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MAJOR ARTICLE

Sustained Malaria Control Over an 8-Year Period in Papua New Guinea: The Challenge of Low-Density Asymptomatic Plasmodium Infections

Cristian Koepfli,[1,2,5,a] Maria Ome-Kaius,[1,2,7,a] Shadrach Jally,[7] Elisheba Malau,[1,2] Samuel Maripal,[7] Jason Ginny,[7] Lincoln Timinao,[7] Johanna Helena Kattenberg,[1,7] Thomas Obadia,[8,9] Michael White,[1,10] Patricia Rarau,[3,7] Nicolas Senn,[7,11] Alyssa E. Barry,[1,2] James W. Kazura,[6] Ivo Mueller,[1,2,8,12,b] and Leanne J. Robinson[1,2,4,7,b]

[1]Population Health and Immunity Division, Walter and Eliza Hall Institute, Melbourne, Australia; [2]Department of Medical Biology, University of Melbourne, Parkville, Australia; [3]School of Population and Global Health, University of Melbourne, Parkville, Australia and; [4]Burnet Institute, Melbourne, Australia; [5]University of California–Irvine; [6]Case Western Reserve University, Cleveland, Ohio; [7]Papua New Guinea Institute of Medical Research, Madang; [8]Malaria: Parasites & Hosts Unit, Department of Parasites and Insect Vectors, Institut Pasteur, Paris, France and [9]Bioinformatics and Biostatistics Hub, Center for Bioinformatics, Biostatistics, and Integrative Biology, Institut Pasteur, Paris, France; [10]Medical Research Council Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College London, United Kingdom; [11]Swiss Tropical and Public Health Institute, Basel, Switzerland; and [12]Barcelona Center for International Health, Barcelona, Spain.

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 was introduced in 2012. Cross-sectional surveys were conducted in Madang Province in 2006 (with 1280 survey participants), 2010 (with 2117 participants), and 2014 (with 2516 participants). Infections were quantified by highly sensitive quantitative polymerase chain reaction (PCR) analysis, and gametocytes were quantified by reverse-transcription qPCR analysis.

Results. *Plasmodium falciparum* prevalence determined by qPCR decreased from 42% in 2006 to 9% in 2014. The *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 malaria 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; gametocyte.

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 toward elimination [3–5]. Clinical malaria episodes that are light microscopy (LM) and/or rapid diagnostic test positive 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-naive individuals experiencing less frequent exposure will acquire immunity more slowly. In combination, these effects are expected to result in changes in treatment frequency, parasite distribution and gametocyte density, and infection duration. Yet, little is known about the extent and time frame of such changes.

Owing to differences in the biology of *P. falciparum* and *P. vivax*, the effects of control are often remarkably different for

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[a]C. K. and M. O.-K. contributed equally to this report.

[b]I. M. and L. J. R. contributed equally to this report.

Correspondence: L. Robinson, PhD, MPH, Walter and Eliza Hall Institute, 1G Royal Parade, Parkville VIC 3052, Australia (robinson@wehi.edu.au).

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the 2 species, and in many countries *P. vivax* has proven more resilient to control [5, 12, 13]. *P. vivax* densities determined by microscopy are generally 5–10 times lower than *P. falciparum* densities [14–16], making diagnosis more difficult. Latent liver-stage parasites (hypnozoites) escape diagnosis, and standard treatment against blood-stage parasites does not affect them [17]. In regions where malaria is highly endemic, up to 80% of all blood-stage *P. vivax* infections in children are due to relapses [18, 19]. If the transmission level 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. Thus, the proportion of all blood-stage parasite infections in the population that are caused by relapsed *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 on the north coast of Papua New Guinea (PNG), *P. falciparum* and *P. vivax* prevalence determined by polymerase chain reaction (PCR) analysis had reached 30%–60% in the general population during 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 2008/2009 and again in 2011/2012, long-lasting insecticidal nets were distributed in PNG. Rapid diagnostic tests to test all febrile cases in health centers, as well as artemisinin-based combination therapy with artemether-lumefantrine as first-line treatment, were implemented in 2012. Surveys conducted after the first round of long-lasting insecticidal net distribution found considerable decreases in entomological inoculation rate [23] and parasite prevalence detected 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). Blood samples were collected from 5913 individuals, and highly sensitive molecular assays were used to diagnose malarial 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 the PNG Institute of Medical Research Institutional Review Board (IMR IRB) (1116/1204), the PNG Medical Research Advisory Committee (MRAC) (11.21/1206), the Walter and Eliza Hall Institute

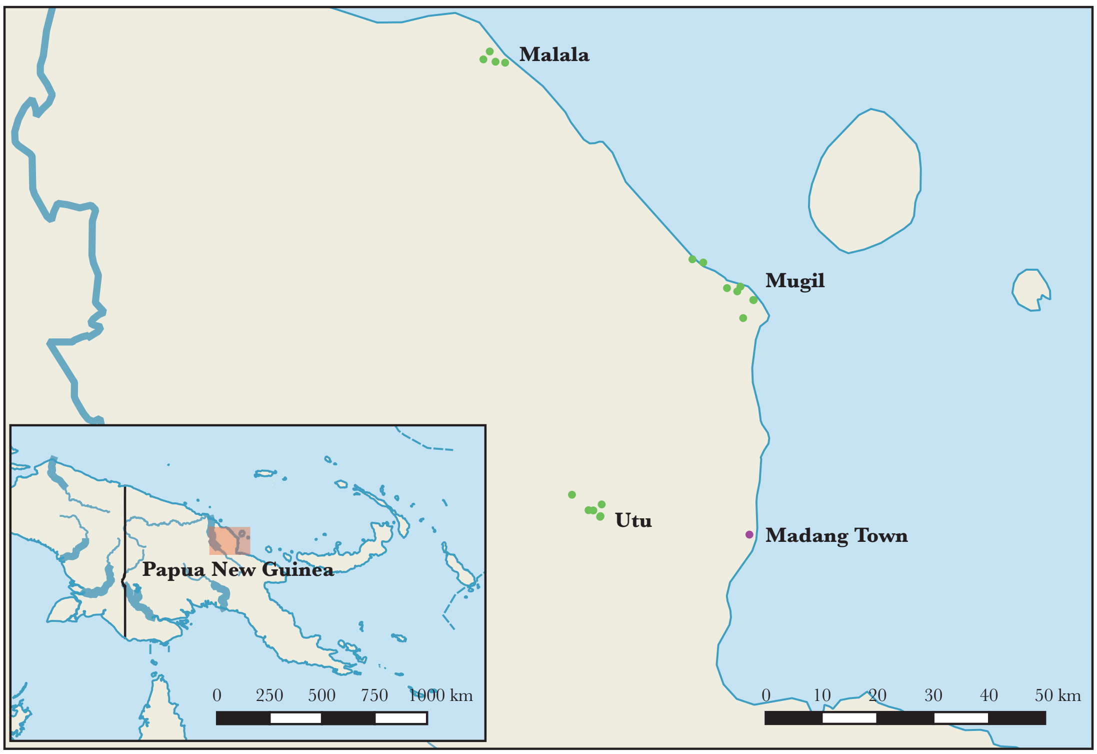

Figure 1. Map of study sites. Green dots represent study villages in the Malala, Mugil, and Utu catchments surveyed in 2014. As a reference, Madang Town is shown (purple dot).

Human Research Ethics Committee (WEHI HREC) (12/09), and the Case Western Reserve University University Hospitals of Cleveland Medical Center(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 1 inland catchment (Utu). The climate is tropical, with a rainy season from December to April. Samples were collected during March–April in 2006 and between mid-May and early July during 2010 and 2014. A convenience sampling strategy including individuals aged >6 months was used. In 2014, among villages, 8.3%–45.1% of residents were sampled (Supplementary Table 1).

From each participant, a 250-µL blood sample obtained by finger prick was collected into ethylenediaminetetraacetic acid–lined tubes. For gametocyte detection, 50 µL of blood was transferred into tubes containing 250 µL of RNAprotect (Qiagen; performed during 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 methods described elsewhere were used [25]. In brief, DNA was extracted from 200 µL of pelleted blood, using the Favorgen 96-well genomic DNA extraction kit and eluted in 200 µL of buffer. *P. falciparum* and *P. vivax*, as well as *Plasmodium malariae* and *Plasmodium ovale* (during the 2010 and 2014 surveys only), were quantified by highly sensitive qPCR assays, using 4 µL of DNA, corresponding to 4 µL of blood [26]. A dilution of plasmids containing the target sequence of the PCR was run as an external standard for absolute quantification. *P. falciparum*–positive samples were genotyped by *msp2* [22, 27], and *P. vivax*–positive samples were genotyped by *msp1*F3 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 reverse-transcription qPCR protocols [30] and were 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 analysis. Densities (determined by LM or qPCR analysis) are given as geometric means. Fever or history of fever was defined as measured fever >37.5°C or reported febrile illness in the past 2 days. Clinical malaria was defined as fever or history of fever and detection of parasites by microscopy. Logistic regression was used to assess risk factors of infection, and χ^2 tests were used to compare rates of infection between age groups and catchments. Only in rare cases was an individual included in >1 survey, because the 3 surveys did not necessarily involve the same villages. These cases were treated as independent observations.

Model for Age-Prevalence Curves

The nonlinear 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 by the work of Smith et al [31]. The host population was described using a compartmental model similar to that of a classical SIRS model, using a set of 3 ordinary differential equations (ODEs). Instead of modeling the proportions of susceptible (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 did not 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 models.

RESULTS

Parasite Prevalence and Density

A total of 5913 individuals were surveyed over the study period, with 1280 participating in 2006, 2117 participating in 2010, and 2516 participating in 2014 (Supplementary Table 2). By LM, *P. falciparum* prevalence decreased from 34.0% in 2006 to 7.3% in 2010 and 2.8% in 2014 ($P < .001$). By qPCR analysis, *P. falciparum* prevalence decreased from 42.1% in 2006 to 18.7% in 2010 and 9.0% in 2014 ($P < .001$; Table 1 and Figure 2A). Prevalence peaked at 9 years in 2006, at 12.5 years in 2010, and at 19.5 years in 2014 (ODE model, $P < .001$; Figure 3A).

P. vivax prevalence by LM similarly decreased from 17.4% in 2006 to 6.9% in 2010 and 2.7% in 2014 ($P < .001$). By qPCR analysis, *P. vivax* prevalence decreased from 41.7% in 2006 to 12.7% in 2010 but increased to 19.7% in 2014 ($P < .001$; Table 1 and

Table 1. ***Plasmodium falciparum* and *Plasmodium vivax* Prevalence, Submicroscopic Infection Percentage, Gametocyte Carriage Percentage, and Parasite Density, by Survey Year**

Parasite, Year	Parasite Prevalence		LM Positive/ qPCR Negative	Submicroscopic Infection	Gametocyte Carriage by RT-qPCR	Parasite Density[a]	
	By qPCR	By LM				By qPCR	By LM
P. falciparum							
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)
P. vivax							
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)

Data are percentage (proportion) of samples, unless otherwise indicated.

Abbreviations: CI, confidence interval; LM, light microscopy; NA, not available; qPCR, quantitative polymerase chain reaction; RT, reverse transcription.

[a]Data are the number of samples positive by qPCR and by LM (95% CI).

Figure 2A). In all surveys, it peaked in children aged approximately 6 years (Figure 3B).

In 2006, 20.7% of individuals (265 of 1280) carried *P. falciparum*/*P. vivax* coinfection, 3.9% (82 of 2117) carried both parasites in 2010, and 1.6% (41 of 2517) carried both parasites in 2014 ($P < .001$). *P. malariae* prevalence was 1.3% (28 of 2117 individuals) in 2010 and 1.4% (36 of 2117 individuals) in 2014 ($P = .758$). *P. ovale* prevalence was 0.01% (2 of 2117 individuals) in 2010, and in 2014 no *P. ovale* was detected ($P = .123$). Thirty-five out of 64 *P. ovale* carriers, and both *P. ovale* carriers were coinfected with other species.

Mean *P. falciparum* and *P. vivax* gene copy numbers decreased 10-fold ($P < .001$) and 5-fold ($P < .001$),

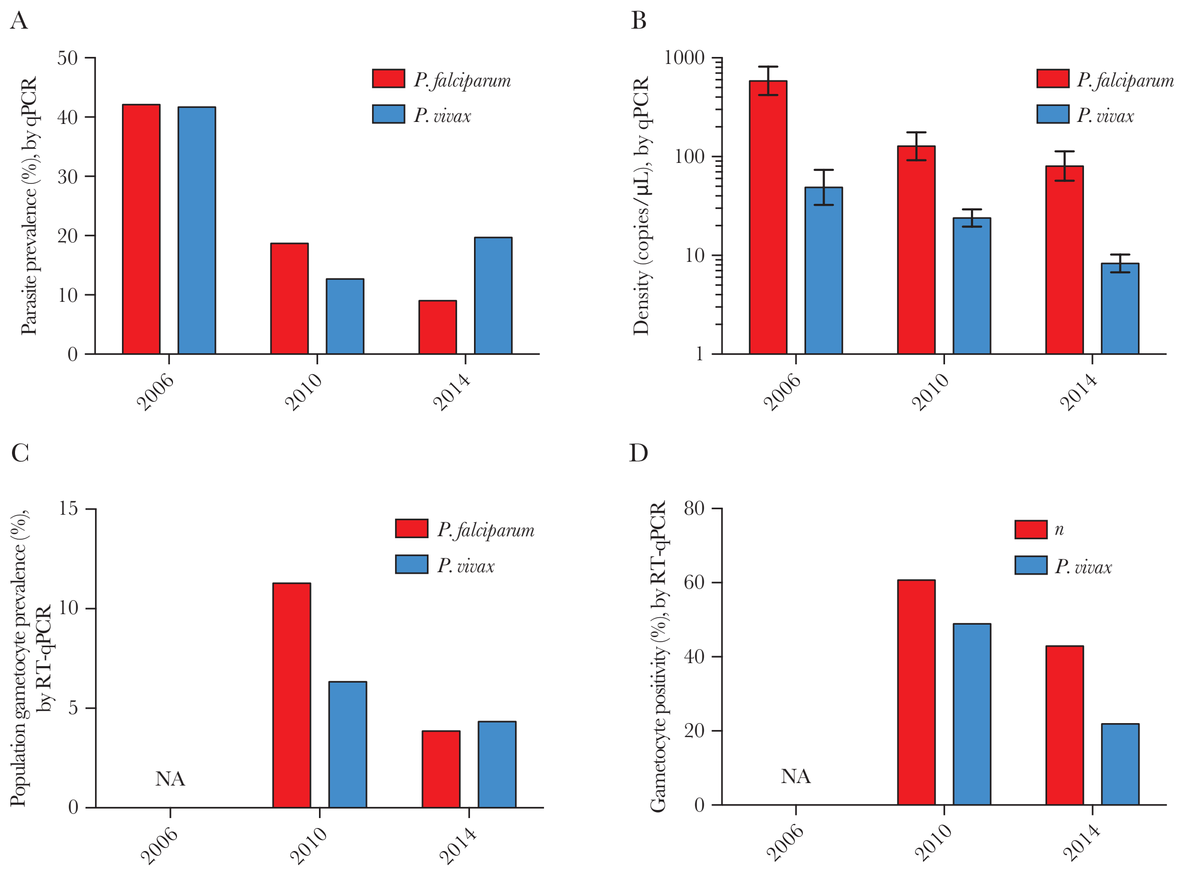

Figure 2. *Plasmodium falciparum* and *Plasmodium vivax* prevalence (*A*) and density (with 95% confidence intervals; *B*), by quantitative polymerase chain reaction (qPCR) analysis, in 2006, 2010, and 2014; and population gametocyte prevalence (*C*) and proportion of all individuals with infection detected by qPCR who were positive for gametocytes (*D*), by reverse-transcription qPCR. NA, no data available.

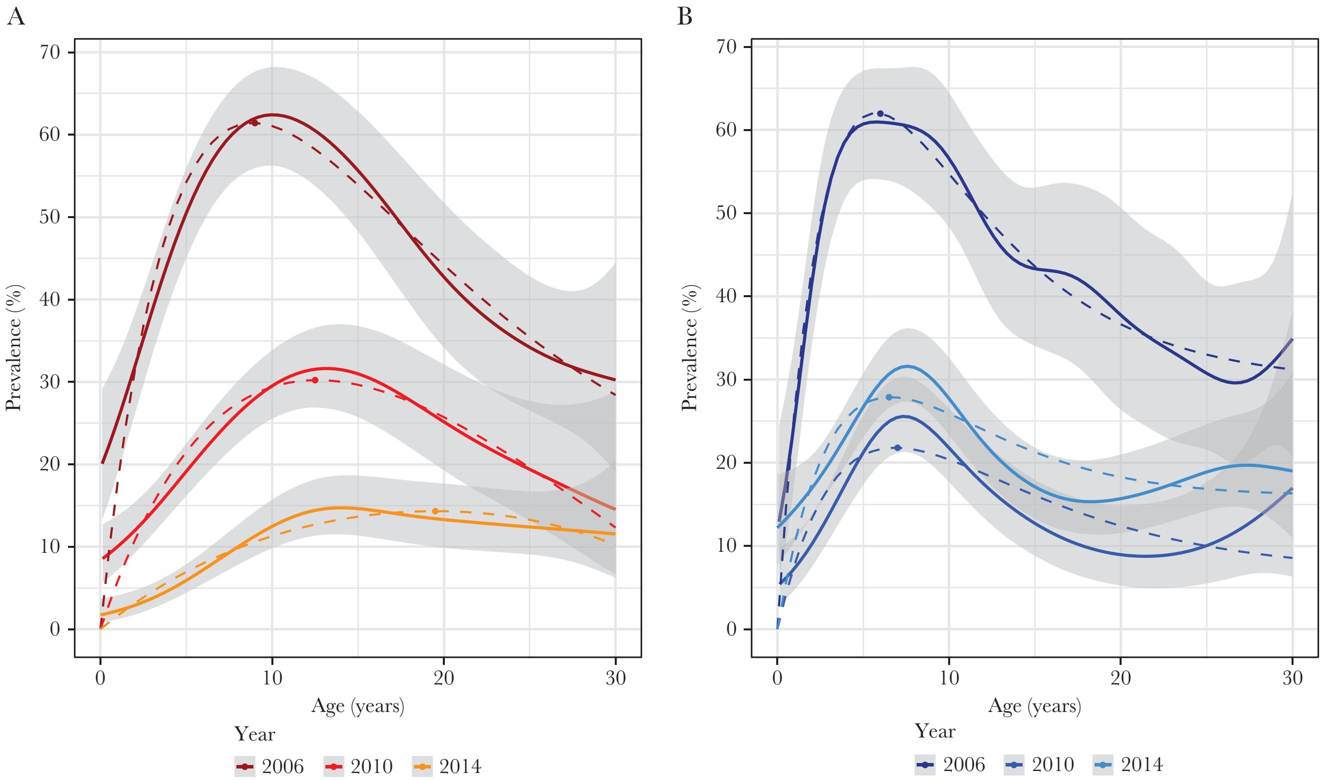

Figure 3. Age trends in *Plasmodium falciparum* (*A*) and *Plasmodium vivax* (*B*) prevalence by quantitative polymerase chain reaction analysis. Solid lines denote general additive model predictions (with 95% confidence intervals), and the dotted lines denote the ordinary differential equations model. *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.

respectively, from 2006 to 2014 (Table 1 and Figure 2B). In all surveys, the mean *P. falciparum* density was 5–10-fold higher than the mean *P. vivax* density. As a result of lower parasite densities, the proportion of submicroscopic infections increased between 2006 and 2014, from 37.2% to 72.1% for *P. falciparum* ($P < .001$) and from 62.0% to 86.7% for *P. vivax* ($P < .001$; Table 1). A generalized additive model indicated that in response to increased training, LM-based diagnosis had become more sensitive over time (Supplementary Figure 1). 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 (interaction of $\log_{10}$ age with $\log_{10}$ density: $P = .042$; Figure 4A). This effect was even more pronounced

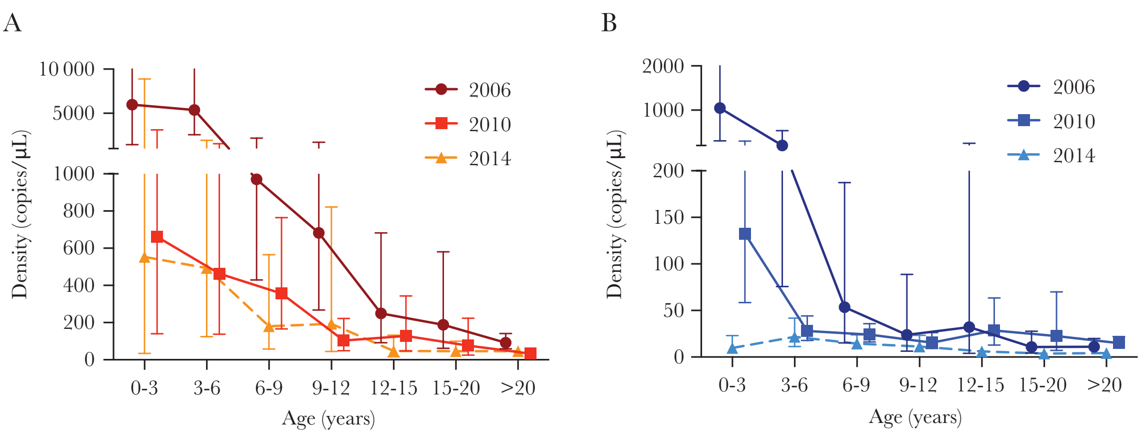

Figure 4. Geometric mean copy numbers across age groups for *Plasmodium falciparum* (*A*) and *Plasmodium vivax* (*B*), by quantitative polymerase chain reaction analysis. Error bars show 95% confidence intervals.

for *P. vivax*, with little change of densities with age in 2014 ($P < .001$; Figure 4B).

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 ($P < .001$). For *P. vivax*, the proportions were 50.9%, 61.3%, and 78.7% in 2006, 2010, and 2014, respectively ($P < .001$).

In multivariable analysis, age was highly associated with the risk of infection in all surveys and for both species (Supplementary Table 3). The *P. falciparum* prevalence differed between catchments in all surveys, but not the *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 ($P < .001$). No such association was observed in 2010 and 2014 ($P \geq .079$).

Clinical Symptoms

Improvements in morbidity indicators were observed over the 8-year period (Table 2). The proportion of individuals who reported having experienced a malaria episode in the past 2 weeks or who had received antimalarials in the previous 2 months decreased 12-fold and 6-fold, respectively ($P < .001$ for both comparisons; Table 2). The proportion of qPCR-positive infections defined as clinical malaria decreased 2–3-fold ($P \leq .039$; Table 2), and the population attributable fraction of fever or history of fever caused by LM-positive infections decreased substantially ($P < .001$; Table 2). In contrast, measured fever did not change significantly ($P = .843$).

There was no significant association between measured fever and *P. falciparum* infection in 2006 (odds ratio [OR], 1.56; 95% confidence interval [CI], .60–4.01; $P = .37$). In 2010, this association was weak (OR, 2.24; 95% CI, 1.04–4.83; $P = .039$), and it was very strong in 2014 (OR, 4.46; 95% CI, 2.02–9.88; $P < .001$). 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, having an infection approximately doubled the odds of presenting with an enlarged spleen (2006: OR, 2.36 [$P < .001$]; 2010: OR, 1.65 [$P = .06$]); in 2014, this association was even stronger (OR, 7.99; $P < .001$). The proportion of participants with moderate-to-severe anemia (defined as a hemoglobin level of <8 g/dL) halved between 2006 and 2014 ($P < .001$; Table 2).

Transmission Potential

P. falciparum gametocytes were detected in 60.7% of individuals with blood-stage parasitemia during 2010 and in 43.3% during 2014 ($P < .001$). This resulted in a population gametocyte prevalence of 11.1% in 2010 and 3.9% in 2014 ($P < .001$; Table 1 and Figure 2C and 2D). *P. vivax* gametocytes were detected in 48.9% of infected individuals during 2010 and in 22.6% during 2014 ($P < .001$), resulting in a population prevalence of 6.2% and 4.4%, respectively ($P = .009$; Table 1 and Figure 2C and 2D). *P. falciparum* gametocyte densities decreased 5-fold between 2010 and 2014 (2010: 85.5 transcripts/µL [95% CI, 58.2–125.4]; 2014: 18.2 transcripts/µL [95% CI, 9.9–33.5]). Little change of *P. vivax* gametocyte densities was observed (2010: 13.6 transcripts/µL [95% CI, 10.0–18.4]; 2014: 23.7 transcripts/µL [95% CI, 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 of detecting gametocytes 1.64-fold (95% CI, 1.42–1.90; $P < .001$) for *P. falciparum* and 3.77-fold (95% CI, 2.98–4.78; $P < .001$) for *P. vivax* (Figure 5). Among gametocyte-positive samples, each 10-fold increase in parasite density

Table 2. Clinical Characteristics of Study Participants

Variable	2006	2010	2014	*P*
Self-reported malaria episode in past 2 wk	17.0 (217/1278)	8.0 (168/2107)	1.4 (34/2477)	<.001
Self-reported antimalarial use in past 2 mo	17.7 (225/1280)	1.8 (39/2117)	2.8 (71/2498)	<.001
Measured fever	1.3 (17/1280)	1.4 (30/2092)	1.2 (30/2430)	.843
Clinical infection[a]				
P. falciparum	6.9 (38/539)	7.6 (26/396)	2.7 (6/226)	.039
P. vivax	3.2 (17/534)	4.8 (13/271)	1.0 (5/496)	.005
Fever, PAF, %[b]				
P. falciparum	24.1	8.2	3.7	<.001
P. vivax	15.6	0	0.8	<.001
Anemia[c]	7.2 (92/1274)	6.1 (113/1844)	3.5 (88/2514)	<.001
Hemoglobin level, g/dL (95% CI)	10.55 (10.46–10.66)	10.64 (10.56–10.72)	10.86 (10.79–10.93)	<.001[d]
Enlarged spleen	30.2 (368/1279)	3.2 (1928/2112)	1.3 (62/2516)	<.001

Data are percentage (proportion) of samples, unless otherwise indicated.

Abbreviations: CI, confidence interval; LM, light microscopy; *P. falciparum*, *Plasmodium falciparum*; *P. vivax*, *Plasmodium vivax*.

[a]Defined as the proportion of qPCR-positive infections that were defined as clinical malaria (based on measured or self-reported fever and LM positivity).

[b]Population attributable fraction (PAF) of measured or self-reported fever caused by LM-positive infections

[c]Defined as a hemoglobin level of <8 g/dL.

[d]Adjusted for age and sex.

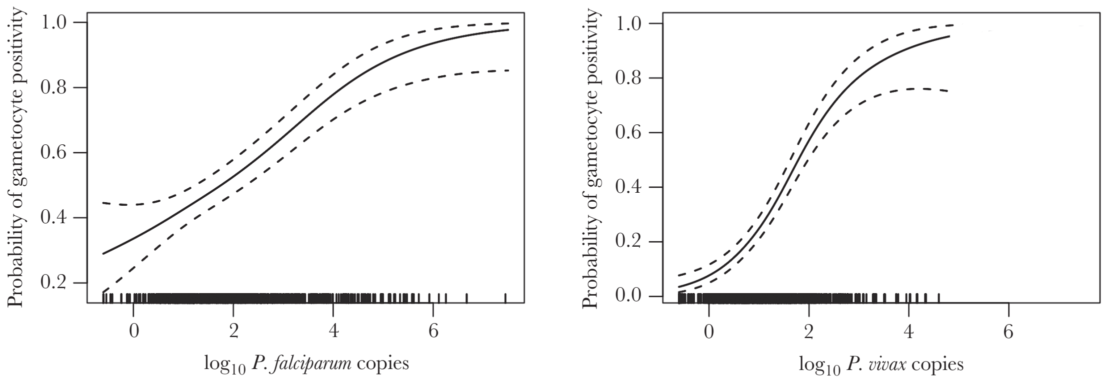

Figure 5. Probability to detect *Plasmodium falciparum* (left) and *Plasmodium vivax* (right) gametocytes versus copy numbers (by quantitative polymerase chain reaction analysis). Data are general additive model predictions with 95% confidence intervals.

resulted in a 1.66-fold (95% CI, 1.33–2.08) and 3.77-fold (95% CI, 2.98–4.77) increase in *P. falciparum* and *P. vivax* gametocyte densities, respectively ($P < .001$).

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, and only 37.1% were positive in 2014 ($P = .004$). Among *P. vivax* gametocyte carriers, 84.1% were LM positive for asexual blood-stage parasites in 2010, but only 39.7% were positive in 2014 ($P < .001$). A total of 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 3 surveys (Supplementary Table 3). In 2006, *P. falciparum* prevalence ranged from 35.1% to 45.5% ($P = .005$). More-pronounced differences were observed in 2010. Prevalence was lowest in Utu (8.0%) but 2-fold higher in Mugil (15.1%) and 3-fold higher in Malala (25.5%; $P < .001$).

In 2014, *P. falciparum* prevalence was 4.7% in Utu as compared to 8.6% and 12.3% in Mugil and Malala, respectively ($P < .001$). At the village level, substantial *P. falciparum* spatial heterogeneity was observed (Supplementary Table 1). In 9 villages, *P. falciparum* prevalence was low (range, 0%–5.6%), while in 8 villages, it ranged from 8.3% to 22.2%. The diversity of parasite populations remained high, even when prevalence was low. In 6 of the low-prevalence villages, ≥2 isolates were genotyped by *pfmsp2*, and within each village, different clones were detected (Supplementary Table 4).

Catchment was not associated with *P. vivax* prevalence in either survey (Supplementary Table 3). In 2006, prevalence was 40.8% in Utu, 43.9% in Mugil, and 39.6% in Malala ($P = .386$). In 2010, prevalence was lowest in Utu (10.6%) and Malala (11.7%) but higher in Mugil (15.1%; $P = .130$). In 2014, prevalence was 15.7% in Utu, 19.7% in Mugil, and 21.8% in Malala ($P = .0502$).

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, detected by LM. Using a highly sensitive qPCR to diagnose infections, the continuous decrease in *P. falciparum* prevalence was confirmed, whereas the *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, because of the increase in the proportion of submicroscopic infections, remaining gametocyte carriers became more difficult to identify. For both species, the majority of gametocyte carriers (determined by reverse-transcription 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 such individuals thus present a challenge for malaria control and elimination. In PNG, most febrile cases presenting at health centers are diagnosed by LM or rapid diagnostic test 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 [32], and in parallel individuals acquire immunity gradually, with the speed of acquisition depending on the transmission intensity. As a result, clinical malaria and parasite prevalence peaks in children and then decreases as immunity is acquired [33]. 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 [34]. 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 determined not only by acquired immunity, but also by the age of the infection on the day of sampling (Supplementary Figure 2). 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 [35] 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 as 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 [36], Solomon Islands [37], and South America [38], the parasite populations in the 2014 survey remained genetically diverse, even in villages with very low prevalences. 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 has 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 [39–41], and often consist of clones that are homologous or related to the initial blood-stage parasite infection [40, 42]. 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 [43]. An increasing proportion of infections caused by relapses of 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 captured a phase during which the vast *P. vivax* hypnozoite reservoir that had accumulated in the population from years of high transmission had not yet been exhausted and that the prevalence had thereby 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*, in which 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.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature **2015**; 526:207–11.
2. Ferreira MU, Castro MC. Challenges for malaria elimination in Brazil. Malar J **2016**; 15:284.
3. Okell LC, Bousema T, Griffin JT, Ouédraogo AL, Ghani AC, Drakeley CJ. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nat Commun **2012**; 3:1237.
4. Cheng Q, Cunningham J, Gatton ML. Systematic review of sub-microscopic P. vivax infections: prevalence and determining factors. PLoS Negl Trop Dis **2015**; 9:e3413.
5. Imwong M, Nguyen TN, Tripura R, et al. The epidemiology of subclinical malaria infections in South-East Asia: findings from cross-sectional surveys in Thailand-Myanmar border areas, Cambodia, and Vietnam. Malar J **2015**; 14:381.
6. World Health Organization. Guidelines for the treatment of malaria. 3rd ed. Geneva: World Health Organization, **2015**.
7. Kiattibutr K, Roobsoong W, Sriwichai P, et al. Infectivity of symptomatic and asymptomatic *Plasmodium vivax* infections to a Southeast Asian vector, Anopheles dirus. Int J Parasitol **2017**; 47:163–70.
8. Ouédraogo AL, Gonçalves BP, Gnémé A, et al. Dynamics of the human infectious reservoir for malaria determined by mosquito feeding assays and ultrasensitive malaria diagnosis in Burkina Faso. J Infect Dis **2016**; 213:90–9.
9. Vallejo AF, García J, Amado-Garavito AB, Arévalo-Herrera M, Herrera S. Plasmodium vivax gametocyte infectivity in sub-microscopic infections. Malar J **2016**; 15:48.
10. Coleman RE, Kumpitak C, Ponlawat A, et al. Infectivity of asymptomatic Plasmodium-infected human populations to Anopheles dirus mosquitoes in western Thailand. J Med Entomol **2004**; 41:201–8.
11. Galatas B, Bassat Q, Mayor A. Malaria parasites in the asymptomatic: looking for the hay in the haystack. Trends Parasitol **2016**; 32:296–308.
12. Barbosa S, Gozze AB, Lima NF, et al. Epidemiology of disappearing *Plasmodium vivax* malaria: a case study in rural Amazonia. PLoS Negl Trop Dis **2014**; 8:e3109.
13. Waltmann A, Darcy AW, Harris I, et al. High rates of asymptomatic, sub-microscopic *Plasmodium vivax* infection and disappearing *Plasmodium falciparum* malaria in an area of low transmission in Solomon Islands. PLoS Negl Trop Dis **2015**; 9:e0003758.
14. Lin E, Kiniboro B, Gray L, et al. Differential patterns of infection and disease with *P. falciparum* and *P. vivax* in young Papua New Guinean children. PLoS One **2010**; 5:e9047.
15. Harris I, Sharrock WW, Bain LM, et al. A large proportion of asymptomatic Plasmodium infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting. Malar J **2010**; 9:254.
16. Michon P, Cole-Tobian JL, Dabod E, et al. The risk of malarial infections and disease in Papua New Guinean children. Am J Trop Med Hyg **2007**; 76:997–1008.
17. Baird KJ, Maguire JD, Price RN. Diagnosis and treatment of *Plasmodium vivax* malaria. Adv Parasitol **2012**; 80:203–70.
18. Betuela I, Rosanas-Urgell A, Kiniboro B, et al. Relapses contribute significantly to the risk of *Plasmodium vivax* infection and disease in Papua New Guinean children 1-5 years of age. J Infect Dis **2012**; 206:1771–80.
19. Robinson LJ, Wampfler R, Betuela I, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Med **2015**; 12:e1001891.
20. Kasehagen LJ, Mueller I, McNamara DT, et al. Changing patterns of Plasmodium blood-stage infections in the Wosera region of Papua New Guinea monitored by light microscopy and high throughput PCR diagnosis. Am J Trop Med Hyg **2006**; 75:588–96.
21. Mueller I, Widmer S, Michel D, et al. High sensitivity detection of Plasmodium species reveals positive correlations between infections of different species, shifts in age distribution and reduced local variation in Papua New Guinea. Malar J **2009**; 8:41.
22. Barry AE, Schultz L, Senn N, et al. High levels of genetic diversity of Plasmodium falciparum populations in Papua New Guinea despite variable infection prevalence. Am J Trop Med Hyg **2013**; 88:718–25.
23. Reimer LJ, Thomsen EK, Koimbu G, et al. Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea. Malar J **2016**; 15:25.
24. Hetzel MW, Morris H, Tarongka N, et al. Prevalence of malaria across Papua New Guinea after initial roll-out of insecticide-treated mosquito nets. Trop Med Int Health **2015**; 20:1745–55.

25. Koepfli C, Robinson LJ, Rarau P, et al. Blood-stage parasitaemia and age determine *Plasmodium falciparum* and *P. vivax* gametocytaemia in Papua New Guinea. PLoS One **2015**; 10:e0126747.
26. Rosanas-Urgell A, Mueller D, Betuela I, et al. Comparison of diagnostic methods for the detection and quantification of the four sympatric Plasmodium species in field samples from Papua New Guinea. Malar J **2010**; 9:361.
27. Falk N, Maire N, Sama W, et al. Comparison of PCR-RFLP and Genescan-based genotyping for analyzing infection dynamics of *Plasmodium falciparum*. Am J Trop Med Hyg **2006**; 74:944–50.
28. Koepfli C, Ross A, Kiniboro B, et al. Multiplicity and diversity of Plasmodium vivax infections in a highly endemic region in Papua New Guinea. PLoS Negl Trop Dis **2011**; 5:e1424.
29. Arnott A, Barnadas C, Senn N, et al. High genetic diversity of Plasmodium vivax on the north coast of Papua New Guinea. Am J Trop Med Hyg **2013**; 89:188–94.
30. Wampfler R, Mwingira F, Javati S, et al. Strategies for Detection of Plasmodium species Gametocytes. Plos One **2013**; 8:e76316.
31. Smith T, Hii JL, Genton B, et al. Associations of peak shifts in age–prevalence for human malarias with bednet coverage. Trans R Soc Trop Med Hyg **2001**; 95:1–6.
32. Port GR, Boreham PFL, Bryan JH. The Relationship of Host Size to Feeding by Mosquitos of the Anopheles-Gambiae Giles Complex (Diptera, Culicidae). B Entomol Res **1980**; 70:133–44.
33. Smith T, Beck HP, Kitua A, et al. Age dependence of the multiplicity of Plasmodium falciparum infections and of other malariological indices in an area of high endemicity. Trans R Soc Trop Med Hyg **1999**; 93 Suppl 1:15–20.
34. Mogeni P, Williams TN, Fegan G, et al. Age, Spatial, and Temporal Variations in Hospital Admissions with Malaria in Kilifi County, Kenya: A 25-Year Longitudinal Observational Study. PLoS Med **2016**; 13:e1002047.
35. Koepfli C, Colborn KL, Kiniboro B, et al. A high force of plasmodium vivax blood-stage infection drives the rapid acquisition of immunity in papua new guinean children. PLoS Negl Trop Dis **2013**; 7:e2403.
36. Mueller I, Kaiok J, Reeder JC, Cortés A. The population structure of *Plasmodium falciparum* and *Plasmodium vivax* during an epidemic of malaria in the Eastern Highlands of Papua New Guinea. Am J Trop Med Hyg **2002**; 67:459–64.
37. Ballif M, Hii J, Marfurt J, et al. Monitoring of malaria parasite resistance to chloroquine and sulphadoxine-pyrimethamine in the Solomon Islands by DNA microarray technology. Malar J **2010**; 9:270.
38. Baldeviano GC, Okoth SA, Arrospide N, et al. Molecular Epidemiology of Plasmodium falciparum Malaria Outbreak, Tumbes, Peru, 2010–2012. Emerg Infect Dis **2015**; 21:797–803.
39. Chen N, Auliff A, Rieckmann K, Gatton M, Cheng Q. Relapses of *Plasmodium vivax* infection result from clonal hypnozoites activated at predetermined intervals. J Infect Dis **2007**; 195:934–41.
40. Imwong M, Boel ME, Pagornrat W, et al. The first *Plasmodium vivax* relapses of life are usually genetically homologous. J Infect Dis **2012**; 205:680–3.
41. Lin JT, Juliano JJ, Kharabora O, et al. Individual Plasmodium vivax msp1 variants within polyclonal P. vivax infections display different propensities for relapse. J Clin Microbiol **2012**; 50:1449–51.
42. Kim JR, Nandy A, Maji AK, et al. Genotyping of Plasmodium vivax reveals both short and long latency relapse patterns in Kolkata. PLoS One **2012**; 7:e39645.
43. Cole-Tobian JL, Michon P, Biasor M, et al. Strain-specific duffy binding protein antibodies correlate with protection against infection with homologous compared to heterologous plasmodium vivax strains in Papua New Guinean children. Infect Immun **2009**; 77:4009–17.