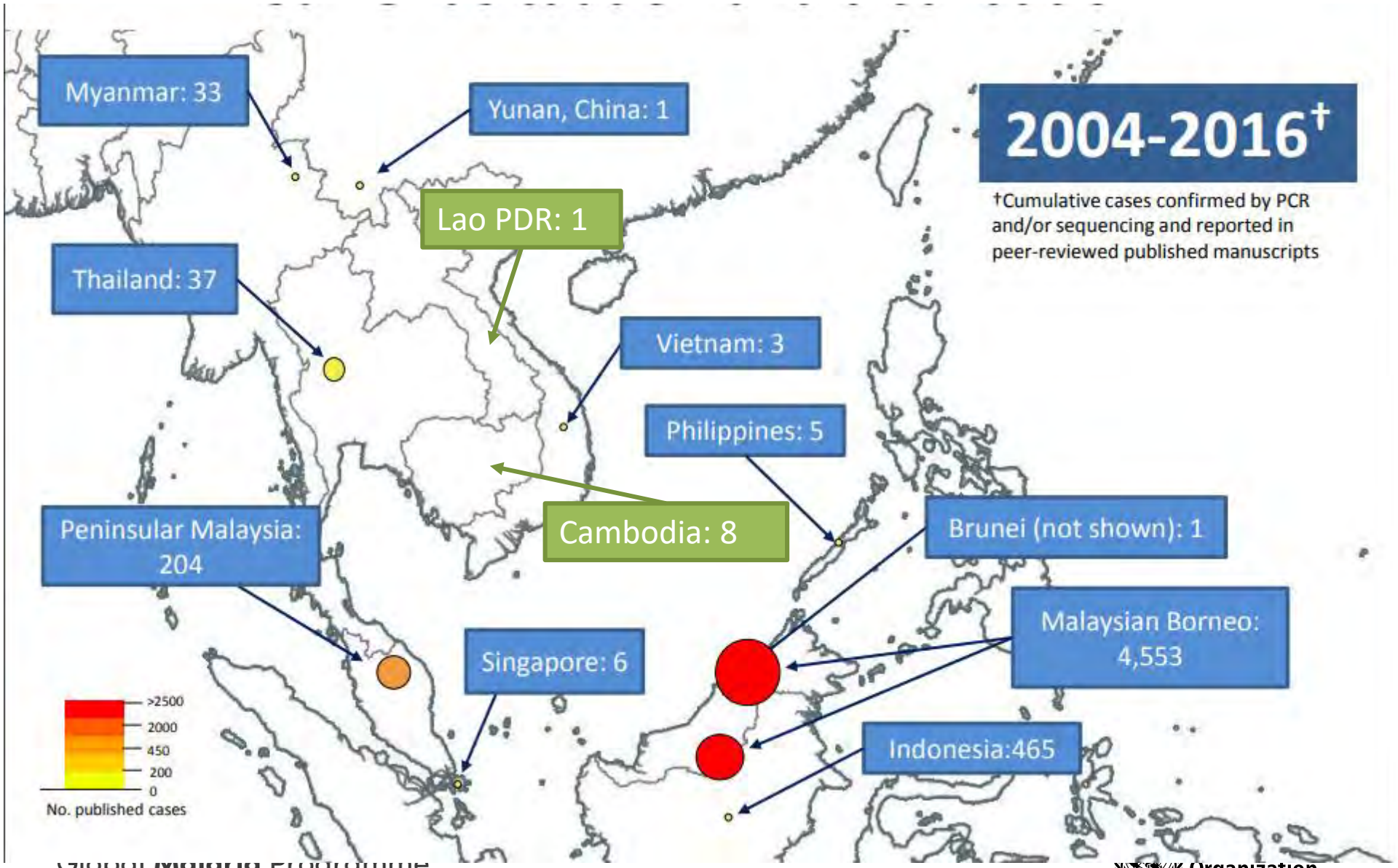
Putative distribution of *P. knowlesi*

- Panel A shows range of evidence for human parasite reservoir from strong (red) to weak (light blue)
  - Subnational areas scored on:
    - Presence of the parasite
    - Presence of monkey host
    - Presence of vector
- Panel B is based on extrapolations from locations of identified cases





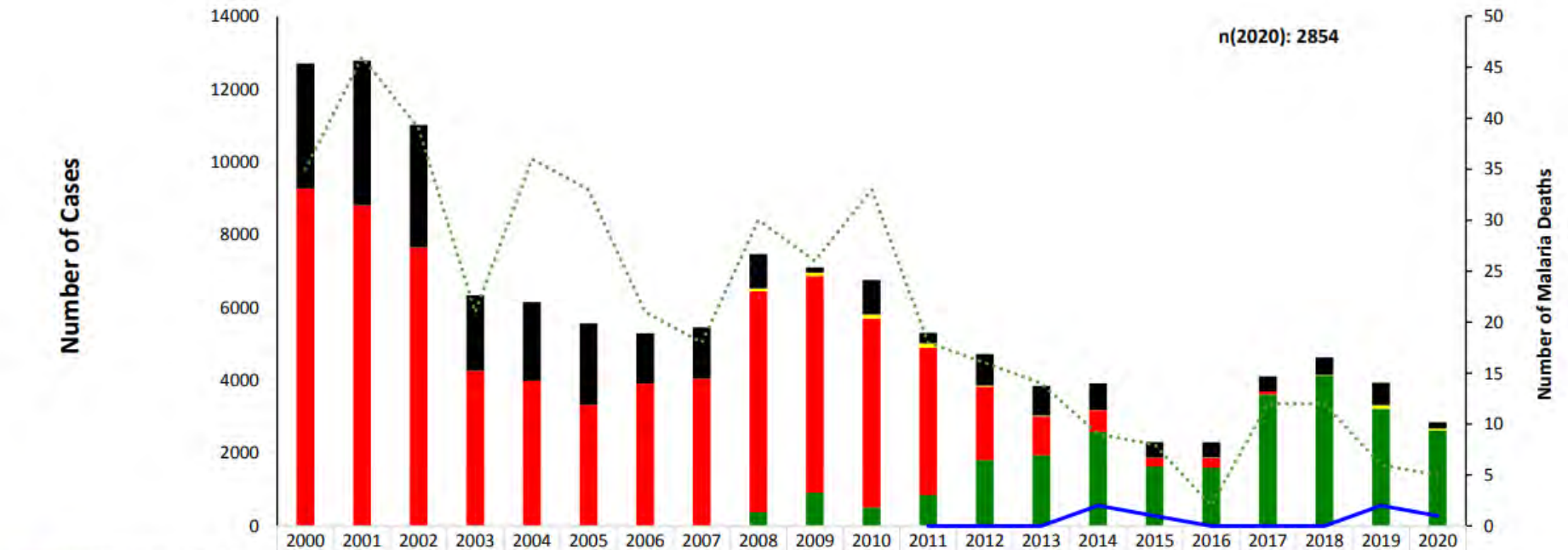
Geographic distribution of confirmed cases



Factors potentially contributing to increase in reported Pk cases

- Improved diagnostic capacity – use of PCR
- Change in land-use patterns creating increased interaction between humans, vectors and zoonotic reservoirs, leading to spillover
- Loss of forest cover increasing densities of vector mosquitoes
- Loss of relative immunity in humans due to decreasing Pf and Pv malaria
- Human-vector-human transmission

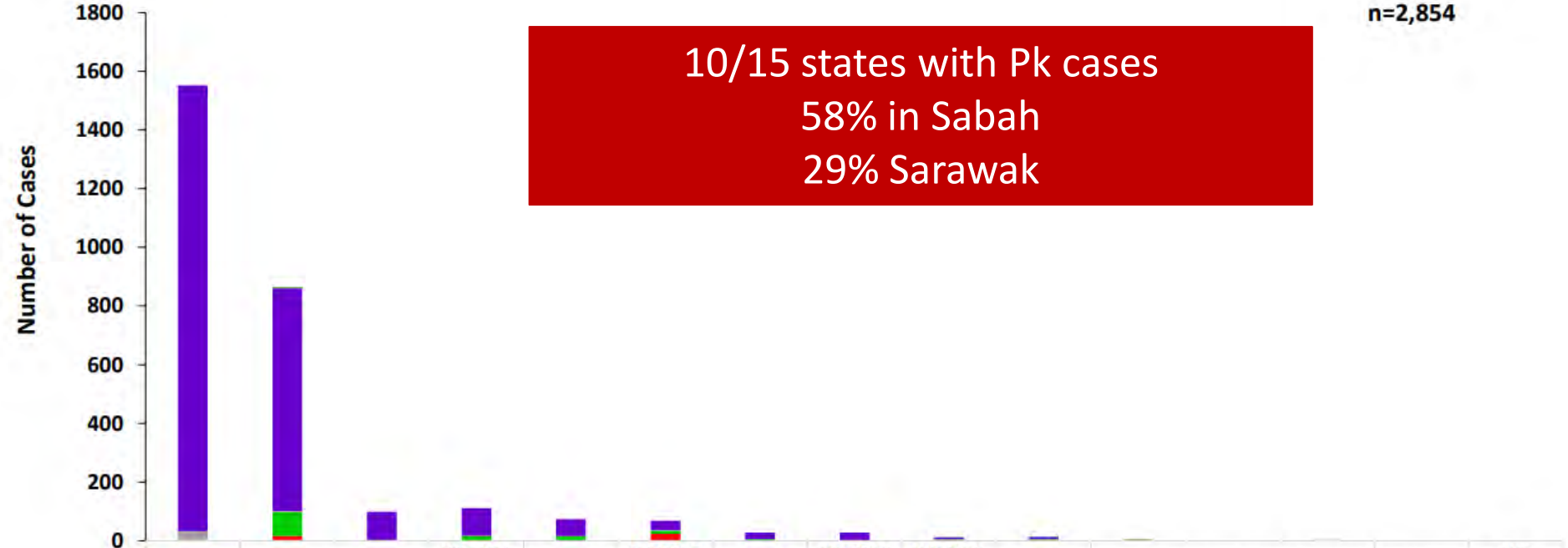
Malaysia has eliminated 4 species of malaria



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Imported Human Malaria	3432	3972	3367	2074	2165	2240	1377	1408	943	143	947	288	862	816	730	428	420	415	478	620	175
Introduced									78	95	107	119	33	26	7	2	16	0	21	96	54
Indigenous Human Malaria	9273	8808	7652	4264	3989	3329	3917	4048	6071	5955	5194	4045	2017	1066	600	240	266	85	0	0	0
Zoonotic malaria (Pk)	0	0	0	0	0	0	0	0	376	912	509	854	1813	1942	2584	1640	1600	3614	4131	3222	2624
Induced												0	0	0	2	1	0	0	0	2	1
Malaria Death	35	46	39	21	36	33	21	18	30	26	33	18	16	14	9	8	2	12	12	6	5

But has reported between 2600 and 4100 cases of Pk since 2018

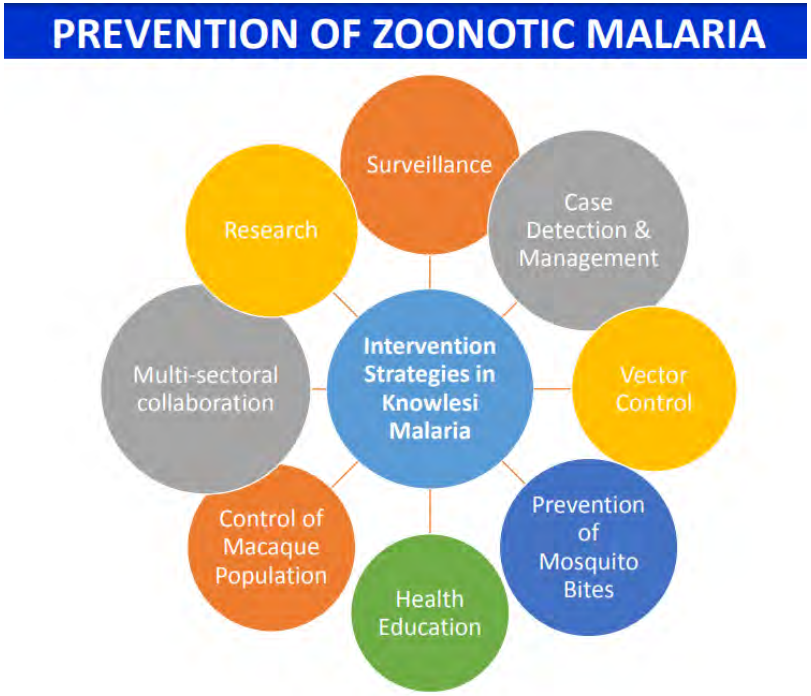
P. knowlesi distribution within Malaysia in 2020



10/15 states with Pk cases
58% in Sabah
29% Sarawak

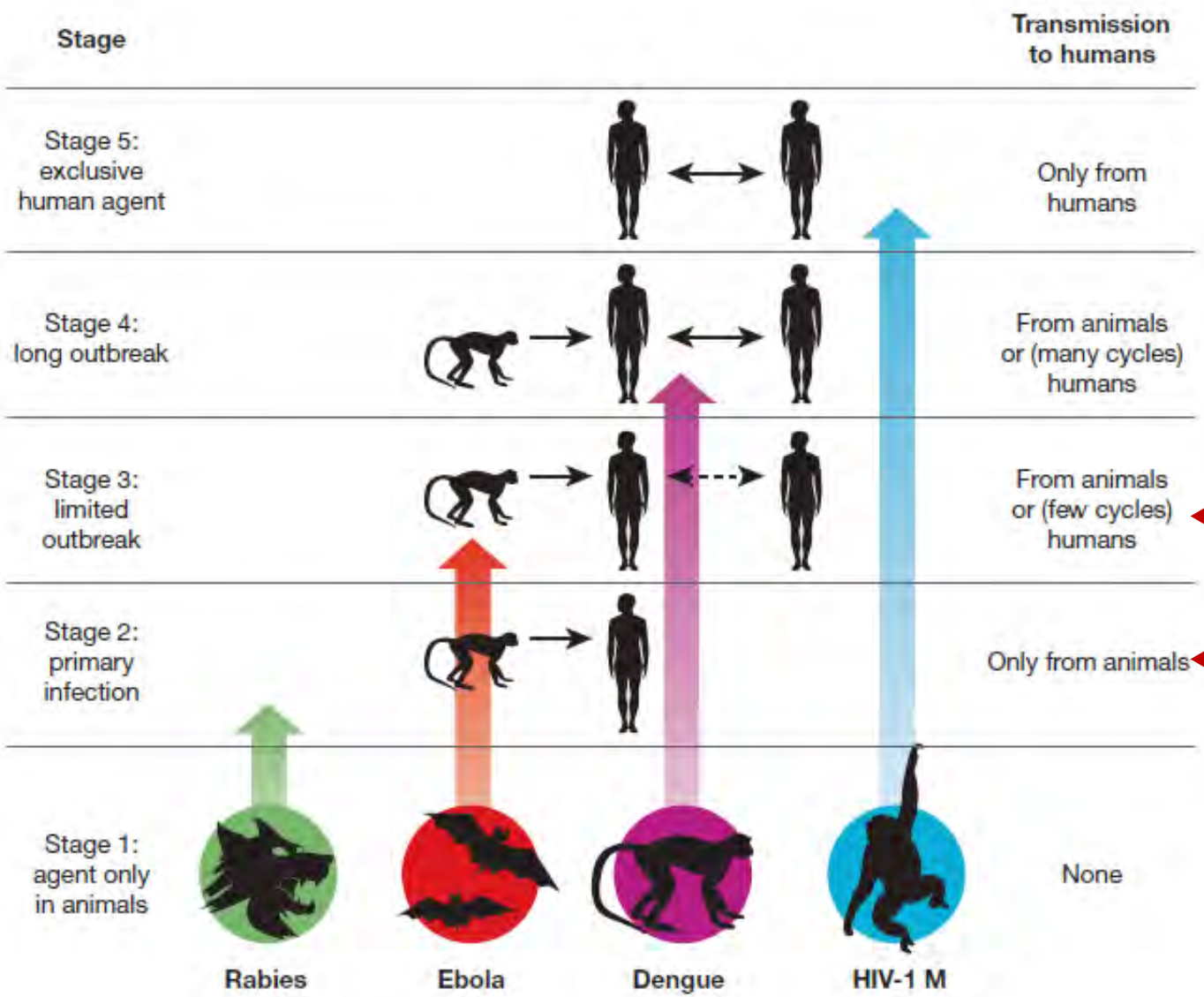
	SABAH	SARAWAK	PERAK	KELANTAN	PAHANG	SELANGOR	JOHOR	TERENGGANU	N.SEMBILAN	KEDAH	WPKL	MELAKA	P.PINANG	PERLIS	WP LABUAN
Mixed Infection	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
P. knowlesi	1519	759	97	94	58	32	22	27	8	8	0	0	0	0	0
P. ovale	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0
P. malariae	31	2	0	0	0	1	0	0	1	0	0	0	0	0	0
P. vivax	2	81	0	17	14	11	4	0	0	2	2	0	0	0	0
P. falciparum	0	17	2	0	1	24	2	1	2	2	3	0	2	0	0

Malaysia taking steps to defeat Pk



- Specific objectives:
  - Reduce Pk incidence to <1/10 000
  - Reduce case fatality ratio to <0.2%
- High level committees
  - Interministerial High Level Committee on Zoonotic Diseases
  - National Technical Committee on Control of Zoonotic Malaria

Five stages of evolution from animal to human diseases



2017 ERG conclusion?

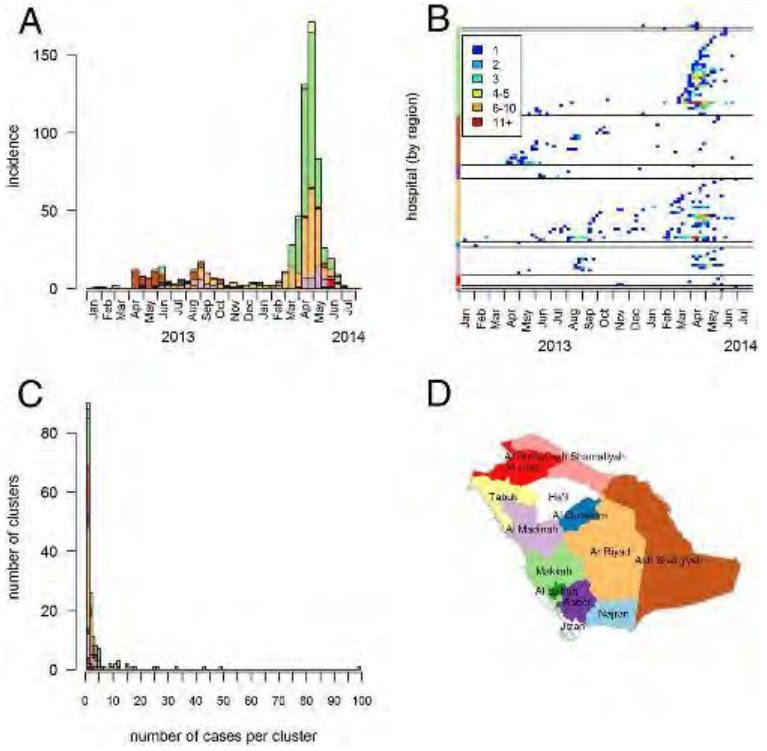
2017 ERG conclusion?

Systematic Literature Review

- Re-examining evidence for/ against human to human transmission of Pk, such as:
  - Laboratory studies of human infections transmitting to mosquitoes
  - Molecular barriers to infection and genetic studies
  - Epidemiological studies, including
    - Mixed or single infections in mosquitoes
    - Spatiotemporal clusters of knowlesi cases
    - Transmission models
- 7229 studies identified for preliminary screening
  - 456 full-text articles assessed for eligibility

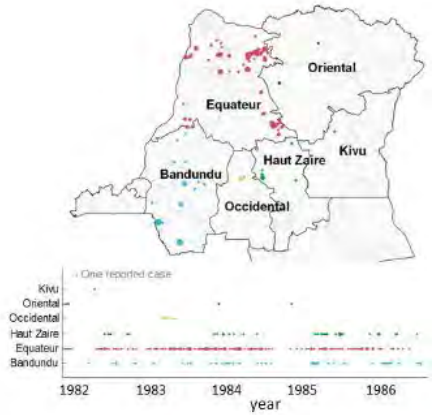
Identify key model parameters

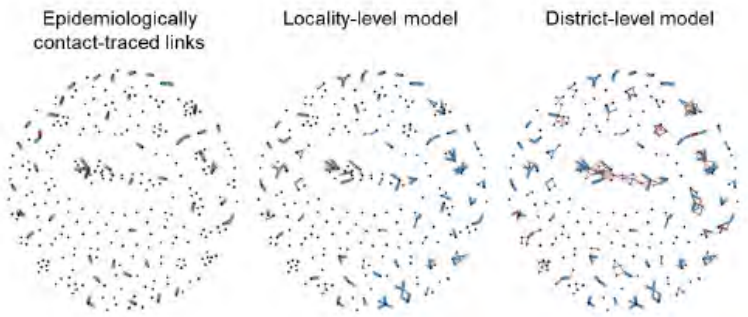
Examples from other zoonotic diseases



Cauchemez et. al, 2016, *PNAS*

- MERS
- Avian Influenza
- Monkeypox

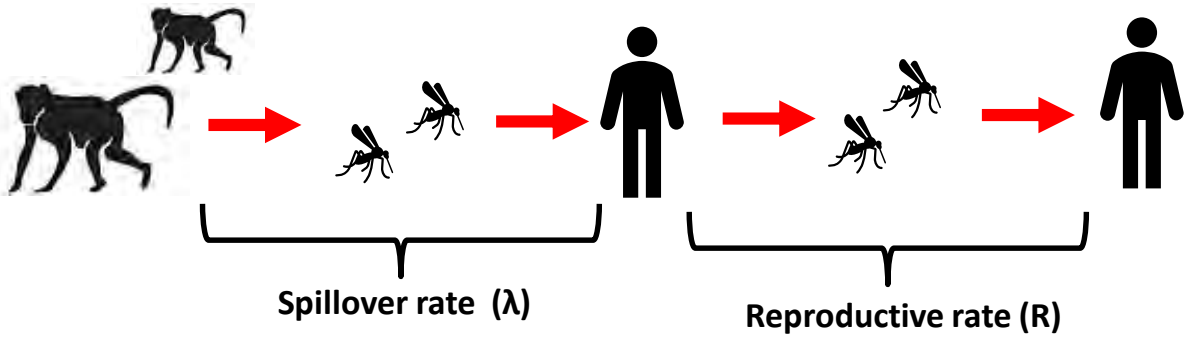




Ambrose et. al, 2019, *medRvix*

Spatiotemporal analysis of surveillance data

- Reconstructing transmission trees of *P. knowlesi* using spatiotemporal data



- Determining probability of human-mosquito-human transmission of Pk using temporal data on cases reported

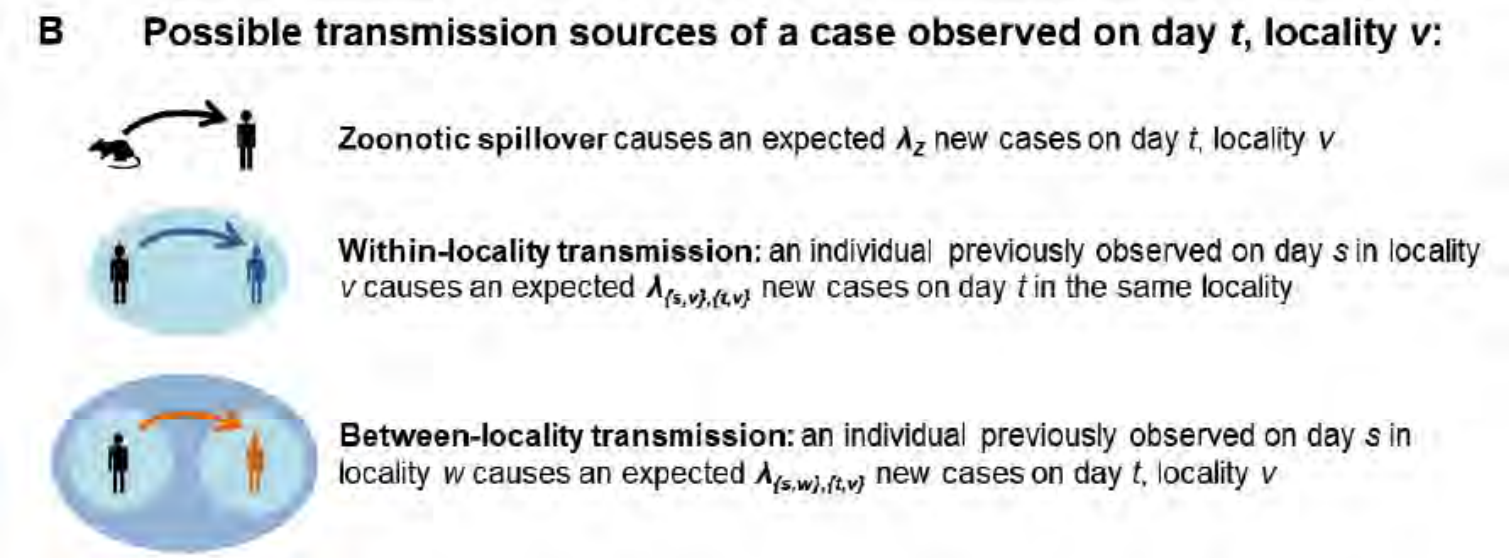
Time determined by:

Duration of sporogony (mosquito)
Mosquito life span

Parasite development rates
Duration of infectiousness (human)
Incubation period and clinical reports

Spatiotemporal analysis of surveillance data

- Extending to include fine-scale spatial data



Monkeypox
Ambrose et. al, 2019, bio*Rvix*

Spatiotemporal analysis of surveillance data

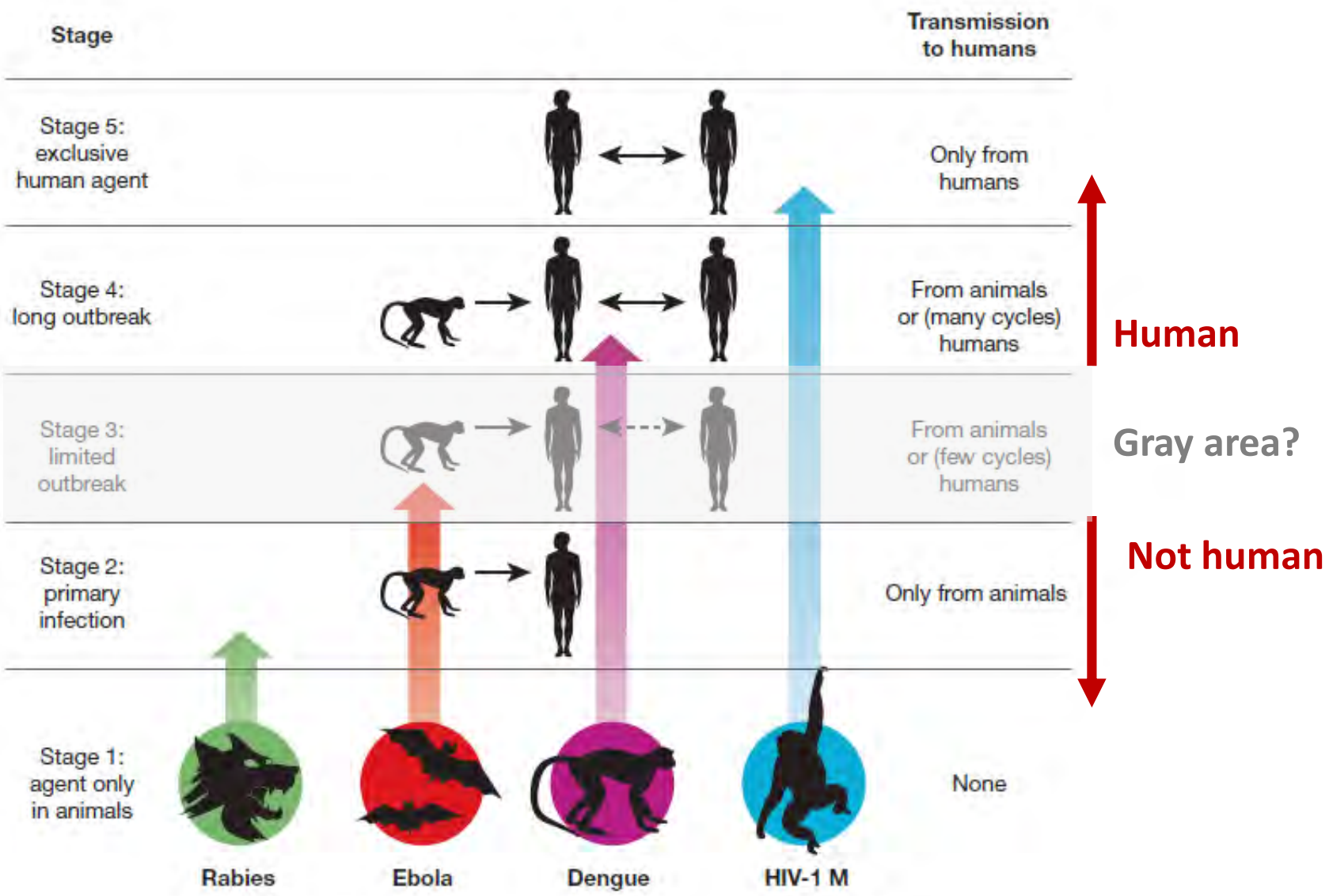
- Key outputs:
  - Identification of clusters which could have resulted from human-to-human transmission
  - Estimation of spillover rates and reproductive rates

  - Cases in location/date = Spillover events + Infections from humans
    - Spillover: dependent on macaque and mosquito habitats (e.g. forest)
    - Infections from humans: dependent on proximity and connectivity to infectious individuals, parasite incubation periods
    - Can extend to reflect surveillance detection probabilities, etc.

Technical Consultation

- To review the evidence from a systematic review of the literature on *P. knowlesi* to determine if human-mosquito-human transmission is occurring and whether sustained transmission is possible.
- To review the results of spatiotemporal analyses of *P. knowlesi* case data from Malaysia that attempt to identify clusters of cases that could have arisen from human-mosquito-human transmission.
- To recommend to WHO a current staging of *P. knowlesi* on the zoonotic continuum based on the evidence reviewed.
- To outline a research and surveillance plan to monitor for emergent changes in the human transmission potential of *P. knowlesi*.

Where does Pk fall?



Implications for elimination, certification and eradication

- Walter Dowdle in 1998:
  - An effective intervention is available to interrupt transmission of the agent
  - Practical diagnostic tools with sufficient sensitivity and specificity are available to detect levels of infection that can lead to transmission
  - Humans are essential for the life-cycle of the agent, *which has no other vertebrate reservoir* and does not amplify in the environment

Questions for MPAG

- What additional evidence should be presented at the technical consultation?
- Do you agree with the approach taken for spatiotemporal analysis of cases in Malaysia?
- Any suggestions for types of expertise to be included among the participants in the technical consultation?
- Any thoughts on approaches to determining the staging of *P. knowlesi?*
- Can MPAG recommend to WHO how PK should be considered, and subsequently, advise on the implications for elimination/eradication?

Thank You