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Sustained malaria control over an eight-year period in Papua New Guinea: the challenge of low-density asymptomatic infections

Running title:

Sustained malaria control in PNG

Summary

Continuous malaria control in Papua New Guinea has resulted in a marked decline of *Plasmodium falciparum* and *P. vivax* prevalence. Yet, an increasing proportion of submicroscopic infections, many of them carrying gametocytes, demands for novel strategies to target residual transmission.

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Conflict of Interest statement

The authors declare no conflict of interest.

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Abstract

Background: The scale-up of effective malaria control in the last decade has resulted in a substantial decline in the incidence of clinical malaria in many countries. The effects on the proportions of asymptomatic and submicroscopic infections, and on transmission potential are yet poorly understood.

Methods: In Papua New Guinea, vector control has been intensified since 2008, and improved diagnosis and treatment introduced in 2012. Cross-sectional surveys were conducted in Madang Province in 2006 (n=1280), 2010 (n=2117) and 2014 (n=2516). Infections were quantified by highly sensitive qPCR and gametocytes by RT-qPCR.

Results: *P. falciparum* prevalence by qPCR decreased from 42% in 2006 to 9% in 2014. *P. vivax* prevalence decreased from 42% in 2006 to 13% in 2010, but then increased to 20% in 2014. Parasite densities decreased 5-fold from 2006 to 2010; 72% of *P. falciparum* and 87% of *P. vivax* infections were submicroscopic in 2014. Gametocyte density and positivity correlated closely with parasitemia, and population gametocyte prevalence decreased 3-fold for *P. falciparum* and 29% for *P. vivax* from 2010 to 2014.

Conclusions: Sustained control has resulted in reduced transmission potential but an increasing proportion of gametocyte carriers are asymptomatic and submicroscopic and represent a challenge to malaria control.

Keywords: Malaria control, temporal trend, submicroscopic, asymptomatic, qPCR, gametocyte, transmission

Introduction

While increased malaria control has led to declining transmission in many countries [1, 2], an increasing proportion of asymptomatic and submicroscopic infections represent a major challenge to further progress towards elimination [3-5]. Clinical and light microscopy (LM) or rapid diagnostic test (RDT)-positive infections can be diagnosed with tools that are now available in the field, but asymptomatic infections are not targeted by programs relying on passive case detection [6]. Asymptomatic and submicroscopic infections have been shown to carry gametocytes and to be infective to mosquitos [7-10]. Data on their frequency is crucial for the design and evaluation of strategies to interrupt malaria transmission.

After roll out of malaria control interventions, such as the distribution of bed nets, naturally acquired immunity in the population may remain high for a number of years [11]. Thus, parasite densities are likely to remain low and few people will present with clinical malaria. After an extended period of lower transmission, however, immunity is expected to wane, resulting in more high-density and clinical infections. In parallel, malaria-naïve individuals experiencing lower exposure will acquire immunity more slowly. In combination, these effects are expected to result in a change of treatment frequency, the distribution of parasite and gametocyte densities, and duration of infection. Yet, little is known about the extent and time frame of such changes.

Due to differences in the biology of *P. falciparum* and *P. vivax*, the effects of control are often remarkably different for the two species, and in many countries *P. vivax* has proven more resilient to control [5, 12, 13]. *P. vivax* densities by microscopy are generally 5-10 times lower than *P. falciparum* densities [14-16], making diagnosis more difficult. Latent liver stages (hypnozoites) escape diagnosis, and standard treatment against blood-stages does not affect them [17]. In highly endemic regions, up to 80% of all *P. vivax* blood-stage infections in children are due to relapses [18, 19]. If transmission declines, individuals who have

experienced high levels of transmission may harbor a large reservoir of hypnozoites, which will result in relapses for an extended period of time. Thus, the proportion of all blood-stage infections in the population that are caused by *P. vivax* relapses as compared to primary infections might temporarily increase.

Few in-depth studies have assessed the effect of intensified control on parasite prevalence, clinical malaria, the proportion of asymptomatic and submicroscopic *P. falciparum* and *P. vivax* infections, and gametocyte carriage over several years in the same population. In the Madang area at the north coast of Papua New Guinea (PNG), *P. falciparum* and *P. vivax* prevalence by PCR had reached 30-60% in the general population in 2001-2006 [14, 16, 20-22]. As a consequence of the corresponding high transmission intensity, children in PNG acquired natural immunity against clinical malaria during early childhood, and 78-97% of infections in the general population were asymptomatic [14, 16]. In 2008/09 and again in 2011/12, long-lasting insecticidal nets (LLINs) were distributed in PNG. Rapid diagnostic tests (RDTs) to test all febrile cases in health centers as well as ACT with Artemether-Lumefantrine as first line treatment were implemented in 2012. Surveys conducted after the first round of LLIN distribution found considerable decreases in entomological inoculation rate [23] and parasite prevalence by LM [24], suggesting these interventions had a pronounced effect on transmission.

To understand the full impact of intensified control, repeated cross-sectional surveys were conducted in Madang Province in 2006, 2010 and 2014 (Figure 1) at the end of the rainy season. Blood samples were collected from 5913 individuals and highly sensitive molecular assays were applied to diagnose malaria infections and gametocytes in the same population during distinct phases of malaria control.

Methods

Ethics statement

Informed written consent was obtained from participants, or, if participants were <18 years, from their parents or legal guardians. This study was approved by PNG IMR IRB (1116/1204), PNG MRAC (11.21/1206), WEHI HREC (12/09) and CWRU UHCMC (05-11-11).

Study site and sample collection

Blood samples were collected in Madang Province (Figure 1), in coastal catchments for 2 health centers (Mugil and Malala), and one inland catchment (Utu). The climate is tropical with a rainy season from December to April. In 2006 samples were collected in March-April, in 2010 and 2014 between mid-May and early July. A convenience sampling strategy including individuals >6 months was applied. In 2014, among villages, 8.3%-45.1% of residents were sampled (Supplementary Table S1).

From each participant, a 250µL finger prick blood sample was collected into EDTA tubes. For gametocyte detection, 50µL blood was transferred into tubes containing 250µL RNeasy Protect (Qiagen, 2010 and 2014 only). In the field samples were stored at 4°C and transferred every night to the laboratory at -20°C (for DNA extraction) or -80°C (for RNA extraction).

Parasite quantification by qPCR and LM

Laboratory methodology as described was applied [25]. In brief, DNA was extracted from 200µL pelleted blood using the Favorgen 96-well Genomic DNA Extraction Kit and eluted in 200µL buffer. *P. falciparum* and *P. vivax*, as well as *P. malariae* and *P. ovale* (2010 and 2014 surveys only) were quantified by highly sensitive qPCR assays screening 4 µL DNA,

corresponding to 4 μ L blood [26]. A dilution of plasmids containing the target sequence of the PCR was run as external standard for absolute quantification. *P. falciparum* positive samples were genotyped by *msp2* [22, 27], *P. vivax* positive samples by *msp1F3* and MS2 [28, 29].

For gametocyte detection, RNA was extracted using the Qiagen RNeasy 96-kit, with additional DNase treatment to remove residual DNA (Qiagen RNase-Free DNase Set). *pfs25* and *pvs25* transcripts were detected using published RT-qPCR protocols [30] and quantified using plasmids to generate an external standard curve. A genus-specific qPCR assay [30] was run to ensure absence of DNA.

Data analysis

Data were analyzed using Stata 12.1. Unless otherwise stated, results are based on qPCR. Densities (by LM or qPCR) are given as geometric mean. Fever or history of fever was defined as measured fever $>37.5^{\circ}\text{C}$ or reported febrile illness in the past 2 days. Clinical malaria was defined as fever or history of fever and parasites detected by microscopy. Logistic regression was used to assess risk factors of infection, and chi-square tests to compare rates of infection between age groups and catchments. Only in rare cases an individual was included in >1 survey, as not the exact same villages were included in the 3 surveys. These cases were treated as independent observations.

Model for age-prevalence curves

The non-linear association between parasite prevalence and age was first assessed using Generalized Additive Models with thin plate smoothing splines, for each survey. The shift in age-prevalence peaks across surveys was then investigated using a likelihood-based model inspired from [33]. The host population was described using a compartmental model similar to

that of classical S-I-R-S model using a set of three Ordinary Differential Equations (ODEs). Instead of modeling the proportions of susceptibles (s_k), infected (i_k) and retired (r_k) individuals in survey k according to time, these were modeled according to age a .

$$\theta(\lambda_k, \gamma_k, \nu_k) = \begin{cases} \frac{ds_k}{da} = \nu_k r_k - \lambda_k s_k \\ \frac{di_k}{da} = \lambda_k s_k - \gamma_k i_k \\ \frac{dr_k}{da} = \gamma_k i_k - \nu_k r_k \end{cases}$$

Hence, this ODE model didn't represent actual transmission events but rather provided an estimate of age-prevalence curves. A binomial likelihood function was used to fit the model to survey data: $\mathcal{L}(\theta|I_k) = \prod_a \binom{N_a}{I_a} p_a^{I_a} (1 - p_a)^{N_a - I_a}$, where p_a denoted the expected fraction of infectious individuals aged a . Constraining the same model by keeping values of λ , γ and ν fixed across surveys yielded the null model where age-prevalence remained constant between 2006, 2010 and 2014. A likelihood ratio test was used to assess the statistical significance between the null and alternate model.

Results

Parasite prevalence and density

A total of 5913 individuals were surveyed over the entire study period: 1280 in 2006, 2117 in 2010, and 2516 in 2014 (supplementary Table S2). By LM, *P. falciparum* prevalence decreased from 34.0% in 2006 to 7.3% in 2010 and 2.8% in 2014 ($P<0.001$). By qPCR, *P. falciparum* prevalence decreased from 42.1% in 2006 to 18.7% in 2010 and 9.0% in 2014 ($P<0.001$, Table 1, Figure 2A). In 2006 prevalence peaked at 9 years, in 2010 at 12.5 years and in 2014 at 19.5 years (ODE model, $P<0.001$, Figure 3A).

P. vivax prevalence by LM similarly decreased from 17.4% in 2006 to 6.9% in 2010 and 2.7% in 2014 ($P<0.001$). By qPCR, *P. vivax* prevalence decreased from 41.7% in 2006 to 12.7% in 2010, but since increased to 19.7% ($P<0.001$, Table 1, Figure 2A). In all surveys it peaked in children of approximately 6 years (Figure 3B).

In 2006, 20.7% (265/1280) of all individuals carried *P. falciparum*/*P. vivax* co-infection, 3.9% (82/2117) in 2010 and 1.6% (41/2517) in 2014 ($P<0.001$). *P. malariae* prevalence was 1.3% (28/2117) in 2010 and 1.4% (36/2117) in 2014 ($P=0.758$). *P. ovale* prevalence was 0.01% (2/2117) in 2010, and in 2014 no *P. ovale* was detected ($P=0.123$). 35/64 *P. malariae* and both *P. ovale* carriers were co-infected with other species.

Mean *P. falciparum* and *P. vivax* gene copy numbers decreased 10-fold ($P<0.001$) and 5-fold ($P<0.001$) from 2006 to 2014 (Table 1, Figure 2B). In all surveys mean *P. falciparum* density was 5-10-fold higher than *P. vivax* density. As a result of lower parasite densities, the proportion of submicroscopic infections increased from 2006 to 2014, for *P. falciparum* from 37.2% to 72.1% ($P<0.001$), and for *P. vivax* from 62.0% to 86.7% ($P<0.001$, Table 1). A

generalized additive model indicated that in response to increased training, LM diagnosis had become more sensitive over time (Supplementary Figure S1). Assuming identical LM sensitivity in all 3 surveys, the increase in the proportion of submicroscopic infections would have been even more pronounced.

Densities of both species decreased with age. The decrease in *P. falciparum* densities was slower in 2010 and 2014 as compared to 2006 (Figure 4A, interaction of \log_{10} -age with \log_{10} -density: $P=0.042$). This effect was even more pronounced for *P. vivax*, with little change of densities with age in 2014 (Figure 4B, $P<0.001$).

By genotyping, a pronounced increase in the proportion of single clone infections was observed. The proportion of *P. falciparum* single clone infections was 57.0% in 2006, 80.1% in 2010 and 82.3% in 2014 ($P<0.001$). For *P. vivax*, the proportions were 50.9%, 61.3% and 78.7% in 2006, 2010 and 2014, respectively ($P<0.001$).

In multivariable analysis, age was highly associated with the risk of infection in all surveys and for both species (Supplementary Table S3). *P. falciparum* prevalence differed between catchments in all surveys, but not *P. vivax* prevalence. Treatment with antimalarials in the past 2 months resulted in an approximately 50% reduction of the odds of *P. falciparum* or *P. vivax* infection in 2006 ($P<0.001$). No such association was observed in 2010 and 2014 ($P\geq 0.079$).

Clinical symptoms

Improvements in morbidity indicators were observed over the eight-year period (Table 2). The proportion of individuals that reported having experienced a 'malaria' episode in the past 2 weeks, or that had received antimalarials in the previous 2 months, decreased 12- and 6-fold ($P<0.001$, Table 2). The proportion of qPCR-positive infections defined as clinical malaria

decreased 2-3 fold ($P \leq 0.039$, Table 2), and the population attributable fraction of fever or history of fever caused by LM-positive infections decreased substantially ($P < 0.001$, Table 2). In contrast, measured fever did not change significantly ($P = 0.843$).

There was no significant association between measured fever and *P. falciparum* infection in 2006 (OR=1.56, CI95 0.60-4.01, $P = 0.37$), in 2010 this association was weak (OR=2.24, CI95 1.04-4.83, $P = 0.039$), and very strong in 2014 (OR=4.46, CI95 2.02-9.88, $P < 0.001$). Measured fever was not associated with *P. vivax* infection.

The proportion of participants presenting with an enlarged spleen decreased from 30.2% to 1.3% (Table 2). In 2006 and 2010, an infection approximately doubled the odds of presenting with an enlarged spleen (2006: OR=2.36, $P < 0.001$, 2010: OR=1.65, $P = 0.06$), in 2014 this association was even stronger (OR=7.99, $P < 0.001$). The proportion of participants with moderate-to-severe anemia (<8 g/dL) halved between 2006 and 2014 ($P < 0.001$, Table 2).

Transmission potential

In 2010, *P. falciparum* gametocytes were detected in 60.7% of individuals with blood-stage parasitemia, and in 43.3% in 2014 ($P < 0.001$). This resulted in a population gametocyte prevalence of 11.1% in 2010 and 3.9% in 2014 ($P < 0.001$), Table 1, Figure 2C, 2D). *P. vivax* gametocytes were detected in 48.9% of infected individuals in 2010, and 22.6% in 2014 ($P < 0.001$), resulting in a population prevalence of 6.2% and 4.4% ($P = 0.009$, Table 1, Figure 2C, 2D). *P. falciparum* gametocyte densities decreased 5-fold between 2010 and 2014 (2010: 85.5 transcripts/ μ L [CI95: 58.2-125.4]; 2014: 18.2 transcripts/ μ L [CI95: 9.9-33.5]). Little

change of *P. vivax* gametocyte densities was observed (2010: 13.6 transcripts/ μL [CI95: 10.0-18.4]; 2014: 23.7 transcripts/ μL [CI95: 15.2-37.0]).

Both the proportion gametocyte positive and gametocyte densities closely correlated with blood-stage parasite densities, especially for *P. vivax*. Each 10-fold increase in parasite density increased the odds to detect gametocytes 1.64-fold [CI95 1.42-1.90, $P<0.001$] for *P. falciparum*, and 3.77-fold [CI95 2.98-4.78, $P<0.001$] for *P. vivax* (Figure 5). Among gametocyte-positive samples, each 10-fold increase in parasite density resulted in a 1.66-fold [CI95 1.33-2.08] and 3.77-fold [CI95 2.98-4.77] increase in *P. falciparum* and *P. vivax* gametocyte densities ($P<0.001$).

The proportion of gametocyte carriers that had blood-stage parasites detected by LM decreased from 2010 to 2014. In 2010, 54.3% of *P. falciparum* gametocyte carriers were LM-positive for blood-stage parasites, in 2014, only 37.1% ($P=0.004$). Among *P. vivax* gametocyte carriers, 84.1% were LM positive for asexual blood-stages in 2010, but only 39.7% in 2014 ($P<0.001$). 90.4% of *P. falciparum* and 92.6% of *P. vivax* gametocyte carriers were asymptomatic.

Spatial heterogeneity

In multivariate analysis, catchment was associated with *P. falciparum* infection in all three surveys (Supplementary Table S3). In 2006, *P. falciparum* prevalence ranged from 35.1%-45.5% ($P=0.005$). More pronounced differences were observed in 2010. Prevalence was lowest in Utu (8.0%), but 2- and 3-fold higher in Mugil (15.1%) and Malala (25.5%, $P<0.001$).

In 2014, *P. falciparum* prevalence was 4.7% in Utu, as compared to 8.6% and 12.3% in Mugil and Malala ($P<0.001$). On the village level, substantial *P. falciparum* spatial heterogeneity

was observed (Supplementary Table S1). In nine villages *P. falciparum* prevalence was low (ranging from 0-5.6%), while in eight villages it ranged from 8.3-22.2%. The diversity of parasite populations remained high, even when prevalence was low. In six of the low-prevalence villages ≥ 2 isolates were genotyped by *pfmsp2*, and within each village different clones were detected (Supplementary Table S4).

Catchment was not associated with *P. vivax* prevalence in either survey (Supplementary Table S3). In 2006 prevalence was 40.8% in Utu, 43.9% in Mugil, and 39.6% in Malala ($P=0.386$). In 2010 prevalence was lowest in Utu (10.6%) and Malala (11.7%), but higher in Mugil (15.1%, $P=0.130$). In 2014, prevalence was 15.7% in Utu, 19.7% in Mugil and 21.8% in Malala ($P=0.0502$).

Discussion

Along the north coast of PNG, continuous control of malaria over 8 years has led to a 12- and 6-fold decrease of *P. falciparum* and *P. vivax* prevalence respectively by LM. Using a highly sensitive qPCR to diagnose infections, the continuous decrease in *P. falciparum* prevalence was confirmed, whilst *P. vivax* prevalence increased between 2010 and 2014. Parasite densities of both species have decreased considerably, and thus an increasing proportion of infections were asymptomatic and submicroscopic.

Gametocyte densities and the probability to detect gametocytes – and thus human-to-mosquito transmission potential – were closely correlated to blood-stage parasite density. Because of the lower parasite densities, gametocytes were detected in a lower proportion of infections in 2014 than in 2010. However, due to the increase in the proportion of submicroscopic infections, remaining gametocyte carriers became more difficult to identify. For both species, the majority of gametocyte carriers (by RT-qPCR) were LM-positive for asexual parasites in 2010, but in 2014 approximately two-thirds of gametocyte carriers presented with submicroscopic infections. Over 90% of gametocyte carriers were asymptomatic, and thus present a challenge for malaria control and elimination. In PNG, most febrile cases presenting at health centers are diagnosed by LM or RDT and antimalarial treatment is given to positive individuals. The increasing proportion of asymptomatic and submicroscopic infections thus remain untreated, yet such infections of both *P. falciparum* and *P. vivax* have been shown to frequently infect mosquitos [8-10].

Decreasing levels of transmission also appeared to have an impact on acquisition of immunity to *P. falciparum*. In malaria endemic countries, the attack rate in children increases with age [34], in parallel individuals acquire immunity gradually, with the speed of acquisition depending on transmission intensity. As a result, clinical malaria and parasite prevalence peaks in children and then decreases as immunity is acquired [35]. In 2006, very high

parasite densities were observed in young children, followed by a rapid decline with increasing age. This age-associated decline was less marked in 2014, and the peak *P. falciparum* prevalence shifted from children to adolescents, reflecting delayed acquisition of immunity, similar to trends observed for clinical malaria in Africa [36]. In parallel, the odds of presenting with fever when infected with *P. falciparum* increased 4-fold between 2006 and 2014, further suggesting a reduced level of (clinical) immunity.

Individuals with low levels of immunity are expected to present with higher parasite densities, and the risk of developing clinical malaria increases. However, over the 8-year period, parasite densities for both species decreased considerably, and the proportion of individuals with clinical malaria decreased 3-fold. Parasite densities are not only determined by acquired immunity, but also by the age of the infection on the day of sampling (Supplementary Figure S2). Densities in the peripheral blood peak in the first phase of the infection, and when not treated they can persist for weeks or months at low densities [3]. When transmission is lower, fewer new infections are acquired and persisting infections are thus on average older and densities lower. For example, the molecular force of *P. vivax* blood-stage infection in PNG children decreased from 15 clones/year [37] to 5 clones/year between 2006 and 2010 [19]. In 2014, the contribution of older low-density infections appeared to be far greater than the contribution of infections with possibly higher initial parasite densities caused by lower levels of immunity. In addition, 6-fold less treatment was administered in 2014 compared to 2006; further contributing to a large number of old, low-density “chronic” infections.

The overall decrease in prevalence was accompanied by increasing heterogeneity of *P. falciparum* prevalence at the village level, indicating foci of residual transmission. In contrast to clonal *P. falciparum* outbreaks observed in the highlands of PNG [38], Solomon Islands [39], and South America [40], the parasite populations in the 2014 survey remained genetically diverse, even in very low prevalence villages. This could indicate that residual infections were imported from villages with higher transmission where a genetically diverse population is maintained.

In contrast to the constant decline in *P. falciparum* prevalence, *P. vivax* prevalence increased since 2010. In PNG, in 2008-2010, 80% of all blood-stage *P. vivax* infections in children were caused by relapses [18, 19]. As a consequence, for *P. vivax*, mosquito-to-human transmission levels and parasite prevalence rates in the population are less correlated than for *P. falciparum*. In 2014, *P. vivax* densities were very low, and almost 80% were single-clone infections. Both factors suggest high proportions of relapses. Relapses often consist of a single clone [41-43], and often consist of clones that are homologous or related to the initial blood-stage infection [42, 44]. They thus carry a reservoir of antigens the immune system has been exposed to recently, and even young children with limited acquired immunity may be able to control such infections [45]. An increasing proportion of infections caused by relapses from previously acquired infections could thus explain the low *P. vivax* densities across all ages in 2014, and the increase in *P. vivax* prevalence from 2010 to 2014. It is possible that the 2014 survey has captured a phase during which the vast *P. vivax* hypnozoite reservoir accumulated in the population from years of high transmission has not yet been 'exhausted' and thereby prevalence has increased temporarily.

In conclusion, the rapid decline in transmission in a population maintaining a relatively high level of clinical immunity resulted in a large proportion of very low density infections. Increasing proportions of submicroscopic infections when prevalence is lower has been found across countries both for *P. falciparum* and *P. vivax* [3, 4]. Few studies have assessed the speed of change in the same population. In the Brazilian Amazon, a 9-fold decrease of *P. vivax* prevalence over 3 years was accompanied by an increase in the proportion of submicroscopic infections from 44% to 73%, and almost all of them carried gametocytes [12]. The present finding of an increasing proportion of gametocyte carriers being submicroscopic – despite an overall lower proportion gametocyte positive – is thus likely a general pattern in countries where transmission levels are decreasing.

Novel strategies are therefore needed to effectively target the asymptomatic low-density reservoir of *Plasmodium* infections. For *P. falciparum*, mass screen and treat (MSAT) approaches would require highly sensitive diagnostics tools. For *P. vivax*, where hypnozoite carriers cannot be detected with any current diagnostic test, MSAT is not an appropriate intervention [19], and other approaches will need to be developed. The large asymptomatic and submicroscopic reservoirs thus represent a challenge to the goal of a malaria-free Asia-Pacific for the foreseeable future. An in-depth understanding of their contribution to maintaining transmission and better tools and surveillance strategies to efficiently identify and target these infections are thus urgently needed.

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Figure 1: Map of study sites. Green dots represent study villages in the Malala, Mugil and Utu catchments surveyed in 2014. As a reference, Madang Town is shown (purple).

Figure 2: *P. falciparum* and *P. vivax* prevalence (A) and density (with 95% CI) (B) by qPCR in 2006, 2010 and 2014, population gametocyte prevalence (C) and proportion of all infected individuals (by qPCR) positive for gametocytes by RT-qPCR (D). NA = no data available.

Figure 3: Age trends in parasite prevalence by qPCR. Solid lines: GAM predictions (with 95% confidence intervals), dotted lines: ODE model. *P. falciparum* prevalence peaks in older individuals in 2010 and 2014 as compared to 2006, while no change for *P. vivax* peak prevalence was observed.

Figure 4: Geometric mean copy numbers across age groups for *P. falciparum* (A) And *P. vivax* (B). Error bars show 95% confidence intervals.

Figure 5: Probability to detect gametocytes vs. copy numbers by qPCR (GAM prediction with 95% confidence intervals).

Table 1: Prevalence by qPCR and LM, proportion submicroscopic infections, gametocyte carriage (by RT-qPCR), and geometric mean parasite densities by LM and genome copy numbers by qPCR in each survey. NA = not available.

<i>P. falciparum</i>							
Survey year	Prevalence by qPCR %	Prevalence by LM %	LM positive/qPCR negative % ¹	Proportion submicroscopic %	Proportion gametocyte positive %	Density by qPCR [CI95]	Density by LM [CI95]
2006	42.1 (539/1280)	34.0 (435/1280)	11.2% (59/435)	36.2 (195/539)	NA	584.0 [419.4-813.2]	378.3 [315.7-453.3]
2010	18.7 (396/2117)	7.3 (156/1094)	7.1% (11/156)	62.7 (224/389)	60.6 (235/387)	127.4 [91.9-176.7]	808.0 [579.0-1127.0]
2014	9.0 (226/2517)	2.8 (69/2513)	8.7% (6/69)	72.1 (163/226)	43.3 (97/224)	80.3 [56.9-113.3]	346.6 [217.6-552.2]
<i>P. vivax</i>							
2006	41.7 (534/1280)	17.4 (223/1280)	8.5% (19/223)	62.0 (331/534)	NA	48.9 [32.5-73.7]	260.6 [212.6-319.2]
2010	12.7 (271/2117)	6.9 (147/2094)	4.8% (7/147)	48.2 (130/270)	48.9 (132/270)	23.9 [19.6-29.2]	118.3 [97.9-142.8],
2014	19.7 (496/2517)	2.7 (68/2513)	2.9% (2/68)	86.7 (430/496)	22.6 (111/492)	8.3 [6.8-10.2]	168.3 [118.2-240.0]

¹ Percentage and number of samples positive by LM and negative by qPCR

Table 2: Clinical symptoms of study participants.

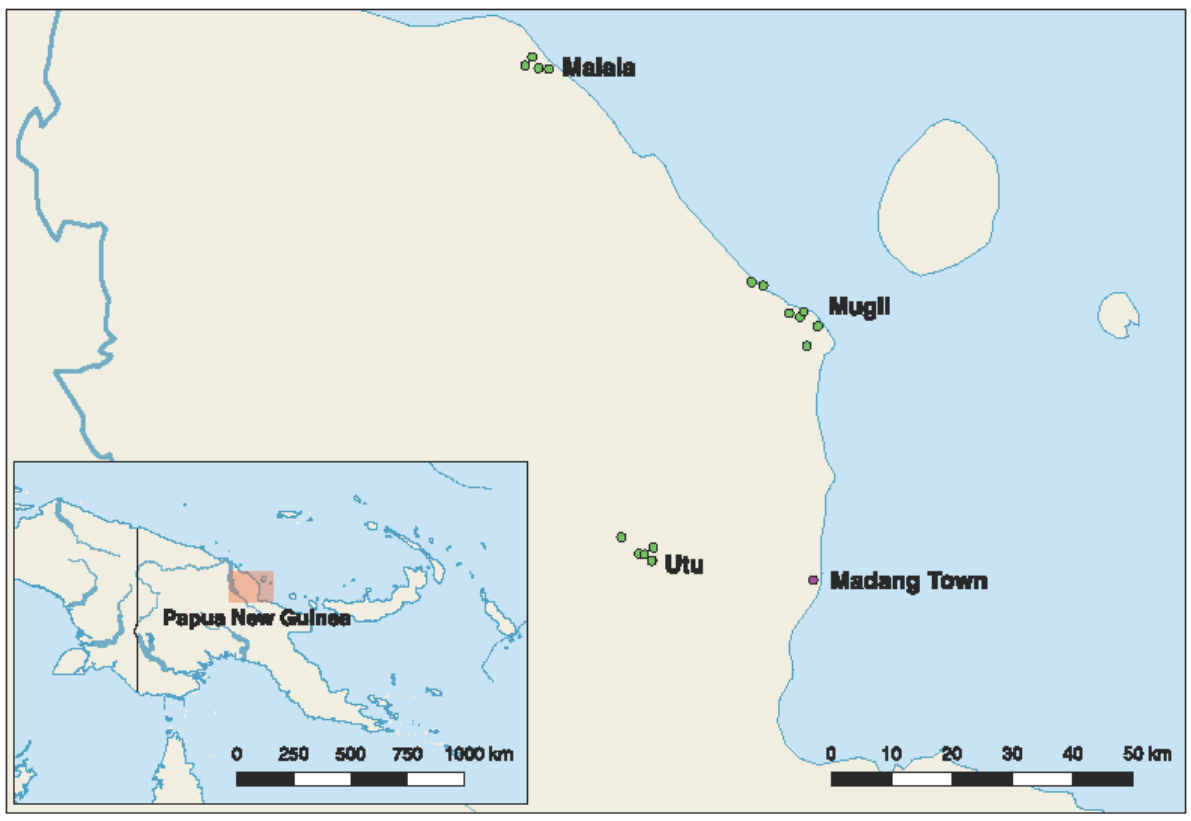
¹ Proportion of all individuals positive by PCR that were defined as clinical cases (measured or reported fever and LM positive).

² Population attributable fraction of measured or reported fever due to LM-positive infections

* *P*-value adjusted for age and gender

	2006	2010	2014	<i>P</i>
'Malaria episode' in past 2 weeks	17.0% (217/1278)	8.0% (168/2107)	1.4% (34/2477)	<0.001
Antimalarials in past 2 months	17.7% (225/1280)	1.8% (39/2117)	2.8% (71/2498)	<0.001
Measured fever	1.3% (17/1280)	1.4% (30/2092)	1.2% (30/2430)	0.843
<i>P. falciparum</i> clinical ¹	6.9% (38/539)	7.6% (26/396)	2.7% (6/226)	0.039
<i>P. vivax</i> clinical ¹	3.2% (17/534)	4.8% (13/271)	1.0% (5/496)	0.005
Proportion of fever attributable to Pf ²	24.1%	8.2%	3.7%	<0.001
Proportion of fever attributable to Pv ²	15.6%	-1.9%	0.8%	<0.001
Anemia (hemoglobin <8 g/dL)	7.2% (92/1274)	6.1% (113/1844)	3.5% (88/2514)	<0.001
Hemoglobin g/dL [CI95]	10.55 [10.46-10.66]	10.64 [10.56-10.72]	10.86 [10.79-10.93]	<0.001*
Enlarged spleen	30.2% (368/1279)	3.2% (1928/2112)	1.3% (62/2516)	<0.001

Figure 1



Accepted

Figure 2

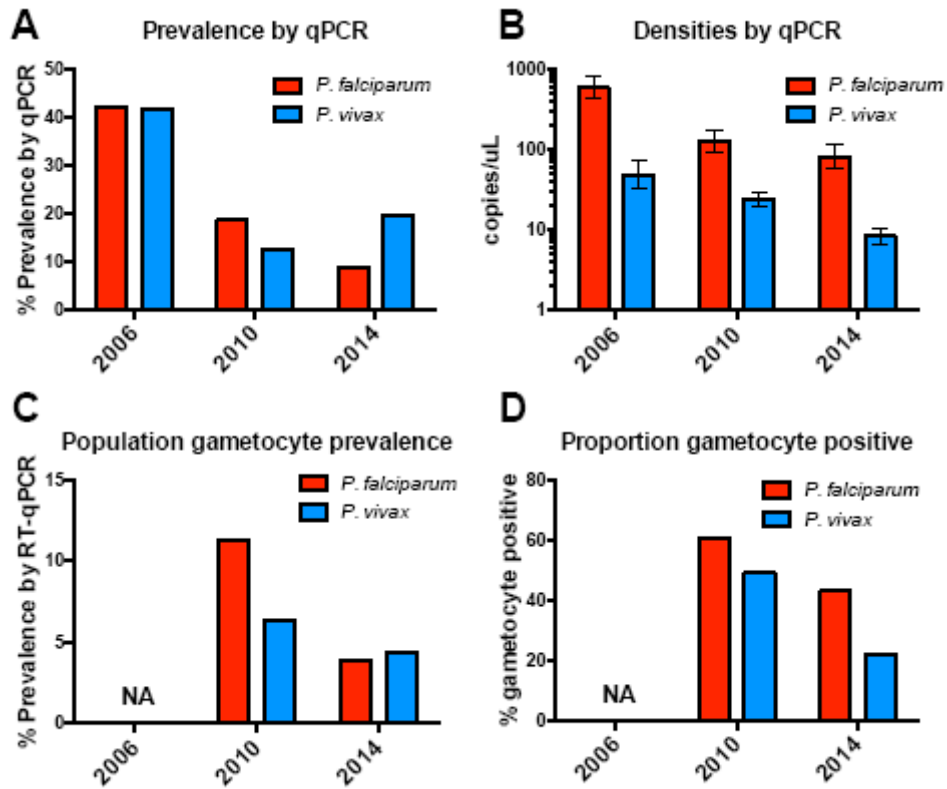
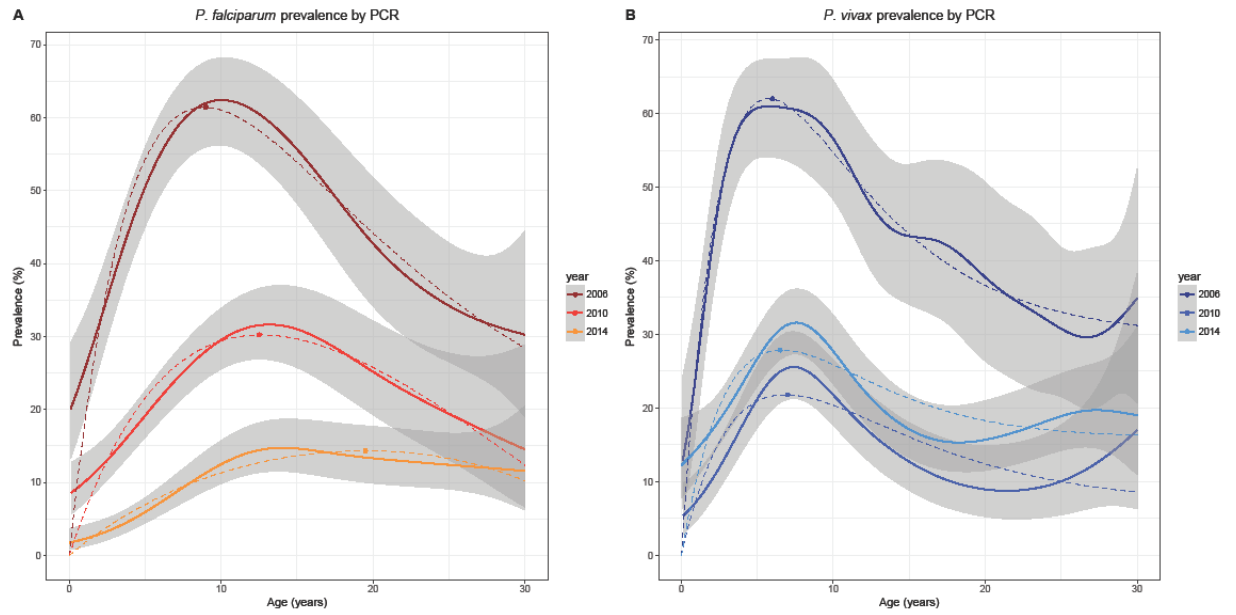
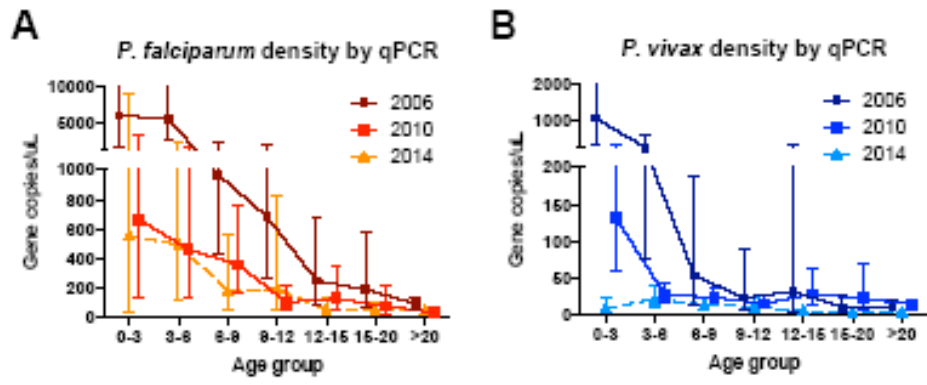


Figure 3



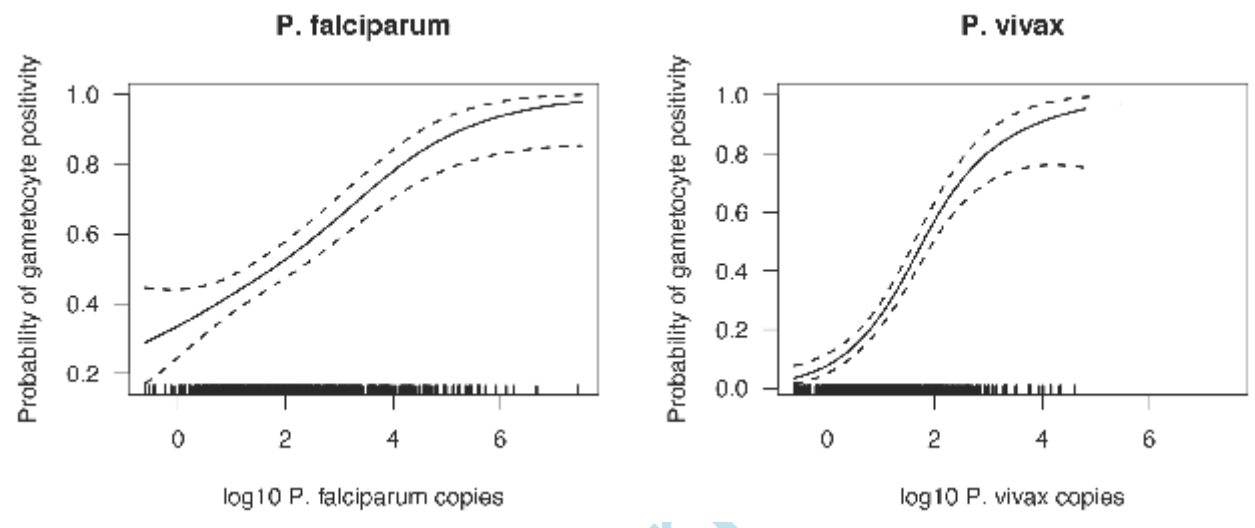
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Figure 4



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Figure 5



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