Estimation of Malaria-Attributable Fever in Malaria Test–Positive Febrile Outpatients in Three Provinces of Mozambique, 2018

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Abstract. Like most malaria-endemic countries, Mozambique relies on tabulation of confirmed malaria test–positive febrile patients to track incidence of malaria. However, this approach is potentially biased by incidental malaria parasitemia in patients with fever of another etiology. We compared pan-*Plasmodium* aldolase and lactate dehydrogenase and *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) antigen concentrations measured using a laboratory bead-based assay of samples collected from 1,712 febrile and afebrile patients of all ages in Maputo, Zambézia, and Cabo Delgado provinces. We used a Bayesian latent class model to estimate the proportion of malaria-attributable fevers in malaria test–positive febrile patients. Depending on the antigen, estimated rates of malaria-attributable fever in malaria test–positive febrile patients were 100% in Maputo, 33–58% in Zambézia, and 63–74% in Cabo Delgado. Our findings indicate that most malaria test–positive febrile patients in the three provinces of Mozambique had a fever that was likely caused by the concurrent malaria infection. Counting malaria test–positive febrile patients for estimation of malaria incidence appears to be appropriate in this setting.

INTRODUCTION

Malaria infection is highly prevalent in Mozambique and is a significant cause of morbidity and mortality for much of the population. In the most recent nationally representative household survey, 40% of children aged 6–59 months carried malaria antigen in their blood based on *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) rapid diagnostic test (RDT) results,¹ indicating current or very recent *P. falciparum* infection. Malaria is also a common diagnosis among patients seeking medical care. The national policy in Mozambique recommends testing for all febrile patients by RDT or microscopy and treatment with an artemisinin-based combination therapy if positive. Roughly 24% of outpatients seeking care in the public sector in 2017 were reported as diagnostically confirmed malaria cases.²

High background rates of asymptomatic concurrent malaria infection can complicate attribution of fever etiology.³ In a malaria-endemic setting, a certain proportion of febrile patients would be expected to have incidental malaria parasitemia and/or antigenemia but with a different etiology for the presenting fever. Estimating the number of febrile patients in whom the patient's malaria infection is the cause of fever is important for determining the burden of malaria and for informing differential fever management needs. In Mozambique, as in many other endemic countries, the National Malaria Control Program (NMCP) tracks the number of reported malaria test–positive (confirmed) febrile patients as its main measure of incidence. This approach for estimating incidence is incompatible with the model-based approaches for measuring incidence used by the WHO, which uses mathematical models to estimate incidence from community prevalence data.⁴ Because the latter is intended to estimate incidence

of malaria-attributable malaria test–positive fevers, it would inherently provide lower estimates and cannot be directly compared with incidence of all malaria test–positive fevers.⁵

At a population level, attribution of etiology of fever can be estimated using statistical methods. In the simplest analysis, rates of malaria test positivity can be compared between febrile and afebrile individuals to calculate the population-attributable fraction. However, this method tends to systematically underestimate the malaria-attributable fraction,³ and a superior approach is to compare either density of parasites⁶ or concentration of malaria antigen⁷ in febrile and afebrile individuals.

We analyzed data on antigen concentration from a recent health facility survey conducted in three provinces in Mozambique in 2018.⁸ Because both febrile and afebrile patients had been included in the survey and systematically tested for the presence and concentration of malaria antigen in their blood, attribution of fever etiology to malaria could be modeled for each of the three provinces included in the survey. The objective of this study was to estimate the proportion of malaria test–positive febrile patients attending the surveyed facilities who had malaria-attributable fever.

METHODS

Study design. We analyzed results of previous testing of samples collected in the health facility survey.⁹ We first estimated the proportion of malaria-attributable fever among febrile patients using a latent class model that identified malaria as a cause of fever based on the concentration of three malaria antigens. Next, we derived and used a formula to estimate the proportion of malaria-attributable fever among malaria test–positive febrile patients who participated in the survey.

Data collection. In April–May 2018, a survey was conducted in 117 randomly selected public health facilities of all levels in Maputo, Zambézia, and Cabo Delgado provinces in Mozambique.⁸ Maputo is in the south of the country, where

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malaria transmission rates are low, whereas Zambézia and Cabo Delgado are in the center and north of the country, where transmission rates are high.¹⁰ As part of the survey, randomly selected patients of all ages and symptoms were invited to undergo an exit interview and re-examination by surveyors. The exit re-examination included systematic fever screening, testing by RDTs detecting PfHRP2 (SD Bioline Pf, Yongin, Republic of Korea), and collection of dried blood spots (DBSs) on filter paper (Whatman 903; GE Healthcare, Chicago, IL). The DBSs were later analyzed in the laboratory using a bead-based immunoassay for three malaria antigens^{11,12}: PfHRP2, pan-*Plasmodium* aldolase (pAldo), and pan-*Plasmodium* lactate dehydrogenase (pLDH). The detection antibodies for PfHRP2 cross-react with the closely related *P. falciparum* histidine-rich protein 3 (PfHRP3), and the PfHRP2 signal reported here can be considered a conflation of the PfHRP2 and PfHRP3 responses. Measures of the median fluorescence intensity minus background were converted to concentrations using standard curves. The lowest level of detection of antigen in the DBS samples was 0.6 ng/mL for PfHRP2, 0.5 ng/mL for pAldo, and 224 ng/mL for pLDH.

Data analysis. Patients were classified as having a febrile illness if they reported fever as a symptom of their illness during the exit interview and/or had an axillary temperature > 37.5°C measured during the re-examination.

The standard algebraic formula for population-attributable fraction was applied to rates of fever among antigen-positive and antigen-negative patients,⁷ stratifying by province, to provide crude estimates of λ , the proportion of fever attributable to presence of malaria antigenemia.

Next, a latent class Bayesian model^{6,7} was also used to estimate λ . Briefly, this approach compared the distribution of antigen concentrations between febrile and afebrile patients to estimate the posterior distribution of λ as well as the incidental parameters θ (the distribution of antigen concentration in afebrile individuals), ϕ (the distribution of antigen concentration in febrile individuals), and λ_i (the malaria-attributable fraction as a function of antigen concentration). The posterior probabilities were sampled using a Monte Carlo Markov Chain algorithm coded in WinBUGS.¹³ As with the approach for the crude rate, the Bayesian analysis was run separately by province and by antigen.

Next, a formula for the proportion of malaria test-positive patients with malaria-attributable fever, denoted as μ , was derived.

$$\begin{aligned}\mu &= P(F_m|F, T, O) \\ &= \frac{P(F_m, T, O)}{P(F, T, O)} \\ &= \frac{P(T, O|F_m)P(F_m)}{P(F, T, O|F_m)P(F_m) + P(F, T, O|F_o)P(F_o)} \\ &= \frac{P(T|O, F_m)P(O|F_m)P(F_m)}{P(O|F_m)P(F_m) + P(T|O, F_o)P(O|F_o)P(F_o)}.\end{aligned}$$

Here, F denotes any fever, F_m denotes malaria-attributable fever, F_o fever of other etiology, T malaria test positivity, and O outpatient. The conditional probabilities, thus, denote the following: $P(O|F_m)$ the probability of being an outpatient (i.e., seeking care) given malaria-induced fever, $P(O|F_o)$ the probability of being an outpatient given non-malaria-induced fever,

$P(T|O, F_m)$ the test positivity rate in outpatients with malaria-induced fever, and $P(T|O, F_o)$ the test positivity rate in febrile outpatients without malaria-induced fever.

We assumed no difference in care seeking by malaria and non-malaria-induced fever, so $P(O|F_m) = P(O|F_o)$. Recent rounds of RDT product testing have shown current RDT sensitivity to be very close to 100% at densities of 2,000 parasites/ μ L,¹⁴ a threshold purposely chosen to correspond to published estimates of the pyrogenic threshold.¹⁵ We therefore, assumed that everyone with malaria-induced fever would test malaria positive, so $P(T|O, F_m) = 1$. Finally, we used the following substitution derived from the definition of λ :

$$\lambda = P(F_m|F) = \frac{P(F_m)}{P(F_m) + P(F_o)}.$$

Thus, the final formula for μ was as follows:

$$\mu = \frac{\lambda}{\lambda + P(T|O, F_o)(1 - \lambda)}.$$

This formula was separately applied using the crude and latent class estimates of λ for RDT positivity and PfHRP2, pLDH, and pAldo concentration. The term $P(T|O, F_o)$, the test positivity rate in febrile outpatients without malaria-induced fever, was approximated as the rate of RDT positivity in afebrile patients. Point estimates and 95% credible intervals (CIs) were calculated.

Data analysis was conducted in R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical review. The survey protocol, including antigen analysis of blood samples, was reviewed and approved by the Mozambique National Health Bioethics Committee (protocol number: 338/CNBS/17) and the Office of the Associate Director for Science in the Center for Global Health at the CDC (CGH2017-517).

RESULTS

A total of 1,712 samples were analyzed from the three provinces of Mozambique. Rates of RDT and antigen positivity among febrile and afebrile patients ranged widely across the provinces, with Maputo Province having substantially lower rates than the other two provinces (Table 1). Consequently, the crude estimates of λ , the proportion of malaria-attributable fever among all febrile individuals, ranged from 1.9% to 4.1% in Maputo, 7.0–16% in Zambézia, and 7.6–16% in Cabo Delgado, depending on the definition of malaria positivity (Table 1). In general, the crude estimates for λ were smallest when based on pLDH positivity, whereas the estimates based on RDT, PfHRP2, and pAldo positivity were higher and similar to each other.

Using the latent class model, estimates of λ based on PfHRP2 concentration were 20% (95% CI: 8–31%) in Zambézia and 25% (15–34%) in Cabo Delgado (Table 1). Because there were a small number of test-positive samples in Maputo, the latent class model provided unstable estimates of λ in that province and modeled estimates were lower than the crude estimates. Latent class estimates of λ using pLDH and pAldo ranged from 11–25% in Zambézia and 20–29% in Cabo Delgado. As with the crude estimates, latent class model estimates of λ were generally larger for PfHRP2 and pAldo compared with pLDH. The distribution of the observed antigen

TABLE 1

Crude and latent-class model estimates of the proportion of fever attributable to malaria, by antigen positivity, in three provinces surveyed in Mozambique, 2018

	Maputo, <i>N</i> = 492		Zambézia, <i>N</i> = 589		Cabo Delgado, <i>N</i> = 631	
PfHRP2 RDT test positivity rate in febrile patients	7.8% (24/307)		53% (226/425)		54% (26/493)	
PfHRP2 RDT test positivity rate in afebrile patients	0.0% (0/185)		24% (40/164)		14% (20/138)	
Crude estimates of proportion of fever attributable to malaria (λ)†	%		%		%	
PfHRP2 RDT	3.1		15		16	
PfHRP2 bead-based assay	4.1		16		15	
pAldo bead-based assay	3.2		14		12	
pLDH bead-based assay	1.9		7.0		7.6	
Latent class model estimates of proportion of fever attributable to malaria (λ)‡	%	95% CI	%	95% CI	%	95% CI
PfHRP2 bead-based assay	0.7	0.03–3	20	8–31	25	15–34
pAldo bead-based assay	1.8	0.2–5	25	14–34	29	21–35
pLDH bead-based assay	0.9	0.04–4	11	2–19	20	10–27

CI = credible interval; pAldo = pan-*Plasmodium* aldolase; PfHRP2 = *Plasmodium falciparum* histidine-rich protein 2; pLDH = pan-*Plasmodium* lactate dehydrogenase; RDT = rapid diagnostic test.

* Estimates of λ for Maputo Province unstable because of low absolute numbers of malaria-positive patients.

† Comparison of test positivity in febrile and afebrile patients.

‡ Comparison of antigen concentration in febrile and afebrile patients.

concentration and the incidental parameters estimated by the latent class model are presented in the Supplemental Figures 1–4.

The point estimates of μ , the proportion of malaria-attributable fever among malaria test-positive febrile patients, were 100% in Maputo using the crude and latent class estimates of λ for all antigens (Table 2). This was because there were no malaria test-positive afebrile cases observed in the study. In Zambézia and Cabo Delgado, where malaria test positivity among afebrile patients was 24% and 14%, respectively, the estimates of μ were lower. In Zambézia, estimates of μ ranged from 33% (8–51%) for pLDH to 58% (42–70%) for pAldo. In Cabo Delgado, estimates ranged from 63% (46–79%) for pLDH to 74% (62–84%) for pAldo. As before, pLDH provided the lowest estimates compared with PfHRP2 and pAldo, but the 95% CIs overlapped for all three antigens.

DISCUSSION

There was substantial variability in the proportion of fevers estimated to be due to malaria infection in this Mozambican study population, largely driven by the difference in transmission intensity between the included provinces. There was also substantial variation in the proportion

of malaria-attributable fever among malaria test-positive patients by province. In low-transmission Maputo Province, which enrolled only 24 malaria test-positive febrile patients during the survey, only 1–4% of all fevers were estimated to be attributable to malaria. However, because there were no malaria test-positive afebrile patients, the estimate of the proportion of malaria test-positive fevers for which malaria would be expected to be the etiology of the fever was 100%, albeit with wide CIs. This is consistent with the view that in areas with low transmission and consequently a population without robust immunity to malaria, fever in a malaria test-positive patient is most likely due to the malaria infection. Importantly, the variability in the proportion of malaria-attributable fever among malaria-positive febrile individuals depending on the method of analysis—by antigen, by province, and by crude versus latent class model—suggests that there is likely no single-parameter correction factor that can be used to convert routine incidence data to only reflect malaria-attributable fever.

Despite variability, the estimates of malaria-attributable fever among malaria test-positive patients were also high in Zambézia and Cabo Delgado, provinces with some of the highest intensity malaria transmission in the world. Previous work has suggested that the estimates of malaria-attributable fraction using PfHRP2 concentration are the most robust of

TABLE 2

Crude and latent class model estimates of the proportion of fever attributable to malaria in malaria test-positive febrile patients (μ), by antigen positivity, in three provinces surveyed in Mozambique, 2018

	Maputo		Zambézia		Cabo Delgado	
	%	95% CI	%	95% CI	%	95% CI
Crude estimates of proportion of fever attributable to malaria in test-positive febrile patients (μ)*						
PfHRP2 rapid diagnostic test	100	—	41	—	57	—
PfHRP2 bead-based assay	100	—	44	—	57	—
pAldo bead-based assay	100	—	40	—	53	—
pLDH bead-based assay	100	—	24	—	34	—
Latent class model estimates of proportion of fever attributable to malaria in test-positive febrile patients (μ)†						
PfHRP2 bead-based assay	100	1–100	50	27–67	70	53–82
pAldo bead-based assay	100	7–100	58	42–70	74	62–84
pLDH bead-based assay	100	1–100	33	8–51	63	46–79

CI = credible interval; pAldo = pan-*Plasmodium* aldolase; PfHRP2 = *Plasmodium falciparum* histidine-rich protein 2; pLDH = pan-*Plasmodium* lactate dehydrogenase.

* Comparison of test positivity in febrile and afebrile patients.

† Comparison of antigen concentration in febrile and afebrile patients.

the three antigen-based estimates provided here.⁷ According to the estimates based on PfHRP2 concentration, at least 50% of malaria test-positive outpatients likely had fever that could be attributed to the malaria infection in these high-transmission areas. Estimates derived from pAldo concentration were even higher, at 58% for Zambézia and 74% for Cabo Delgado, possibly reflecting pAldo's better performance as a marker of active infection.¹⁶ Estimates for λ and μ for the LDH antigen were consistently lower than those based on PfHRP2 and pAldo or on the RDT result. Of the three assays used in this study, the pLDH assay is by far the least sensitive, so it is likely that many true infections simply had pLDH levels that were below the limit of reportability for the antigen immunoassay. The level of detection for each antigen is a major factor in the robustness of the λ and μ estimates, with lower levels of detection (higher sensitivities) more likely to provide accurate and unbiased estimates. Variation among λ and μ estimates among different antigens should be considered with appropriate interpretation for both the biological expression levels in human infections as well as the limitations of the assay being used to detect the antigens.

The survey was performed during the end of the rainy season in Mozambique. Pyrogenic thresholds, the concentration of parasites that lead to fever, are known to vary by season,^{17,18} and it is unknown how the results would differ if samples had been collected during the dry season, when malaria transmission is less intense. We did not have sufficient sample size to stratify by age, another factor known to influence the pyrogenic threshold.¹⁹ Rates of non-falciparum malaria mono-infections in the sample set were previously shown to be low (< 1%), and it was not possible to stratify the analysis by species. A fundamental underlying assumption of the model is that fever is a marker of symptomatic malaria infection, although it has been well documented that the spectrum of malaria symptoms is wide and does not always include fever.²⁰ As such, the model might underestimate the proportion of malaria test-positive outpatients whose symptoms are etiologically linked to malaria. Finally, the results around attribution of etiology can only be applied at a population level, with no patient-level inference possible.

Nevertheless, these findings can inform how the NMCP in Mozambique interprets malaria incidence data. The results suggest that, at least in the study area and period, most malaria test-positive fevers in outpatients were due to the patients' malaria infection, and this attributable fraction of fevers was inversely related to transmission intensity in a province as would be expected. This in turn suggests that incidence estimated from routine health facility visits could largely reflect the true malaria burden among patients seeking care, conditional on universal testing of febrile patients. The Mozambique NMCP should be encouraged to continue to use malaria-positive outpatients as the main data source for malaria incident cases in the country. Finally, the findings here highlight the utility of rigorous health facility surveys with systematic screening and malaria testing of both febrile and afebrile patients. Characterization of malaria infection status among afebrile patients is crucial for building the evidence base around attribution of fever etiology in malaria-infected individuals.

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