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## YA-UMPHAN AND OTHERS

### *P. FALCIPARUM* MALARIA RISK ON THE TMB

## *Anopheles* Salivary Biomarker as a Proxy for Estimating *Plasmodium falciparum* Malaria Exposure on the Thailand–Myanmar Border

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### Abstract.

Timely identification and treatment of malaria transmission “hot spots” is essential to achieve malaria elimination. Here we investigate the relevance of using an *Anopheles* salivary biomarker to estimate *Plasmodium falciparum* malaria exposure risk along the Thailand–Myanmar border to guide malaria control. Between May 2013 and December 2014, > 9,000 blood samples collected in a cluster randomized control trial were screened with serological assays to measure the Ab responses to *Anopheles* salivary antigen (gSG6-P1) and *P. falciparum* malaria antigens (CSP, merozoite surface protein 119 [MSP-1<sub>19</sub>]). *Plasmodium falciparum* infections were monitored through passive and active case detection. Seroprevalence to gSG6-P1, MSP-1<sub>19</sub>, and CSP were 71.8% (95% Confidence interval [CI]: 70.9, 72.7), 68.6% (95% CI: 67.7, 69.5), and 8.6% (95% CI: 8.0, 9.2), respectively. Multivariate analysis showed that individuals with the highest Ab response to gSG6-P1 had six times the odds of being positive to CSP antigens ( $P < 0.001$ ) and two times the odds of *P. falciparum* infection compared with low gSG6-P1 responders ( $P = 0.004$ ). Spatial scan statistics revealed the presence of clusters of gSG6-P1 that partially overlapped *P. falciparum* infections. The gSG6-P1 salivary biomarker represents a good proxy for estimating *P. falciparum* malaria risk and could serve to implement hot spot–targeted vector control interventions to achieve malaria elimination.

### INTRODUCTION

Malaria along the Thailand–Myanmar border (TMB) is characterized by foci of high prevalences of asymptomatic and submicroscopic *Plasmodium* spp. carriage.<sup>1</sup> Over the past several decades malaria caused by *Plasmodium falciparum* infection has continuously declined

in populations living along the TMB that has mainly been attributed to the use of rapid diagnostic tests (RDTs) for early malaria detection and treatment of malaria with highly effective artemisinin-based combination antimalarial therapies and, to a lesser extent to prevention of mosquito bites.<sup>2,3</sup> The recent emergence of *P. falciparum* parasites that are resistant to artemisinin and artemisinin partner drugs along the TMB raised concern about the potential spread of artemisinin resistance to India and Africa and threatens recent gains in the reduction in the burden of malaria in the region and globally.<sup>4,5</sup> To contain *P. falciparum* artemisinin resistance, the Shoklo Malaria Research Unit and partners conducted a mass drug administration (MDA) pilot trial with the aim to eliminate submicroscopic reservoirs of *Plasmodium* spp. parasites and interrupting malaria transmission.<sup>6,7</sup> Along the TMB, local malaria transmission is heterogeneously distributed and the risk of malaria may be confined to geographically small high-risk areas and subsets of the population.<sup>3</sup> Identifying and targeting these malaria transmission “hot spots” and “hot-pops” is therefore essential to remove the remaining sources of transmission and to achieve malaria elimination.<sup>8</sup>

Measuring local malaria transmission poses considerable challenges because of the lack of sensitivity of commonly used entomology methods.<sup>9,10</sup> Assessing malaria transmission intensity by determining the entomological inoculation rate (EIR) is challenging considering the low frequency of mosquitoes positive for infection and spatial and temporal variations in mosquito densities and composition necessitate long-term intensive sampling.<sup>11</sup> Serological biomarkers using antibodies specific for *Plasmodium* spp. are increasingly used to estimate changes in malaria transmission in areas of low endemicity.<sup>12–16</sup> Measuring antimalarial antibodies to detect malaria transmission offer several advantages (compared with entomological and parasite outcomes) because of the longer duration of specific antibody responses, they are indicative of recent malaria exposure rather than point prevalence.<sup>12,17</sup> Recently, new serological biomarkers that measure the intensity of human exposure to mosquito salivary antigens have been identified (reviewed in Doucoure and Drame<sup>18</sup>). Compared with other serological tools, salivary markers offer great potential for measuring small-scale variations in the exposure to malaria vectors as they provide shorter lived antibody responses.<sup>18</sup> In a previous study, we demonstrated that antibodies specific for the salivary biomarker gSG6-P1 are relevant to quantify human–vector contact and estimate the malaria transmission risk at the TMB.<sup>19</sup> We showed that the risk of malaria transmission strongly varied in space and time and was influenced by the environment and human behavior. Nevertheless, the relationship between human exposure to *Anopheles* bites and the risk of being infected by *P. falciparum* malaria was not elucidated.

The aim of the present study was to address whether *Anopheles* salivary biomarker can detect small-scale variations in human exposure to *P. falciparum* malaria in a context of malaria elimination. This information is pivotal in public health programs to improve malaria surveillance and guide vector control programs.

## MATERIAL AND METHODS

### Study site and survey procedure.

Four villages, Htoo Pyin Nyar (TPN, 17°14'N, 98°29'E), Tar Au Ta (TOT, 16°36'N, 98°57'E), Ka Nu Hta (KNH, 17°18'N, 98°24'E), and Htee Kaw Taw (HKT, 16°85'N, 98°47'E), with > 10% *P. falciparum* prevalence by high-volume ultrasensitive qPCR (uPCR) were selected after the engagement of the community.<sup>1</sup> A community-based malaria clinic (“malaria post” or

MP) was set up in each village to monitor malaria infections with SD Bioline Pf/Pv RDT.<sup>20</sup> A census was performed before the surveys and demographic information was collected.<sup>19</sup> Two villages (TOT and KNH) were randomly assigned to MDA intervention immediately and two (HKT, TPN) were followed for 9 months (control period) before receiving MDA as described in Landier et al.<sup>20</sup> Briefly a 3-day treatment course of dihydroartemisinin–piperaquine and a single 0.25-mg base/kg dose of primaquine was administered orally under supervision every month for 3 months to all participants. Long-lasting impregnated nets (LLIN) were distributed to all households at the start of the study (M0). Seven household surveys were carried in each village every 3 months (month 0, 3, 6, 9, 12, 15, and 18) from May 2013 to December 2014. At each survey, dried blood spots on Whatman filter papers (3MM, 0.34 mm thickness) were collected during surveys of the entire village population for enzyme-linked immunosorbent assay (ELISA). *Plasmodium* spp. infections were also recorded by uPCR according to methods described previously.<sup>1,21</sup>

### **Antigen selection.**

The gambiae salivary peptide gSG6-P1 was selected because it is highly antigenic and highly specific to the *Anopheles* genus, with no relevant cross-reactivity with epitopes from other proteins or vectors of parasites.<sup>22,23</sup> The synthetic nature largely ensures high reproducibility of the assay and it induces short-term (up to 2 months) and host-specific humoral response.<sup>18</sup> The *P. falciparum* antigens merozoite surface protein 119 (MSP-1<sub>19</sub>) and CSP were selected for their capacity to detect different life cycle stages of the *Plasmodium* (sporozoite versus merozoite) and for their difference in immunogenicity and persistence.<sup>15,24</sup>

### **Enzyme-linked immunosorbent assay: Human antibody response to *P. falciparum* antigens.**

Serologic testing of human exposure to MSP-1<sub>19</sub> and CSP antigens was carried out by using ELISA as described in the study by Drakeley et al.,<sup>15</sup> but with some modifications (Supplemental Text 1). Individual responses were expressed as the  $\Delta OD$  value:  $\Delta OD = OD_x - OD_n$ .  $OD_x$  and  $OD_n$  represent the mean of individual optical density (OD) in two antigen wells and one blank well containing no Pf MSP-1<sub>19</sub>/CSP peptide, respectively. Specific anti-MSP-1<sub>19</sub>/CSP IgG responses were also determined by ELISA in nonmalaria-exposed individuals (negative samples from France:  $N = 18$ ) to quantify the nonspecific background Ab level and to calculate the cut-off value for seropositivity (mean [ $\Delta OD_{\text{neg}}$ ] + 3 SD). Procedures for testing human exposure to gSG6-P1 salivary biomarker have previously been described.<sup>19</sup>

### **Statistical analysis.**

To investigate the association between the intensity of Ab response to *Anopheles* salivary antigen (gSG6-P1; categorical variable: low, medium, high, very high; based on quartiles) and Ab responses specific for *P. falciparum* CSP and MSP-1<sub>19</sub> (binary variable: positive, negative), we used a multivariate multilevel logistic mixed regression model with adjustment for relevant covariates, which represented proxies for social, demographic, or environmental status. At the individual level, covariates included Ab response to gSG6-P1, age group, gender, and MDA treatment (a time-dependent individual binary variable, 0 as long as the individual has not taken any drugs, 1 when individuals have received 1, 2, or 3 doses). Household-level covariates included LLIN use (never, sometimes, and every night). At the village level, the population size at each survey, the temperature, and relative humidity were taken into account in the model (i.e.,

second order polynomial) and season was defined according to the Thai Meteorological Department. In a second analysis, we investigated the relationship between the intensity of Ab response to *Anopheles* salivary antigens (four categories) and *P. falciparum* malaria infections (binary variable: positive and negative) using a multivariate mixed logistic regression adjusted for covariates described previously. Statistical analyses were carried out with Stata version 13.0 (StataCorp LP, College Station, TX). Graphs were constructed using GraphPad Prism 5 software (San Diego, CA). A sensitivity analysis was conducted using ellipsoid rather than spherical windows.

### **Spatial analysis.**

Geographic references (latitude and longitude) were recorded for all households in the study villages. Each house was given an identification code and all study participants could be linked back to their respective houses using the identification code. Scan statistics were used to test for statistically significant clusters of salivary biomarker (gSG6-P1) and *P. falciparum* malaria infections as measured by uPCR (surveys) and RDT (MP). A discrete space-time Poisson model was used to test for statistically significant clusters for each of these outcomes and for each study village across all screenings (M0–M18).<sup>25,26</sup> The scan statistic uses a moving window (a spherical kernel) that centers on each point (house) in the village and calculates the number of cases (*P. falciparum* infections or biomarker positives) within the window and the number of expected cases within the window, given the population within the window and the distribution of cases and population across the entire village. The window increases in size until half of the village population is contained and then moves to the next point. Likelihood ratios are calculated for each window location, size, and time point and *P* values are calculated using Monte Carlo simulations for the largest ranking clusters. Scan statistics were calculated using SaTScan software (<https://www.satscan.org/>).

### **Ethical approval.**

This study was part of a multicenter cluster randomized control trial conducted in several sites in the Greater Mekong subregion and registered on ClinicalTrials.gov: NCT01872702. Study protocol was reviewed and approved by OxTREC (reference no. 1017–13 and 1015–13).

## **RESULTS**

### **Study populations and outcomes.**

Participants consisted of 2,602 people followed up every 3 months over 18 months and are described in Table 1. The compositions of the four study villages were comparable in age and gender. The overall prevalence of *P. falciparum* infections over the surveys ranged from 0.88% (17/1,934) in TPN to 3.14% (64/2,036) in KNH. The incidence of *P. falciparum* recorded at MP ranged from 1 per 1,000 people per year at HKT to 45.5 at TOT. A total of 9,373 and 9,401 dried spots were analyzed for CSP and MSP-1<sub>19</sub>, respectively. Overall, Ab seroprevalence was higher for MSP-1<sub>19</sub> (range: 23.9–100%) than for CSP (range: 0.4–42%) and increased with age in each village, with the exception of TNP where CSP seroprevalence was lower in subjects > 60 years than in other age groups (Table 1). The seroprevalence to gSG6-P1 ranged from 59% at TPN to 86% at HKT.

### **Seroprevalence in antibody response to *Anopheles* spp. and *P. falciparum* antigens.**

The Ab response to the MSP-1<sub>19</sub> and CSP antigens varied according to the village and survey (Figure 1). For MSP-1<sub>19</sub>, seroprevalence greatly increased from M3 to M9 in all sites, except at HKT where it remained constant all along the study ( $\approx 60\%$ ) (Supplemental Table 1). By contrast, seroprevalence to CSP increased by  $\approx 2$ -fold from M0 to M3 in all sites and then continuously declined until M18 (Supplemental Table 2). The seroprevalence to gSG6-P1 was high (71.8%, 95% Confidence interval [CI]: 70.9, 72.7) and strongly varied across villages and surveys (Supplemental Table 3).

### **Relationship between *P. falciparum* malaria and vector exposure.**

The mean number of measurements per individual was 3.4 (range, 1–6). Multivariate logistic mixed analyses showed a highly significant and positive dose–response relationship between Ab response to gSG6-P1 and the odds of a positive Ab response against *P. falciparum* CSP and MSP-1<sub>19</sub> (Table 2). Individuals with the 25% highest Ab response to gSG6-P1 (i.e., “very high” responders) had 6 times the odds of being positive to CSP antigens (OR: 5.94, 95% CI: 3.72–9.48,  $P < 0.001$ ) compared with low gSG6-P1 responders. Increased odds of positive MSP-1<sub>19</sub> responses were also demonstrated but the magnitude of effect was smaller (OR: 2.91, 95% CI: 1.86–3.67,  $P < 0.005$ ). Ad hoc multivariate analysis showed that the very high responders to gSG6-P1 were also associated with increased odds of *P. falciparum* infection after adjustment for other covariates (OR: 2.27, 95% CI: 1.35–3.80,  $P = 0.002$ , Table 3).

Multivariate logistic analysis also showed that the odds of *P. falciparum* Ab prevalence strongly increased with age and was lower in females compared with males (Table 2). The seroprevalence of *P. falciparum* Ab varied according to village ( $P < 0.001$ ) and season ( $P < 0.001$ ) but the strength and direction of the association differed according to antigen. The highest seroprevalence to CSP was in HKT whereas KNH showed the highest seroprevalence to MSP-1<sub>19</sub> (Table 2). The rainy and cool seasons were associated with higher odds of positive CSP responses compared with the hot season (OR: 9.47, 95% CI: 2.69, 33.35,  $P < 0.001$  and OR 8.34, 95% CI: 2.47, 28.11,  $P = 0.001$ ). Mass drug administration was associated with decreased odds of CSP seroprevalence (OR: 0.49, 95% CI: 0.33, 0.74,  $P = 0.001$ ) but increased odds of MSP-1<sub>19</sub> Ab seroprevalence (OR: 4.18, 95% CI: 3.15, 5.54,  $P < 0.001$ ). Finally, there was no strong evidence of an association between bednet use and the seroprevalence of *P. falciparum* Ab (Table 2).

### **Spatial patterns in *P. falciparum* malaria infections.**

We mapped together the very high responders to *Anopheles* salivary bites with *P. falciparum* infections monitored at either surveys or MPs during the rainy season, at baseline (M0, no MDA) and then 15 months after implementation of MDA (M15). At baseline, three villages (KNH, TOT, and HKT) had statistically significant clusters of *P. falciparum* malaria that partially overlapped with clusters of gSG6-P1 at KNH and HKT (Figure 2). In KNH, there were 34 houses in the salivary response cluster and 65 houses in the two Pf clusters and 28 of these houses overlapped. In HKT, there were 78 houses in the salivary response cluster and also 78 houses in the Pf cluster and 75 of these houses overlapped (96%). Htoo Pyin Nyar showed a detectable hotspot of *P. falciparum* infections in the middle of the village, with no apparent hotspot of vector exposure (the number of *P. falciparum* infections were however low). At TOT, *P. falciparum* infections were dispersed within the village (no clustering) and a wide cluster of

vector exposure was seen ( $P < 0.0001$ ; relative risk = 16.15). *P. falciparum* infections were drastically reduced by M15 in all villages receiving MDA (Figure 2), except at TOT were remaining pockets of *P. falciparum* malaria infections and vector exposure persisted in the western part of the village. In this site, there were 64 houses in the salivary response cluster in and 92 in the Pf cluster and 64 of these overlapped. All of the salivary response cluster houses were contained within the Pf cluster (70% of the Pf cluster overlapping with the salivary response cluster).

All hotspot houses detected with spherical windows were also detected using ellipsoid windows, with slight variations in hotspot window shape. We then retained the spherical windows for final visualization.

## DISCUSSION

In the present study, we used antibody responses to demonstrate a strong and significant relationship between *Anopheles* exposure and *P. falciparum* malaria in areas of low endemicity along the TMB. This association was shown for antibody responses to two different *P. falciparum* antigens (MSP-1<sub>19</sub> and CSP) and confirmed by the observation that very high responders to gSG6-P1 were also at higher risk of *P. falciparum* infections. This was further supported by spatial statistics showing that *P. falciparum* clusters partially overlap the gSG6-P1 clusters before and after the use of MDA for malaria elimination. This confirms previous results in the same villages showing a significant correlation between EIR and Ab responses to gSG6-P1 salivary antigen.<sup>19</sup> Altogether, these findings suggest that anti-gSG6-P1 could be used to estimate population vulnerability to malaria vector bites and hence be used as a proxy to estimate malaria transmission risk as part of malaria surveillance and elimination.

The spatial mapping data showed that *P. falciparum* infections clustered in specific parts of the study villages, which were partially geographically correlated to vector mosquito exposure. Although it is likely that some *P. falciparum* malaria are imported in the study villages by migration and population movement,<sup>27</sup> our findings indicate that heterogeneity in *P. falciparum* malaria is positively associated with heterogeneous mosquito-biting behavior, hence reflecting “focal” transmission risk. Spatial autocorrelation was less obvious over time probably because of the successive introduction of MDA that eliminated  $> 95\%$  *P. falciparum* reservoir.<sup>20</sup> Despite that, the salivary biomarker was successful to identify remaining sources of *P. falciparum* transmission at TOT, where low coverage (75%) of MDA was reported.<sup>20</sup> Absence of spatial autocorrelation between vector exposure and *P. falciparum* exposure in some surveys suggest also that a nonnegligible part of malaria transmission probably occurred outside the villages.

Our study showed that the risk of exposure to malaria and the vector vary in space (village) and time (season). The reasons for higher malaria risk in particular locations within villages are presently unknown but we assume that this may reflect different human behavior and socioeconomic conditions, environment (presence of mosquito breeding habitats), vector control practices, etc as demonstrated by others.<sup>3</sup> As expected, a clear age-dependent response in malaria-specific immune responses was seen, which confirms exposure-driven acquisition of antibody responses.<sup>13,28,29</sup> This trend was more pronounced with MSP-1<sub>19</sub> than CSP, probably due to the stronger immunogenic property of this antigen. Interestingly, the higher odds of *P. falciparum* seropositivity to CSP and MSP in older age group correlates well with the age-dependent response to *Anopheles* exposure observed previously in the area.<sup>19</sup> We showed that females were also at a lower risk of *P. falciparum* malaria exposure than males confirming the

predominance of *P. falciparum* infections in adult males along the TMB due to different behavior, movement, and occupation.<sup>2</sup>

Other risk factors associated with sero-reactivity to *P. falciparum* infections was MDA, but the strength and direction of the association differed according to antigen (CSP or MSP-1<sub>19</sub>). The observed differences between antigens may be because of differences in immunogenic properties (e.g., longevity of antibody responses) or differences in exposure of life cycle-specific antigens to the immune system (immunogenicity of antigens); sporozoite antigens, such as CSP, are exposed to the immune system for a shorter duration than blood-stage merozoite antigens, such as MSP-1<sub>19</sub>.<sup>30,31</sup> Finally, potential cross-reactivity between *P. falciparum* and *Plasmodium vivax* antigens cannot be ruled out. In areas co-endemic for *P. falciparum* and *P. vivax*, homologous antigens may elicit cross-reactive antibodies<sup>32</sup> and MSP-1<sub>19</sub> shares ~50% amino acid identity between the two species. Cross-reactivity may also have contributed to the maintenance of specific Ab responses to MSP in areas where *P. vivax* persists after MDA.<sup>20</sup>

Finally, our study revealed that frequent bednet use (“self-reporting”) was not associated with a reduction of *P. falciparum* malaria exposure, which is consistent with previous findings, showing an absence of correlation between net use and the Ab response to *Anopheles* bites.<sup>19</sup> Along the TMB, malaria vectors bite preferentially outdoors and in the early evening when people are not protected by insecticide treated nets.<sup>33,34</sup> This finding suggests that more appropriate personal protection tools (e.g., repellents, treated clothes) should be delivered to people at risk of malaria to strengthen malaria control and elimination efforts. Our results also confirm the great potential of using salivary markers to evaluate the efficacy of vector control interventions, where malaria prevalence and intensity of transmission become low.<sup>35</sup> The development of factory-made rapid diagnostic device for detection of human exposure to *Anopheles* bites would be useful to replace labor-intensive ELISA and be routinely incorporated into elimination programs.

## CONCLUSION

In communities living in areas of low seasonal malaria transmission, where reservoirs of submicroscopic infections have been identified, the biomarker of exposure to vector correlates well with the risk of *P. falciparum* infections. In this study, the relationship between the vector exposure (biomarker) and risk of malaria carriage (Ab) is complex, partly because of MDA and because of the complexities of malaria ecology in the region. Biomarker could be a useful tool to measure the impact of targeted vector control measures deployed as part of an elimination effort that addresses the parasite reservoirs in the “hot spots” villages. These vector control measures are important because LLIN may provide little protective efficacy against *Anopheles* vectors in this region.

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FIGURE 1. Seroprevalence data with 95% confidence interval for antibodies against *Plasmodium falciparum* merozoite surface protein 119 (MSP-1<sub>19</sub>), and *P. falciparum* sporozoite (CSP), according to villages Ka Nu Hta, Tar Au Ta, Htee Kaw Taw, and Htoo Pyin Nyar (KNH, TOT, HKT, and TPN) and surveys (seven surveys over 18 months). Seropositivity for MSP-1<sub>19</sub> and CSP was calculated based on cut-off values ( $\Delta OD$ ) > 0.162 and > 0.115, respectively. OD = optical density.

FIGURE 2. Micogeographical clusters of Pf malaria infections and vector exposure in the study villages at baseline (M0) and then after the implementation of the mass drug administration (MDA) pilot trial (M15). The large red (*Plasmodium falciparum* infections as measured by either ultrasensitive qPCR or rapid diagnostic test) and green (very high responder to gSG6-P1) circles are the statistically significant hotspots detected by spatial scan statistics ( $P < 0.001$ ). Pf infections are shown in red diamonds. Before MDA (M0), three villages Tar Au Ta, Ka Nu Hta, and Htee Kaw Taw (TOT, KNH, and HKT) had multiple clusters of gSG6-P1 responders (Relative Risk [RR] = 16.15, 9.29, 8.83, and,  $P < 0.0001$ , respectively) that partially overlapped with clusters of *P. falciparum* infections at KNH

(RR = 30.20,  $P < 0.0001$ ) and HKT (RR = 19.46,  $P < 0.0001$ ). After MDA (M15 conducted at the end of the rainy season), *P. falciparum* infections almost disappeared in all villages except at TOT where a cluster of *P. falciparum* infections was detected in the western part of the village (RR = 77.23,  $P < 0.0001$ ) that mostly overlapped with the cluster of gSG6-P1 (RR = 5.86,  $P < 0.0001$ ).

TABLE 1

Descriptive statistics of participants, by study sites

Characteristics	Htoo Pyin Nyar		Tar Au Ta		Ka Nu Hta		Htee Kaw Taw	
Age (years), median (range)	21 (0–66)		19 (0–80)		22 (0–73)		19 (0–94)	
Female gender, %	47		52		46		50	
Pf prevalence by uPCR % (no. positive/no. tested)	0.88 (17/1,934)		2.77 (51/1,843)		3.14 (64/2,036)		1.62 (59/3,648)	
Pf incidence by rapid diagnostic test (cases per 1,000 per year)	11.4		45.5		6.6		1.0	
Antibody prevalence to gSG6-P1	59.3 (1,131/1,906)		68.8 (1,356/1,970)		61.4 (1,256/2,046)		86.3 (3,024/3,503)	
Antibody prevalence to Pf antigen, visits (%)	CSP	MSP-1 <sub>19</sub>	CSP	MSP-1 <sub>19</sub>	CSP	MSP-1 <sub>19</sub>	CSP	MSP-1 <sub>19</sub>
All ages	6.0 (113/1,892)	66.7 (1,268/1,902)	14.5 (284/1,958)	73.7 (1,452/1,969)	8.7 (179/2,050)	77.9 (1,591/2,043)	6.7 (231/3,469)	61.3 (2,136/3,484)
Ages 0–4 years	0.4 (1/241)	51.4 (125/243)	3.2 (10/309)	28.0 (87/311)	1.3 (3/225)	44.0 (99/225)	1.5 (7/453)	23.9 (109/456)
Ages 5–15 years	3.6 (20/551)	63.6 (353/555)	5.4 (34/627)	66.4 (420/633)	4.1 (22/540)	68.8 (368/535)	3.4 (431/1,262)	54.0 (683/1,264)
Ages 16–59 years	8.7 (91/1,051)	70.9 (748/1,055)	21.7 (202/932)	91.8 (857/934)	11.9 (145/1,215)	86.9 (1,055/1,214)	9.9 (165/1,661)	76.2 (1,273/1,671)
Ages > 60 years	2.0 (1/49)	85.7 (42/49)	42.2 (38/90)	96.7 (88/91)	12.9 (9/70)	100.0 (69/69)	17.2 (16/93)	76.3 (71/93)

MSP-1<sub>19</sub> = merozoite surface protein 119; uPCR = ultrasensitive qPCR.

TABLE 2

Multivariate logistic regression mixed model showing the relationship between *Plasmodium falciparum* antibody responders (CSP, MSP-1<sub>19</sub>) and the intensity of antibody responses to gSG6-P1 salivary antigen and other covariates

Characteristics	Ab response to CSP			Ab response to MSP-1 <sub>19</sub>		
	OR	95% CI	P value	OR	95% CI	P value
Ab response to gSG6-P1						
Low	1	–	–	1	–	–
Medium	2.08	1.31–3.32	0.002	1.81	1.35–2.44	< 0.001
High	3.74	2.35–5.95	< 0.001	1.71	1.25–2.34	0.001
Very high	5.94	3.72–9.48	< 0.001	2.61	1.86–3.67	< 0.001
Age, years						
< 5	1	–	–	1	–	–
5–15	5.05	1.89–13.47	0.001	28.83	15.67–52.97	< 0.001
16–59	32.90	12.57–86.12	< 0.001	285.60	147.36–553.51	< 0.001
> 59	96.89	26.15–359.03	< 0.001	1,629.94	345.53–7,688.80	< 0.001
Gender						
Male	1	–	–	1	–	–
Female	0.70	0.46–1.05	0.082	0.46	0.31–0.68	< 0.001
Village						
Htee Kaw Taw	1	–	–	1	–	–
Htoo Pyin Nyar	0.03	0.01–0.10	< 0.001	10.16	3.96–26.11	< 0.001
Ka Nu Hta	0.05	0.02–0.17	< 0.001	22.60	8.21–62.15	< 0.001
Tar Au Ta	0.78	0.34–1.81	0.561	3.21	1.46–7.02	0.004
Season						
Hot	1	–	–	1	–	–
Cool	9.48	2.69–33.35	< 0.001	0.59	0.23–1.48	0.268
Rainy	8.34	2.47–28.1	0.001	0.45	0.18–1.09	0.079
MDA						
No MDA	1	–	–	1	–	–
MDA	0.49	0.33–0.74	0.001	4.18	3.15–5.54	< 0.001
Sleep under bednet						
Never	1	–	–	1	–	–
Some night	0.72	0.16–3.27	0.675	2.01	0.45–9.02	0.364
Every night	0.97	0.55–1.72	0.912	1.80	1.02–3.17	0.043

CI = confidence interval; MDA = mass drug administration; MSP-1<sub>19</sub> = merozoite surface protein 119. The multilevel model included Ab response to gSG6-P1, age group, gender, and MDA at the individual level, long-lasting impregnated nets use at the household level and season, the population size at each survey, the temperature, and relative humidity at the village level.

TABLE 3

Multivariate logistic regression mixed model showing the relationship between the *Plasmodium falciparum* infections and the intensity of antibody responses to gSG6-P1 salivary antigen

Characteristics	<i>P. falciparum</i> infections		
	OR	95% Confidence interval	<i>P</i> value
Ab response to gSG6-P1*			
Low	1	—	—
Medium	0.88	0.49–1.55	0.664
High	1.45	0.84–2.52	0.178
Very high	2.27	1.35–3.82	0.002

\* gSG6-P1 classes were optical density < 0.4125 for low responders,  $0.4125 < x < 0.6345$  for medium responders,  $0.6345 < x < 0.9045$  for high HB responders, and  $\geq 0.9045$  for very high responders. Analyses were adjusted for temperature, humidity, age, season, and village.

#### SUPPLEMENTARY TEXT 1

### Operating procedures for ELISA.

The standardized dried blood spots (1 cm diameter containing  $\approx 40$   $\mu$ L of blood) were eluted by incubation in 400 mL of phosphate-buffered saline (PBS-Tween 0.1%) at 4°C for 24 hours. Maxisorp plates (Nunc, Roskilde, Denmark) were coated with the Pf merozoite surface protein 119 (MSP1-19)/Pf CSP peptide (Vaximax, France) at a concentration of 1  $\mu$ g/mL in coating buffer (PBS + Phenol Red 1%) at 4°C overnight. After washing (with a solution of PBS-Tween 0.1% + NaCl), the plates were blocked for 1 hour under stirring at room temperature with 150 mL of saturation buffer (PBS-Tween 0.1% + milk powder 3%). Thereafter, each eluted sample was incubated in duplicate for 2 hours under stirring at room temperature at 1/20 dilution in sample dilution buffer (PBS-Tween 0.1% + milk powder 1% + sodium azide 0.02%). Antihuman IgG coupled to the peroxidase (Invitrogen) was incubated at a 1/3,000 dilution for 1 hour under stirring at room temperature. Substrate TMB one (3,3',5,5'-tetramethylbenzidine) (Promega) was added for 30 minutes at room temperature protected from light then stopping the reaction with 0.2 M H<sub>2</sub>SO<sub>4</sub>, and absorbance was measured at 450 nm. In parallel, each test sample was assessed in a blank well containing no Pf MSP1-19/Pf CSP peptide (ODn) to measure nonspecific reactions. Individual results were expressed as the  $\Delta$ OD value:  $\Delta$ OD = OD<sub>x</sub> – OD<sub>n</sub>. OD<sub>x</sub> and OD<sub>n</sub> represent the mean of individual optical density (OD) in two antigen wells and one blank well containing no Pf MSP1-19/Pf CSP peptide, respectively. Specific anti-Pf MSP1-19/Pf CSP IgG responses were also assayed in nonmalaria-exposed individuals (negative samples from France:  $n = 18$ ) to quantify the nonspecific background Ab level and to calculate the cut-off value (mean [ $\Delta$ OD<sub>oneg</sub>] + 3SD). Based on our findings, a participant was classified as an immune responder to Pf MSP1-19 and Pf CSP if  $\Delta$ OD was > 0.162 and > 0.115, respectively.

All peptide batches were shipped in lyophilized form and then suspended in ultrafiltered water and frozen in aliquots at –20°C until use.

SUPPLEMENTAL TABLE 1

Seroprevalence in antibody response to CSP per age group and surveys

Survey of blood collection	Total population	Characteristic of blood spot					Pf CSP antibody prevalence %							
		Blood collection date	Number of dry blood spot	Male	Female	Median age (range) (years)	Gender			Age (years)				
							Male	Female	Total	0–4	5–15	16–59	60 up	
Village	Htoo Pyin NyarTPN													
M0	373	May 20, 2013	257	145	112	27.4 (1–66)	%	5.5	3.6	4.7	16.7	3.9	4.8	0.0
							(95% CI)	(2.6–11.0)	(1.2–9.4)	(2.6–8.2)	(0.9–63.5)	(1.0–11.7)	(2.2–9.6)	(0.0–43.9)
							(n/N)	(8/145)	(4/112)	(12/257)	(1/6)	(3/77)	(8/167)	(0/7)
M3	406	August 23, 2013	267	143	124	25.1 (1–66)	%	21.7	21.0	21.4	0.0	16.0	28.7	0.0
							(95% CI)	(15.4–29.5)	(14.4–29.4)	(16.7–26.9)	(0.0–15.5)	(8.9–26.7)	(21.9–36.5)	(0.0–40.2)
							(n/N)	(31/143)	(26/124)	(57/267)	(0/27)	(12/75)	(45/157)	(0–8)
M6	434	November 7, 2013	300	159	141	24.6 (0–66)	%	3.1	5.0	4.0	0.0	0.0	7.1	0.0
							(95% CI)	(1.2–7.6)	(2.2–10.3)	(2.2–7.1)	(0.0–11.2)	(0.0–5.4)	(3.9–12.4)	(0.0–43.9)
							(n/N)	(5/159)	(7/141)	(12/300)	(0/39)	(0/85)	(12/169)	(0/7)
M9	464	January 28, 2014	307	160	147	24.0 (0–66)	%	3.1	3.4	3.3	0.0	1.2	5.4	0.0
							(95% CI)	(1.2–7.5)	(1.3–8.2)	(1.7–6.1)	(0.0–10.0)	(0.1–7.2)	(2.7–10.4)	(0.0–37.1)
							(n/N)	(5/160)	(5/147)	(10/307)	(0/46)	(1/86)	(9/166)	(0/9)
M12	473	April 23, 2014	264	138	126	22.2 (0–66)	%	3.6	2.4	3.0	0.0	1.2	5.4	0.0
							(95% CI)	(1.3–8.7)	(0.6–7.3)	(1.4–6.1)	(0.0–9.8)	(0.1–7.3)	(2.4–11.2)	(0.0–43.9)
							(n/N)	(5/138)	(3/126)	(8/264)	(0/45)	(1/85)	(7/130)	(0/7)
M15	492	July 14, 2014	234	127	107	24.4 (0–66)	%	2.4	0.9	1.7	0.0	1.5	2.4	57.1
							(95% CI)	(0.6–7.3)	(0.1–5.8)	(0.6–4.6)	(0.0–13.0)	(0.1–9.3)	(0.6–7.3)	(20.2–88.2)
							(n/N)	(3/127)	(1/107)	(4/234)	(0/33)	(1/66)	(3/127)	(4/7)
M18	501	October 8, 2014	266	139	127	22.8 (0–66)	%	5.0	2.4	3.8	0.0	2.5	5.2	16.7
							(95% CI)	(2.2–10.5)	(0.6–7.3)	(1.9–7.0)	(0.0–9.8)	(0.4–9.7)	(2.3–10.7)	(0.9–63.5)

							(n/N)	(7/139)	(3/127)	(10/266)	(0/45)	(2/79)	(7/136)	(1/6)
Village	Tar Au Ta													
M0	740	June 14, 2013	402	192	210	26.7 (0–80)	%	17.7	9.1	13.2	7.1	0.7	18.5	40.0
							(95% CI)	(2.7–24.0)	(5.7–14.0)	(10.1–17.0)	(3.7–35.8)	(0.0–4.7)	(13.8–24.2)	(20.0–63.6)
							(n/N)	(34/192)	(19/210)	(53/402)	(1/14)	(1/135)	(43/233)	(8/20)
M3	785	September 12, 2013	308	145	163	22.5 (0–70)	%	26.9	23.9	25.3	4.7	17.3	35.5	50.0
							(95% CI)	(20.0–35.0)	(17.8–31.4)	(20.6–30.6)	(0.8–17.1)	(11.0–25.9)	(27.7–44.0)	(24.0–76.0)
							(n/N)	(39/145)	(39/163)	(78/308)	(2/43)	(19/110)	(50/141)	(7/14)
M6	789	November 26, 2013	205	102	103	23.7 (1–70)	%	16.7	9.7	13.2	3.6	7.1	16.0	46.2
							(95% CI)	(10.3–25.6)	(5.0–17.5)	(9.0–18.8)	(0.2–20.2)	(2.7–16.6)	(9.5–25.3)	(20.4–73.9)
							(n/N)	(17/102)	(10/103)	(27/205)	(1/28)	(5/70)	(15/94)	(6/13)
M9	814	February 18, 2014	292	143	149	21.5 (0–80)	%	18.9	14.8	16.8	5.4	6.8	24.8	54.6
							(95% CI)	(13.0–26.5)	(9.7–21.7)	(12.8–21.7)	(1.4–15.8)	(2.8–14.8)	(18.0–33.1)	(24.6–81.9)
							(n/N)	(27/143)	(22/149)	(49/292)	(3/56)	(6/88)	(34/137)	(6/11)
M12	833	May 15, 2014	250	114	136	19.2 (0–70)	%	12.3	5.2	8.4	1.6	0.0	16.5	22.2
							(95% CI)	(7.1–20.1)	(2.3–10.7)	(5.4–12.7)	(0.1–9.7)	(0.0–6.6)	(10.3–25.1)	(4.0–59.8)
							(n/N)	(14/114)	(7/136)	(21/250)	(1/63)	(0/69)	(18/109)	(2/9)
M15	861	August 6, 2014	305	150	155	21.5 (0–80)	%	14.7	10.3	12.5	1.8	2.0	20.7	43.8
							(95% CI)	(9.6–21.6)	(6.2–16.5)	(9.1–16.8)	(0.1–10.8)	(0.4–7.9)	(14.4–28.7)	(20.8–69.5)
							(n/N)	(22/150)	(16/155)	(38/305)	(1/56)	(2/98)	(28/135)	(7/16)
M18	879	October 30, 2014	196	94	102	18.9 (0–70)	%	11.7	6.9	9.2	2.0	1.8	16.9	28.6
							(95% CI)	(6.3–20.4)	(3.0–14.1)	(5.7–14.3)	(0.1–12.2)	(0.1–10.6)	(9.9–27.0)	(5.1–69.7)
							(n/N)	(11/94)	(7/102)	(18/196)	(1/49)	(1/57)	(14/83)	(2/7)
Village	Ka Nu Hta													
M0	348	June 14, 2013	323	178	145	29.0 (1–73)	%	7.9	4.1	6.2	0.0	1.2	8.0	15.4
							(95% CI)	(4.5–13.1)	(1.7–9.2)	(3.9–9.6)	(0.0–34.5)	(0.1–7.1)	(4.9–12.7)	(2.7–46.3)
							(n/N)	(14/178)	(6/145)	(20/323)	(0/10)	(1/87)	(17/213)	(2/13)
M3	417	September 12, 2013	275	148	127	27.9 (1–73)	%	26.4	26.8	26.5	13.0	17.6	32.7	20.0
							(95% CI)	(19.6–34.3)	(19.5–35.5)	(21.5–32.3)	(3.4–34.7)	(10.1–28.5)	(25.8–40.5)	(3.5–55.8)



							(n/N)	(39/148)	(34/127)	(73/275)	(3/23)	(13/74)	(55/168)	(2/10)
M6	435	December 11, 2013	261	140	121	28.0 (0-73)	%	12.9	4.1	8.8	0.0	2.8	12.0	2.0
							(95% CI)	(8.0-19.8)	(1.5-9.9)	(5.8-13.1)	(0-19.2)	(0.5-10.7)	(7.5-18.3)	(3.5-55.8)
							(n/N)	(18/140)	(5/121)	(23/261)	(0/21)	(2/71)	(19/159)	(2/10)
M9	449	March 5, 2014	303	155	148	25.9 (0-73)	%	2.6	4.1	6.6	0.0	2.4	9.5	18.2
							(95% CI)	(0.8-6.9)	(1.7-9.0)	(4.2-10.2)	(0.0-11.4)	(0.4-9.0)	(5.7-15.2)	(3.2-52.2)
							(n/N)	(14/155)	(6/148)	(20/303)	(0/38)	(2/85)	(16/169)	(2/11)
M12	469	May 26, 2014	301	160	141	24.9 (0-73)	%	7.5	2.1	5.0	0.0	1.2	8.3	0.0
							(95% CI)	(4.1-13.0)	(0.6-6.6)	(2.9-8.3)	(0.0-10.7)	(0.1-7.3)	(4.8-13.8)	(0.0-43.9)
							(n/N)	(12/160)	(3/141)	(15/301)	(0/41)	(1/85)	(14/169)	(0/7)
M15	485	August 21, 2014	303	164	139	25.8 (0-70)	%	8.5	2.9	5.9	0.0	4.2	7.9	10.0
							(95% CI)	(4.9-14.2)	(0.9-7.7)	(3.7-9.4)	(0.0-10.2)	(1.1-12.5)	(4.5-13.1)	(0.5-45.9)
							(n/N)	(14/164)	(4/139)	(18/303)	(0/43)	(3/72)	(14/178)	(1/10)
M18	495	November 13, 2014	285	155	130	24.6 (0.70)	%	5.2	1.5	3.5	0.0	0.0	6.3	0.0
							(95% CI)	(2.4-10.3)	(0.3-6.0)	(1.8-6.6)	(0.0-9.1)	(0.0-6.9)	(3.2-11.5)	(0-34.5)
							(n/N)	(8/155)	(2/130)	(10/285)	(0/49)	(0/66)	(10/160)	(0/10)
Village	Htee Kaw Taw													
M0	899	July 4, 2013	487	238	249	26.2 (0-94)	%	8.8	6.4	7.6	0.0	3.0	10.1	22.2
							(95% CI)	(5.7-13.4)	(3.8-10.4)	(5.5-10.4)	(0.0-16.7)	(1.1-7.2)	(6.9-14.4)	(7.4-48.1)
							(n/N)	(21/238)	(16/249)	(37/487)	(0/25)	(5/167)	(28/277)	(4/18)
M3	979	October 7, 2013	524	263	261	24.1 (0-89)	%	19.0	23.4	21.2	10.2	12.0	28.6	55.6
							(95% CI)	(4.6-24.4)	(18.5-29.1)	(17.8-25.0)	(3.8-23.0)	(7.9-17.8)	(23.4-34.4)	(12.1-58.5)
							(n/N)	(50/263)	(61/261)	(111/524)	(5/49)	(22/183)	(79/276)	(5/16)
M6	1,029	January 8, 2014	493	241	252	21.9 (0-78)	%	5.0	4.4	4.7	0.0	2.1	7.2	27.3
							(95% CI)	(2.7-8.8)	(2.3-7.9)	(3.1-7.0)	(0.0-7.1)	(0.1-5.5)	(4.3-11.6)	(7.3-60.7)
							(n/N)	(12/241)	(11/252)	(23/493)	(0/64)	(4/195)	(16/223)	(3/11)
M9	1,070	May 1, 2014	478	241	237	22.6 (0-78)	%	4.2	3.4	3.8	0.0	4.3	5.9	22.2
							(95% CI)	(2.1-7.7)	(1.6-6.8)	(2.3-6.0)	(0.0-6.8)	(0.2-4.7)	(3.4-10.0)	(4.0-59.8)
							(n/N)	(10/241)	(8/237)	(18/478)	(0/67)	(2/166)	(14/236)	(2/9)

M12	1,192	June 24, 2014	512	257	255	21.8 (0–89)	%	3.9	1.6	2.7	0.0	1.1	4.7	7.7
							(95% CI)	(2.0–7.2)	(0.5–4.2)	(1.6–4.7)	(0.0–6.8)	(0.2–4.4)	(2.5–8.5)	(0.4–3.8)
							(n/N)	(10/257)	(4/255)	(14/512)	(0/88)	(2/178)	(11/233)	(1/13)
M15	1,217	September 16, 2014	504	264	240	21.8 (0–78)	%	5.3	1.7	3.6	1.2	3.4	4.7	0.0
							(95% CI)	(3.0–8.9)	(0.5–4.5)	(2.2–5.7)	(0.1–7.6)	(1.4–7.7)	(2.5–8.5)	(0.0–30.1)
							(n/N)	(14/264)	(4/240)	(18/504)	(1/82)	(6/175)	(11/235)	(0/12)
M18	1,236	December 8, 2014	471	233	238	19.9 (0–78)	%	2.6	1.7	2.2	1.3	1.0	3.3	7.1
							(95% CI)	(1.1–5.8)	(0.5–4.5)	(1.1–4.1)	(0.1–7.9)	(0.2–4.0)	(1.4–7.4)	(0.4–35.8)
							(n/N)	(6/233)	(4/238)	(10/471)	(1/78)	(2/198)	(6/181)	(1/14)

CI = confidence interval.

SUPPLEMENTAL TABLE 2

Seroprevalence in antibody response to MSP-1<sub>19</sub> per age group and surveys

Survey of blood collection	Total population	Characteristic of blood spot					Pf MSP1-19 antibody prevalence %							
		Blood collection date	Number of dry blood spot	Male	Female	Median age (range) (years)	Gender			Age (years)				
							Male	Female	Total	0–4	5–15	16–59	60 up	
Village	Htoo Pyin Nyar													
M0	373	May 20, 2013	256	145	111	27.2 (1–66)	%	49.7	46.0	48.1	16.7	30.8	56.6	66.7
							(95% CI)	(41.3–58.0)	(36.5–55.6)	(41.8–54.4)	(0.9–63.5)	(21.1–42.4)	(48.7–64.2)	(24.1–94.0)
							(n/N)	(72/145)	(51/111)	(123/256)	(1/6)	(24/78)	(94/166)	(4/6)
M3	406	August 23, 2013	278	148	130	25.3 (1–66)	%	49.3	44.6	47.1	21.4	40.5	54.0	62.5
							(95% CI)	(41.1–57.6)	(36.0–53.6)	(41.2–53.2)	(9.0–41.5)	(29.8–52.2)	(46.0–61.8)	(25.9–89.8)
							(n/N)	(73/148)	(58/130)	(131/278)	(6/28)	(32/79)	(88/163)	(5/8)
M6	434	November 7, 2013	299	158	141	24.6 (0–66)	%	58.2	55.3	56.9	33.3	56.0	61.5	85.7
							(95% CI)	(50.1–)	(46.7–)	(51.0–)	(19.6–)	(44.7–)	(53.7–)	(42.0–)

							% CI)	65.9)	63.6)	62.5)	50.3)	66.6)	68.8)	99.3)
							(n/N )	(92/158)	(78/141)	(170/299 )	(13/39 )	(47/84)	(104/169 )	(6/7)
M9	464	January 28, 2014	306	160	146	23.9 (0-66)	%	80.0	76.0	78.1	58.7	82.6	80.0	100.0
							(95 % CI)	(72.8- 85.7)	(68.1- 82.5)	(73.0- 82.5)	(43.3- 72.7)	(72.6- 89.6)	(72.9- 85.7)	(62.9- 100.0)
							(n/N )	(128/160 )	(111/146)	(239/306 )	(27/46 )	(71/86)	(132/165 )	(9/9)
M12	473	April 23, 2014	264	138	125	22.2 (0.66)	%	82.6	75.2	78.8	60.0	79.5	83.9	100.0
							(95 % CI)	(75.0- 88.3)	(66.5- 82.3)	(73.3- 83.5)	(44.4- 73.9)	(69.0- 87.3)	(76.1- 89.5)	(51.7- 100.0)
							(n/N )	(114/138 )	(94/125)	(208/264 )	(27/45 )	(66/83)	(109/130 )	(6/6)
M15	492	July 14, 2014	235	137	108	24.3 (0-66)	%	72.3	75.9	77.0	58.8	72.7	82.8	100.0
							(95 % CI)	(63.9- 79.4)	(66.8- 83.4)	(71.0- 82.1)	(40.8- 74.9)	(60.2- 82.6)	(74.9- 88.7)	(56.1- 100.0)
							(n/N )	(99/137)	(82/108)	(181/235 )	(20/34 )	(48/66)	(106/128 )	(7/7)
M18	501	October 8, 2014	266	139	127	22.8 (0-66)	%	82.7	79.5	81.2	68.9	82.3	84.6	83.3
							(95 % CI)	(75.2- 88.4)	(71.3- 86.0)	(75.8- 85.6)	(53.2- 81.4)	(71.7- 89.6)	(77.1- 90.0)	(36.5- 99.1)
							(n/N )	(115/139 )	(101/127)	(216/266 )	(31/45 )	(65/79)	(115/136 )	(5/6)
Village	Tar Au Ta													
M0	740	June 14, 2013	402	193	209	26.7 (0-80)	%	74.6	73.2	73.9	21.4	53.3	87.1	95.0
							(95% CI)	(67.8- 80.5)	(66.6- 79.0)	(69.2- 78.1)	(5.7- 51.2)	(44.6- 61.9)	(82.0- 91.0)	(73.1- 99.7)
							(n/N )	(144/193 )	(153/209 )	(297/402)	(3/14)	(72/135)	(203/233 )	(19/20 )
M3	785	Septembe r 12, 2013	322	151	171	22.1 (0-70)	%	64.2	60.2	62.1	10.6	45.7	88.3	100.0
							(95% CI)	(56.0- 71.8)	(52.5- 67.5)	(56.5- 67.4)	(4.0- 23.9)	(36.5- 55.2)	(81.6- 92.8)	(73.2- 100.0)
							(n/N )	(97/151)	(103/171)	(200/322)	(5/47)	(53/116)	(128/145)	(14/14)

									)			)	)	
M6	789	November 26, 2013	202	100	102	24.0 (1-70)	%	71.0	68.6	69.8	19.2	57.1	89.3	23.1
							(95% CI)	(60.9-79.4)	(58.6-77.3)	(62.9-75.9)	(7.3-40.0)	(44.8-68.7)	(80.7-94.4)	(6.2-54.0)
							(n/N)	(71/100)	(70/102)	(141/202)	(5/26)	(40/70)	(83/93)	(13/13)
M9	814	February 18, 2014	291	142	149	21.5 (0-80)	%	81.0	81.9	81.4	39.3	84.1	96.3	90.9
							(95% CI)	(73.4-86.9)	(74.6-87.5)	(76.4-85.6)	(26.8-53.3)	(74.4-90.7)	(91.2-98.6)	(57.1-99.5)
							(n/N)	(115/142)	(122/149)	(237/291)	(22/56)	(74/88)	(131/136)	(10/11)
M12	833	May 15, 2014	249	113	136	19.2 (0-70)	%	77.0	80.2	78.7	33.3	86.6	98.2	100.0
							(95% CI)	(67.9-84.2)	(72.3-86.3)	(73.0-83.5)	(22.3-46.4)	(75.5-93.3)	(92.9-99.7)	(62.9-100.0)
							(n/N)	(87/113)	(109/136)	(196/249)	(21/63)	(58/67)	(108/110)	(9/9)
M15	861	August 6, 2014	307	150	157	21.6 (0-80)	%	78.7	79.6	79.2	32.1	79.0	96.3	100.0
							(95% CI)	(71.1-84.8)	(72.3-85.5)	(74.1-83.5)	(20.7-46.1)	(69.5-86.3)	(91.1-98.6)	(77.1-100.0)
							(n/N)	(118/150)	(125/157)	(243/307)	(18/56)	(79/100)	(129/134)	(17/17)
M18	879	October 30, 2014	196	94	102	18.9 (0-70)	%	71.3	69.6	70.4	26.5	77.2	90.4	85.7
							(95% CI)	(60.9-79.9)	(59.6-78.1)	(63.4-76.6)	(1.5-41.3)	(63.9-86.8)	(81.4-95.5)	(42.0-99.3)
							(n/N)	(67/94)	(71/102)	(138/196)	(13/49)	(44/57)	(75/83)	(6/7)
Village	Ka Nu Hta													
M0	348	June 14, 2013	321	178	143	29.0 (1-73)	%	75.9	65.7	71.3	20.0	53.5	79.3	100.0
							(95% CI)	(68.8-81.8)	(57.3-73.3)	(66.0-76.2)	(3.5-55.8)	(42.5-64.2)	(73.0-84.4)	(71.7-100.0)
							(n/N)	(135/178)	(94/143)	(229/321)	(2/10)	(46/86)	(168/212)	(13/13)
M3	417	September 12, 2013	272	146	126	28.1 (1-73)	%	76.7	69.8	73.5	18.2	55.6	86.9	100.0
							(95% CI)	(68.9-83.1)	(60.9-77.5)	(67.8-78.6)	(6.0-41.0)	(43.4-67.1)	(80.6-91.4)	(65.6-100.0)
							(n/N)	(112/146)	(88/126)	(200/272)	(4/22)	(40/72)	(146/168)	(10/10)
M6	435	December	260	139	121	28.3	%	81.3	70.3	76.2	45.5	60.9	85.5	100.0

		er 11, 2013				(0-73)	(95% CI)	(73.6- 87.2)	(61.2- 78.0)	(70.4- 81.1)	(25.1- 67.3)	(48.4- 72.2)	(78.9- 90.4)	(65.6- 100.0)
							(n/N)	(113/139 )	(85/121)	(198/260)	(10/22 )	(42/69)	(136/159 )	(10/10)
M9	449	March 5, 2014	304	156	148	25.9 (0-73)	%	90.4	84.5	87.5	68.4	82.4	93.5	100.0
							(95% CI)	(84.4- 94.3)	(77.4- 89.7)	(83.1- 90.9)	(51.2- 82.0)	(72.2- 89.5)	(88.4- 96.6)	(67.9- 100.0)
							(n/N)	(141/156 )	(125/148 )	(266/304)	(26/38 )	(70/85)	(159/170 )	(11/11)
M12	469	May 26, 2014	300	159	141	24.7 (0-73)	%	84.3	80.9	82.7	46.3	82.4	90.5	100.0
							(95% CI)	(77.5- 89.4)	(73.2- 86.8)	(77.8- 86.7)	(31.0- 62.4)	(72.1- 89.5)	(84.8- 94.3)	(51.7- 100.0)
							(n/N)	(134/159 )	(114/141 )	(248/300)	(19/41 )	(70/85)	(153/169 )	(6/6)
M15	485	August 21, 2014	302	163	139	25.7 (0-70)	%	76.1	74.8	75.5	39.5	68.1	85.9	100.0
							(95% CI)	(68.6- 82.2)	(66.6- 81.6)	(70.2- 80.2)	(25.4- 55.6)	(55.9- 78.3)	(79.7- 90.5)	(65.6- 100.0)
							(n/N)	(124/163 )	(104/139 )	(228/302)	(17/43 )	(49/72)	(152/177 )	(10/10)
M18	495	November 13, 2014	285	155	130	24.6 (0-70)	%	79.4	76.2	77.9	42.9	77.3	87.6	100.0
							(95% CI)	(72.0- 85.3)	(67.7- 83.0)	(72.5- 82.5)	(29.1- 57.7)	(65.0- 86.3)	(81.2- 92.1)	(62.9- 100.0)
							(n/N)	(123/155 )	(99/130)	(222/285)	(21/49 )	(51/66)	(141/161 )	(9/9)
Village	Htee Kaw Taw													
M0	899	July 4, 2013	488	237	251	26.3 (0-94)	%	65.0	57.0	60.9	16.7	48.5	70.8	83.3
							(95% CI)	(58.5- 71.0)	(50.6- 63.1)	(56.4- 65.2)	(5.5- 38.2)	(40.8- 56.3)	(65.0- 76.0)	(57.7- 95.6)
							(n/N)	(154/237 )	(143/251 )	(297/488)	(4/24)	(82/169)	(196/277 )	(15/18)
M3	979	October 7, 2013	533	267	266	24.3 (0-89)	%	59.6	56.0	57.8	20.0	43.7	72.5	75.0
							(95% CI)	(53.4- 65.4)	(49.8- 62.0)	(53.5- 62.0)	(10.5- 34.1)	(36.5- 51.2)	(66.9- 77.6)	(47.4- 91.7)
							(n/N)	(159/267 )	(149/266 )	(308/533)	(10/50 )	(80/183)	(206/284 )	(12/16)
M6	1,029	January 8, 2014	496	240	256	22.0 (0-78)	%	64.6	57.4	60.9	20.0	53.9	77.5	81.8
							(95% CI)	(58.1- 70.6)	(51.1- 63.5)	(56.4- 65.2)	(11.5- 32.1)	(46.6- 61.0)	(71.4- 82.7)	(47.8- 96.8)

							(n/N)	(155/240)	(147/256)	(302/496)	(13/65)	(104/193)	(176/227)	(9/11)
M9	1,070	May 1, 2014	479	243	236	22.4 (0-78)	%	67.5	58.9	63.3	19.1	56.0	80.8	77.8
							(95% CI)	(61.2-73.3)	(52.3-65.2)	(58.8-67.6)	(11.0-30.8)	(48.1-63.5)	(75.0-85.5)	(40.2-96.1)
							(n/N)	(164/243)	(139/236)	(303/479)	(13/68)	(94/168)	(189/234)	(7/9)
M12	1,192	June 24, 2014	513	258	255	21.8 (0-89)	%	58.5	51.0	54.8	20.5	49.7	71.2	61.5
							(95% CI)	(52.2-64.6)	(44.7-57.3)	(50.4-59.1)	(12.9-30.7)	(42.2-57.3)	(64.9-76.9)	(32.3-84.9)
							(n/N)	(151/258)	(130/255)	(281/513)	(18/88)	(89/179)	(166-233)	(8/13)
M15	1,217	September 16, 2014	506	264	242	21.7 (0-78)	%	68.6	67.4	68.0	33.7	61.7	83.9	83.3
							(95% CI)	(62.5-74.0)	(61.0-73.1)	(63.7-72.0)	(24.0-45.0)	(54.0-68.9)	(78.4-88.2)	(50.9-97.1)
							(n/N)	(181/264)	(163/242)	(344/506)	(28/83)	(108/175)	(198/236)	(10/12)
M18	1,236	December 8, 2014	469	231	238	19.9 (0-78)	%	67.5	60.9	64.2	29.5	64.0	78.9	71.4
							(95% CI)	(61.0-73.4)	(54.4-67.1)	(59.6-68.5)	(20.0-41.1)	(56.8-70.6)	(72.1-84.5)	(42.0-90.4)
							(n/N)	(156/231)	(145/238)	(301/469)	(23/78)	(126/197)	(142/180)	(10/14)

CI = confidence interval.

SUPPLEMENTAL TABLE 3

Seroprevalence in antibody response to gSG6-P1 per age group and surveys

Survey blood collection	Total population	Characteristic of blood spot					Antibody prevalence %							
		Blood collection date	Number of dry blood spot	Male	Female	Median age (range) (years)	Gender			Age (years)				
							Male	Female	Total	0-4	5-15	15-59	60 up	
Village	Htoo Pyin Nyar													
M0	373	May 20, 2013	258	146	112	27.3 (1-66)	%	45.2	42.9	44.2	33.3	41.0	45.2	66.7
							(95% CI)	(37.0-53.6)	(33.7-52.6)	(38.1-50.0)	(6.0-75.9)	(30.2-52.8)	(37.6-53.1)	(24.1-94.0)
							(n/N)	(66/146)	(48/112)	(114/258)	(2/6)	(32/78)	(76/168)	(4/6)
M3	406	August 23, 2013	283	154	129	24.8 (1-66)	%	57.8	60.5	59.0	51.7	53.7	63.0	57.1
							(95% CI)	(49.6-65.6)	(51.5-68.9)	(53.0-64.8)	(32.9-70.1)	(42.4-64.6)	(55.1-70.3)	(20.2-88.2)
							(n/N)	(89/154)	(78/129)	(167/283)	(15/29)	(44/82)	(104-165)	(4/7)
M6	434	November 7, 2013	300	158	142	24.8 (0-66)	%	49.4	49.3	49.3	43.6	57.8	45.6	71.4
							(95% CI)	(41.4-57.4)	(40.9-57.8)	(43.6-55.1)	(28.2-60.2)	(46.5-68.4)	(38.0-53.4)	(30.3-94.9)
							(n/N)	(78/158)	(70/142)	(148/300)	(17/39)	(48/83)	(78/171)	(5/7)
M9	464	January 28, 2014	307	160	147	23.9 (0-66)	%	50.0	36.1	43.3	34.8	47.7	43.4	44.4
							(95% CI)	(42.0-58.0)	(28.4-44.4)	(37.7-49.1)	(21.8-50.3)	(36.9-58.7)	(35.8-51.3)	(15.3-77.3)
							(n/N)	(80/160)	(53/147)	(133/307)	(16/46)	(41/86)	(72/166)	(4/9)
M12	473	April 23, 2014	260	135	125	22.8 (0-66)	%	60.7	68.0	64.2	65.1	63.4	65.9	33.3
							(95% CI)	(51.9-68.9)	(59.0-75.9)	(58.0-70.0)	(49.0-78.6)	(52.0-73.6)	(57.0-73.9)	(6.0-75.9)

							(n/N )	(82/135)	(85/125)	(167/260 )	(28/43)	(52/82)	(85/129)	(2/6)
M15	492	July 14, 2014	233	127	106	24.3 (0–66)	%	80.3	76.4	78.5	70.6	83.1	77.2	100.0
							(95% CI)	(72.1– 86.6)	(67.0– 83.9)	(72.6– 83.5)	(52.3– 84.3)	(71.3– 90.9)	(68.7– 84.0)	(56.1– 100.0)
							(n/N )	(102/127 )	(81/106)	(183/233 )	(24/34)	(54/65)	(98/127)	(7/7)
M18	501	October 8, 2014	265	139	126	23.0 (0–66)	%	83.5	81.7	82.6	82.2	78.2	85.3	83.3
							(95% CI)	(76.0– 89.0)	(73.7– 87.9)	(77.4– 86.9)	(67.4– 91.5)	(67.2– 86.5)	(78.0– 90.6)	(36.5– 99.1)
							(n/N )	(116/139 )	(103– 126)	(219/265 )	(37/45)	(61/78)	(116/136)	(5/6)
Village	Tar Au Ta													
M0	740	June 14, 2013	404	192	212	26.8 (0–80)	%	68.2	62.7	65.3	42.9	58.1	72.4	50.0
							(95% CI)	(61.1– 74.7)	(55.8– 69.2)	(60.5– 69.9)	(18.8– 70.4)	(49.3– 66.4)	(66.1– 78.0)	(28.8– 71.2)
							(n/N )	(131/192 )	(133/212 )	(264/404 )	(6/14)	(79/136)	(168/232)	(11/22 )
M3	785	Septemb er 12, 2013	322	149	173	22.2 (0–70)	%	77.2	71.7	74.2	63.6	70.3	78.8	92.9
							(95% CI)	(69.5– 83.5)	(28.7– 38.5)	(69.0– 78.8)	(47.7– 77.2)	(61.1– 78.2)	(71.1– 84.9)	(64.2– 99.6)
							(n/N )	(115/149 )	(124/173 )	(239/322 )	(28/44)	(83/118)	(115/146)	(13/14 )
M6	789	Novemb er 26, 2013	201	98	103	24.1 (1–70)	%	75.5	81.6	78.6	72.0	73.2	82.6	92.3
							(95% CI)	(65.6– 83.4)	(72.4– 88.3)	(72.2– 83.9)	(50.4– 87.1)	(61.2– 82.7)	(73.0– 89.4)	(62.1– 99.6)
							(n/N )	(74/98)	(84/103)	(158/201 )	(18/25)	(52/71)	(76/92)	(12/13 )
M9	814	February 18, 2014	292	144	148	21.7 (0–80)	%	46.5	47.3	46.9	40.0	51.1	45.7	63.6
							(95% CI)	(38.3– 55.0)	(39.1– 55.6)	(41.1– 52.8)	(27.3– 54.8)	(40.3– 61.9)	(37.2– 54.3)	(31.6– 87.6)
							(n/N )	(67/144)	(70/148)	(137/292 )	(22/55)	(45/88)	(63/138)	(7/11)
M12	833	May 15, 2014	249	114	135	19.3 (0–70)	%	59.6	56.3	57.8	39.7	59.1	66.7	66.7
							(95% CI)	(50.0– 68.6)	(47.5– 64.7)	(51.4– 64.0)	(27.8– 52.8)	(46.3– 70.8)	(57.0– 75.2)	(30.9– 91.0)
							(n/N )	(68/114)	(76/135)	(144/249)	(25/63)	(39/66)	(74/111)	(6/9)



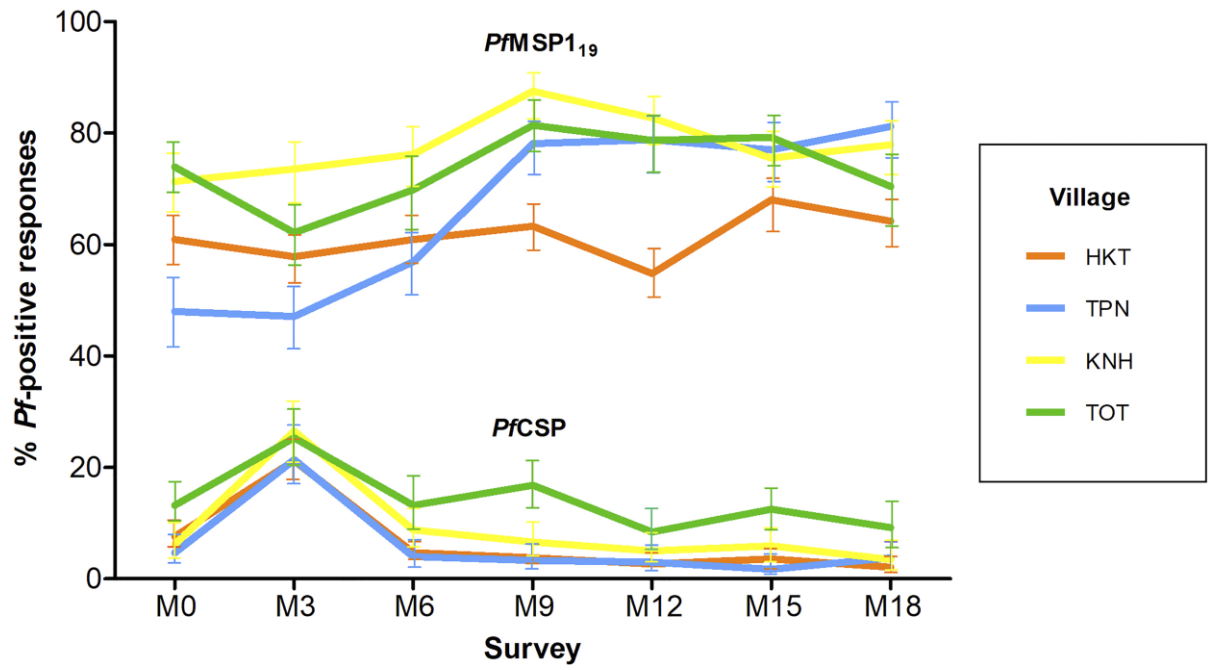
M15	861	August 6, 2014	307	152	155	21.3 (0–80)	% (95% CI) (n/N)	92.1 (86.3– 95.7) (140/152)	93.5 (88.1– 96.7) (145/155)	) (89.2– 95.4) (285/307)	94.7 (84.5– 98.6) (54/57)	92.0 (84.4– 96.2) (92/100)	92.5 (86.4– 96.2) (124/134)	93.8 (67.7– 99.7) (15/16)
M18	879	October 30, 2014	195	93	102	19.5 (0–70)	% (95% CI) (n/N)	57.0 (46.3– 67.1) (53/93)	74.5 (64.8– 82.4) (76/102)	) (59.0– 72.7) (129/195)	47.8 (33.1– 62.9) (22/46)	76.8 (63.3– 86.6) (43/56)	72.1 (61.2– 81.0) (62/86)	28.6 (5.1– 69.7) (2/7)
Village	Ka Nu Hta													
M0	348	June 14, 2013	325	180	145	29.1 (2–73)	% (95% CI) (n/N)	55.6 (48.0– 62.9) (100/180)	51.7 (43.3– 60.0) (75/145)	) (48.3– 59.3) (175/325)	33.3 (9.0– 69.1) (3/9)	40.2 (30.0– 51.3) (35/87)	59.3 (52.4– 65.8) (128/216)	69.2 (38.9– 89.7) (9/13)
M3	417	September 12, 2013	277	147	130	28.1 (1–73)	% (95% CI) (n/N)	47.6 (39.4– 56.0) (70/147)	51.5 (42.7– 60.3) (67/130)	) (43.4– 55.5) (137/277)	30.4 (14.1– 53.0) (7/23)	38.4 (27.4– 50.5) (28/73)	56.7 (49.0– 64.2) (97/171)	50.0 (20.1– 79.9) (5/10)
M6	435	December 11, 2013	263	141	122	28.1 (0–73)	% (95% CI) (n/N)	61.7 (53.1– 69.7) (87/141)	52.5 (43.3– 61.5) (64/122)	) (51.2– 63.4) (151/263)	59.1 (36.7– 78.5) (13/22)	55.7 (43.4– 67.4) (39/70)	56.5 (48.5– 64.2) (91/161)	80.0 (44.2– 96.5) (8/10)
M9	449	March 5, 2014	301	155	146	25.9 (0–73)	% (95% CI) (n/N)	47.7 (39.7– 55.9) (74/155)	49.3 (41.0– 57.7) (72/146)	) (42.8– 54.3) (146/301)	43.2 (27.5– 60.4) (16/37)	45.2 (34.5– 56.4) (38/84)	52.1 (44.3– 59.8) (88/169)	36.4 (12.4– 68.4) (4/11)
M12	469	May 26, 2014	298	160	138	25.1 (0–73)	% (95% CI) (n/N)	41.9 (34.2– 49.9) (67/160)	39.1 (31.1– 47.8) (54/138)	) (35.0– 46.4) (121/298)	31.7 (18.6– 48.2) (13/41)	47.6 (36.7– 58.7) (40/84)	38.4 (31.0– 46.4) (63/164)	55.6 (22.7– 84.7) (5/9)
M15	485	August 21, 2014	300	162	138	26.0 (0–70)	% (95%	94.4 (89.4–	96.4 (91.3–	) (92.1–	81.0 (65.4–	97.1 (89.1–	97.8 (94.0–	100.0 (65.6–

							CI	97.3)	98.7)	97.3)	90.9)	99.5)	99.3)	100.0)
							(n/N)	(153/162)	(133/138)	(286/300)	(34/42)	(68/70)	(174/178)	(10/10)
							%	86.4	83.6	85.1	63.3	84.6	91.2	100.0
							(95% CI)	(79.7–91.2)	(75.8–89.3)	(80.3–89.0)	(48.3–76.2)	(73.1–92.0)	(85.4–94.9)	(62.9–100.0)
							(n/N)	(133/154)	(107/128)	(240/282)	(31/49)	(55/65)	(145/159)	(9/9)
Village	Htee Kaw Taw													
M0	899	July 4, 2013	490	241	249	26.4 (0–94)	%	92.9	94.8	93.9	91.7	92.2	95.0	94.4
							(95% CI)	(88.8–95.7)	(91.0–97.1)	(91.3–95.8)	(71.5–98.6)	(86.8–95.6)	(91.6–97.2)	(70.6–99.7)
							(n/N)	(224/241)	(236/249)	(460/490)	(22/24)	(154/167)	(267/281)	(17/18)
M3	979	October 7, 2013	535	269	266	24.5 (0–89)	%	88.5	86.8	86.2	81.6	85.7	86.8	94.1
							(95% CI)	(80.6–89.4)	(82.0–90.5)	(83.9–89.0)	(67.5–90.8)	(79.6–90.3)	(82.2–90.4)	(69.2–99.7)
							(n/N)	(230/269)	(231/266)	(461/535)	(40/49)	(156/182)	(249/287)	(16/17)
M6	1,029	January 8, 2014	500	243	257	22.1 (0–78)	%	78.2	85.6	82.0	75.8	81.9	84.8	63.6
							(95% CI)	(72.4–83.1)	(80.6–89.5)	(78.3–85.2)	(63.4–85.1)	(75.6–86.9)	(79.3–89.0)	(31.6–87.6)
							(n/N)	(190/243)	(220/257)	(410/500)	(50/66)	(158/193)	(195/230)	(7/11)
M9	1,070	May 1, 2014	478	240	238	22.4 (0–78)	%	87.9	86.6	87.2	77.9	86.9	89.7	100.0
							(95% CI)	(83.0–91.6)	(81.4–90.5)	(83.8–90.0)	(65.9–86.7)	(80.6–91.4)	(84.9–93.2)	(62.9–100.0)
							(n/N)	(211/240)	(206/238)	(417/478)	(53/68)	(146/168)	(209/233)	(9/9)
M12	1,192	June 24, 2014	527	269	258	21.7 (0–89)	%	85.1	89.1	87.1	87.6	88.8	85.7	84.6
							(95% CI)	(80.2–89.0)	(84.6–92.6)	(83.9–89.8)	(78.6–93.4)	(83.1–92.8)	(80.5–89.8)	(53.7–97.3)
							(n/N)	(229/269)	(230/258)	(459/527)	(78/89)	(166/187)	(204/238)	(11/13)
M15	1,217	September 16, 2014	506	266	240	21.6 (0–78)	%	81.2	85.0	83.0	82.1	85.8	81.3	81.8
							(95% CI)	(75.9–85.6)	(79.7–89.1)	(79.4–86.1)	(71.9–89.3)	(79.6–90.4)	(75.6–85.9)	(47.8–96.8)
							(n/N)	(216/266)	(204/240)	(420/506)	(69/84)	(151/176)	(191/235)	(9/11)

M18	1,236	December 8, 2014	467	233	234	20.0 (0–78)	%	82.8	87.2	85.0	84.0	85.4	84.9	85.7
							(95% CI)	(77.2– 87.3)	(82.1– 91.1)	(81.4– 88.1)	(73.3– 91.1)	(79.6– 89.8)	(78.6– 89.7)	(56.2– 97.5)
							(n/N)	(193/233 )	(204/234 )	(397/467 )	(63/75 )	(170/199 )	(152/179 )	(12/14 )

CI = confidence interval; MSP-1<sub>19</sub> = merozoite surface protein 119.

Figure 1



**Figure 2**

