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Prevalence of malaria across Papua New Guinea after initial roll-out of insecticide-treated mosquito nets

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Abstract

OBJECTIVES To assess the population prevalence of malaria in villages across Papua New Guinea (PNG) following the first roll-out of free long-lasting insecticidal nets (LLIN).

METHODS Between October 2008 and August 2009, a household survey was conducted in 49 random villages in districts covered by the LLIN distribution campaign. The survey extended to 19 villages in sentinel sites that had not yet been covered by the campaign. In each village, 30 households were randomly sampled, household heads were interviewed and capillary blood samples were collected from all consenting household members for microscopic diagnosis of malaria.

RESULTS Malaria prevalence ranged from 0% to 49.7% with a weighted average of 12.1% (95% CI 9.5, 15.3) in the national sample. More people were infected with *Plasmodium falciparum* (7.0%; 95% CI 5.4, 9.1) than with *P. vivax* (3.8%; 95% CI 2.4, 5.7) or *P. malariae* (0.3%; 95% CI 0.1, 0.6). Parasitaemia was strongly age-dependent with a *P. falciparum* peak at age 5–9 years and a *P. vivax* peak at age 1–4 years, yet with differences between geographical regions. Individual LLIN use and high community coverage were associated with reduced odds of infection (OR = 0.64 and 0.07, respectively; both $P < 0.001$). Splenomegaly in children and anaemia were common morbidities attributable to malaria.

CONCLUSIONS Malaria prevalence across PNG is again at levels comparable to the 1970s. The strong association of LLIN use with reduced parasitaemia supports efforts to achieve and maintain high country-wide coverage. *P. vivax* infections will require special targeted approaches across PNG.

keywords Insecticide-treated Bednets, *falciparum* malaria, *vivax* malaria, Papua New Guinea, Surveys

Introduction

Over 60% of the 7 million people of Papua New Guinea (PNG) live in areas where malaria transmission is endemic, while most of the remaining population occupies potentially epidemic-prone areas [1, 2]. Due to the country's proximity to the equator, malaria transmission is influenced mainly by altitude [1], but small-area variations in endemicity are common [3, 4]. Areas of perennial transmission can be found in coastal lowlands and islands, while unstable transmission with localised epidemics is common at altitudes between 1300 and 1600 m. Above 1700 m, transmission has been considered unlikely or absent [1]. *Plasmodium falciparum* and *P. vivax* are encountered most frequently and both species have been associated with severe disease in children [5]; *P. malariae* and *P. ovale* are present but these days found only rarely [1, 4]. The mosquitoes of the *Anopheles punctulatus* group are the principal vectors but several other species with considerable differences in ecology and transmission potential are also abundant [6].

Malaria control programmes have heavily influenced the epidemiology of malaria in PNG since the end of World War II. An elimination campaign based chiefly on mass drug administration, environmental management and indoor residual house spraying (IRS) with

dichloro-diphenyl-trichloroethane (DDT) in the 1960s and 1970s [7] led to initial reductions in anopheline populations and parasite prevalence in some communities [8]. However, the aim of eliminating malaria from PNG was abandoned in 1972 as a result of operational challenges [9]. DDT spraying remained a major method of control until the mid-1980s when IRS was stopped and the responsibility for malaria control transferred to the provinces [10].

Increasing resistance of malaria parasites to commonly used drugs became evident with the first cases of chloroquine (CQ)-resistant falciparum malaria reported in 1976 [11]. This progressed rapidly to widespread CQ and amodiaquine (AQ) resistance of *P. falciparum* by the mid-1990s [1], while both drugs retained a considerable degree of efficacy against *P. vivax* until the early 2000s [12]. In studies conducted between 2003 and 2007, combination regimens of CQ/AQ plus sulphadoxine–pyrimethamine (SP) faced up to 29% *in vivo* resistance of *P. falciparum* and 23% of *P. vivax* after PCR-correction [13–15] hampering the effectiveness of malaria control.

Before intensive control began in the early 1960s, *P. vivax* was the generally predominant species, followed by *P. falciparum* and *P. malariae* both in the lowlands [16] and in the highlands where seasonal low level transmission and regular epidemics with significant morbidity and mortality occurred [17, 18]. IRS appeared to increase *P. vivax* predominance up to the 1970s [16, 19] as the shorter extrinsic cycle, rapid gametocytaemia and long-lasting liver stages made it more difficult to interrupt *P. vivax* transmission [1, 20]. The cessation of IRS and the emergence of drug resistance [11] led in parts to a massive resurgence of *P. falciparum* contributing to a shift from *P. vivax* to *P. falciparum* predominance [19, 21–23]. Changes in drug use may also have contributed to this development. In some areas, in which spraying was delayed, mass drug administration (mainly chloroquine and pyrimethamine) shifted the balance to *P. falciparum* even before initiation of vector control [24]. At the end of the 1990s, *P. falciparum* was reported to be predominant throughout the country with the exception of very low-endemic highland areas, where *P. vivax* remained the main species of endemic transmission but *P. falciparum* the main cause of malaria epidemics [4, 25–27].

In 2004, PNG secured a grant from the Global Fund to Fight AIDS, Tuberculosis and Malaria that allowed the first country-wide free distribution of long-lasting insecticidal nets (LLIN). Over the course of 5 years, 2.3 million LLINs were distributed to households across PNG on a district-by-district basis, resulting in 65% household ownership and 33% use of LLINs country-wide [28]. A subsequent Global Fund Round 8 grant has been supporting the continued free LLIN distribution since 2009 and the roll-out of artemisinin-based combination therapy and a test-and-treat policy since 2012 [29].

This study assessed the prevalence of *Plasmodium* spp. parasitaemia in villages across PNG following the first country-wide free LLIN distribution (2005–2008). During the era of the global malaria eradication campaign of the 1950s to early 1980s, malaria surveys were carried out across PNG. After the cessation of the programme, systematic prevalence surveys were conducted primarily as part of research studies, for example in the Maprik area [23, 30, 31], around Madang [22], or across the highlands in preparation for renewed IRS [4]. The survey presented here provides for the first time since cessation of intensive control data on malaria prevalence from across PNG, complementing the highlands malaria surveys of the early 2000s [4] and allowing comparisons with historical and future prevalence assessments.

Methods

Study sites

In the frame of the evaluation of the PNG national malaria control programme, a household survey was undertaken in sixteen of twenty provinces between October 2008 and August 2009 [28]. In 14 provinces, two districts already covered by the free LLIN distribution campaign were randomly selected; in two provinces, only one district was eligible for selection. Per district, two villages were randomly sampled from a georeferenced list of census villages [32]. Within each village, 30 households were randomly selected by the survey team leader from a list of all households *ad hoc* upon arrival. In villages with less than 30 households, all were included. In addition, the survey extended to 19 villages in six purposively selected sentinel sites, which had not yet been covered with the net distribution campaign. In each site, 3–4 villages were randomly sampled from the catchment area of a central health facility.

Data collection

An abridged household head questionnaire following the Malaria Indicator Survey template [33] was applied to record demographic details of all members of sampled households alongside information on malaria control intervention coverage [28]. Village locations and altitudes were recorded using hand-held GPS devices (Garmin etrex, Garmin Ltd., Olathe, KS, USA). Capillary blood samples were collected by finger stick from all available

and consenting household members above 5 months of age. One thick and one thin blood film were prepared on the same glass slide for each participant. An additional dry blood spot was prepared on a Whatman 3 MM filter paper, whenever possible, and stored in individual plastic zip bags with desiccant silica gel. Symptomatic household members were diagnosed using a malaria rapid diagnostic test (ICT Combo, ICT Diagnostics, Cape Town, South Africa), and positive cases were treated according to standard treatment guidelines [34]. Haemoglobin levels were measured with a portable HemoCue Hb 201+ photometric analyser (HemoCue AB, Ängelholm, Sweden) and axillary temperature with an electronic thermometer. A short questionnaire eliciting information on previous malaria treatment and recent travel was administered to each participant.

Malaria diagnosis

Thin films were first fixed with methanol and thick and thin films stained with Giemsa. Thick and thin films were read by light microscopy independently by two microscopists at the PNG Institute of Medical Research. Discordant results were read by a third senior microscopist. A minimum of 200 thick film fields were read before a slide was declared negative. The number of parasites was counted until reaching 200 white blood cells. Densities for each species and for *P. falciparum* gametocytes were converted to the number of parasites per microlitre of blood assuming 8000 white blood cells per microlitre. In case of more than two discordant species read results, the species diagnosis was confirmed using a semi-quantitative post-polymerase chain reaction, ligase detection reaction/fluorescent microsphere assay described in more detail elsewhere [31, 35, 36]. DNA for the assay was extracted from the dried blood spot samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, USA) or the Favorgen 96-Well Genomic DNA Kit (Favorgen Biotech Corp., Taiwan) following the manufacturer's protocols.

Data analysis

A microscopy slide was considered positive if at least two microscopists found parasites. Species-specific results are based on at least two concordant species identifications (microscopy or PCR), and densities are averaged values of concordant microscopy reads. For national and regional estimates, sampling weights were applied, calculated as the inverse of a person's probability of selection, as described in more detail elsewhere [28]. For aggregated subnational estimates, age-standardised prevalence was calculated to account for differences in age distribution between survey locations and the association between age and prevalence. Age standardisation is based on the INDEPTH standard population for Asia [37], which most closely resembles the age distribution found in this survey. Parasite prevalence results are presented separately for the national survey and the sentinel sites, accounting for differences in sampling approaches, while both data sets were pooled for the analysis of predictors of infection and associated morbidity. Splenomegaly was defined as palpable spleen in individuals aged 2–9 years (Hackett score 1–5) and anaemia in accordance with WHO definitions applying age group-specific cut-offs and altitude adjustment [38].

All statistical analyses were carried out using Stata 12 (StataCorp LP, College Station, USA) software. Clustering was accounted for by applying the – svy – command set in Stata [39]. Chi-squared tests and logistic regression were used to compare binary outcomes and analyses of variance and linear regression for continuous measures. Nonparametric Mann–Whitney *U*-tests were used to compare the distributions for non-normally distributed data or unequal standard deviations. The malaria attributable fraction of clinical presentations in parasitaemic individuals was calculated as (OR−1)/(OR)*100 and the population malaria attributable fraction as (P_e(OR−1))/(1+(P_e(OR−1)))*100, where P_e is the proportion parasitaemic in individuals without the respective symptom.

Ethics

Ethical clearance for this survey was granted by the PNG Medical Research Advisory Committee (MRAC No. 07.30, 30 November 2007).

Results

Survey sample

A total of 49 villages located in sixteen provinces across all four geographical regions were surveyed. Forty-four villages (90%) were found at altitudes <1000 m, one (2%) at 1200–1399 m, two (4%) at 1400–1599 m and two (4%) at ≥1600 m. Blood samples were collected from 6,646 individuals (Table S1), 52% of whom were female. There was a significant deficit of females among participants aged 1–15 years (48%, $P < 0.001$) and an excess among adults 20–39 years (58%, $P < 0.001$).

An additional 19 villages were surveyed in six sentinel sites (four in Yapsie and three in each of the other sites). Fifteen of these villages were located below 1000 m altitude; in Tabibuga, two villages were located at 1200–1399 m and one at 1300–1500 m; in Mumeng, one

village was located at 1400–1599 m. A total of 2290 individual blood samples were collected (Table S2), 52% of which were from female participants. Across all sites, an excess of female participants was found in the age group 15–19 years (62%, $P = 0.010$).

Across the two data sets, 8936 slides were diagnosed by at least two microscopists. Species-specific results were not available for 148 slides.

Prevalence of parasitaemia

The weighted overall parasite prevalence in the national sample amounted to 12.1% (95% CI 9.5, 15.3), with 7.0% (95% CI 5.4, 9.1) prevalence of *P. falciparum*, 3.8% (95% CI 2.4, 5.7) *P. vivax*, 0.3% (95% CI 0.1, 0.6) *P. malariae*, no *P. ovale* and 0.4% (95% CI 0.2, 0.7) *P. falciparum*/*P. vivax* mixed infections. *P. falciparum* gametocytes were found in 1.5% (95% CI 0.9, 2.4) of all samples. In infected individuals, parasite density per μl of blood ranged from 40 to 119 760 (geometric mean [GM] 974, 95% CI 816, 1163) for *P. falciparum* infections, from 40 to 115 173 (GM 575, 95% CI 468, 707) for *P. vivax* and from 80 to 5680 (GM 904, 95% CI 532, 1538) for *P. malariae*. The density of *P. falciparum* gametocytes found in 111 blood samples ranged from 40 to 4540 (GM 279, 95% CI 224, 348).

Age-standardised village parasite prevalence in the national survey sample ranged from 0% in six villages to 49.7% (median 4.9%, IQR 1.2, 14.6) (Figure 1), with a maximum of 24.6% *P. falciparum* (median 2.6%, IQR 0.8, 10.1), 24.0% *P. vivax* (median 0.9%, IQR 0.0, 3.8) and 2.7% *P. malariae* (median 0%, IQR 0.0, 0.0). The highest prevalence was found in the Islands region (16.7%; 95% CI 15.2, 18.1), followed by Southern (8.3%; 95% CI 7.2, 9.3), Momase (7.3%; 95% CI 5.8, 8.7) and Highlands (3.5%; 95% CI 2.0, 4.9), yet with considerable heterogeneity within the regions (Figure 1 & Table 1). *P. falciparum* was the exclusive or dominant species in 30 villages, while *P. vivax* was exclusive or dominant in eleven. Prevalence by individual survey location is provided as supporting information (Table S3).

Age-standardised prevalences found in the six sentinel sites are presented in Table 2. Prevalence was highest in Yapsie (34.9%; 95% CI 31.0, 38.9), followed by Sausi (10.8%; 95% CI 6.8, 14.8), Mumeng (10.8%; 95% CI 6.9, 14.7), Tabibuga (9.3%; 95% CI 6.0, 12.6), Finschhafen (8.2%; 95% CI 5.7, 10.6) and Wipim (0.8%; 95% CI 0.0, 1.6). *P. falciparum* was the dominant parasite in all sentinel sites, followed by *P. vivax* and *P. malariae* (found in three sites).

Predictors of village-level prevalence

Age-standardised village parasite prevalence on its own was not significantly associated with altitude ($t = -1.07$, $P = 0.29$) or altitudinal zones (as defined earlier; $F(3,64) = 0.56$, $P = 0.64$). The small number of villages ($N = 9$) surveyed at >1000 m altitude limited the extent to which the effect of altitude could be investigated. In a multivariate model including altitude (below/above 1000 m), proportion of LLIN users and national *vs.* sentinel site sample, increased LLIN use ($\beta = -0.18$, $P = 0.006$) and altitude above 1000 m ($\beta = -9.44$, $P = 0.03$) were associated with reduced malaria prevalence. There was no difference in village prevalence between national and sentinel site samples ($P > 0.05$) beyond that explained by the other predictors. Effect sizes were similar for *P. falciparum* (LLIN $\beta = -0.08$, $P = 0.05$; altitude 1000 m $\beta = -4.55$, $P = 0.08$) and *P. vivax* (LLIN $\beta = -0.07$, $P = 0.04$; altitude 1000 m $\beta = -3.14$, $P = 0.04$).

Individual predictors of *Plasmodium* infection

Malaria infection was strongly age-dependent, being most common in the age group 5–9 years (22.8%) and least common in adults 20–39 (6.6%) and >40 years (6.4%, $P < 0.001$) (Figure 2). *P. falciparum* peaked at age 5–9 years (14.0%) and *P. vivax* at age 1–4 years (9.5%), but differences were observed between geographical regions. In Southern region, a later *P. falciparum* peak was found at 5–9 (10.2%) and 10–14 years (10.6%), and in the Highlands region, *P. falciparum* peaked at age 1–4 years (7.1%) and *P. vivax* at 5–9 years (8.2%). In Southern and Highlands regions, no infections were found in children aged 6 months to 1 year.

In a multivariable model, age, altitude and mosquito net use were all independently associated with malaria infection (Table 3). At the time of the survey, all villages in the national survey sample but not those in sentinel sites had been covered by a free LLIN distribution campaign [28]. Across the national survey villages, 33% of study participants had slept under a LLIN the previous night, whereas in sentinel sites, only 5% had. Individual use of an LLIN the previous night and high community-wide use were both independently associated with significantly reduced odds of infection (OR = 0.64 and OR = 0.07, respectively; Table 3). The self-reported recent intake of an antimalarial medicine did not lower the odds of infection but was associated with a twofold increase in the case of *P. falciparum* infection. Living in sentinel site villages (pre-LLIN distribution) was associated with increased odds of parasitaemia in general

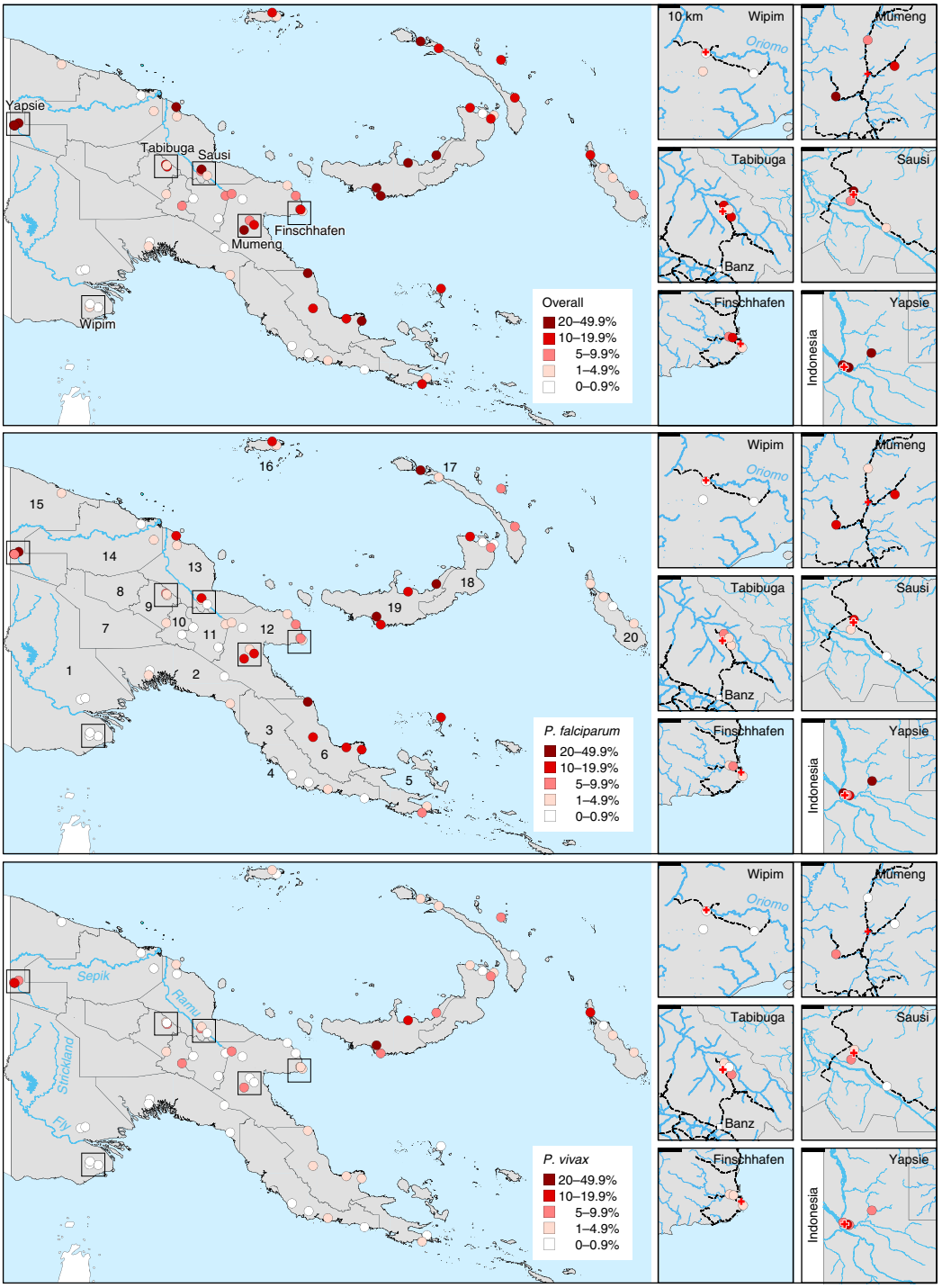

Figure 1 Age-standardised *Plasmodium* spp. prevalence by individual survey location. Blue lines are major rivers, black lines represent borders of provinces: 1 Western (Fly), 2 Gulf, 3 Central, 4 National Capital District, 5 Milne Bay, 6 Northern (Oro), 7 Southern Highlands, 8 Enga, 9 Western Highlands, 10 Chimbu, 11 Eastern Highlands, 12 Morobe, 13 Madang, 14 East Sepik, 15 West Sepik (Sandaun), 16 Manus, 17 New Ireland, 18 East New Britain, 19 West New Britain and 20 Bougainville. Sentinel sites in square frames and enlarged on the right: Red crosses indicate location of sentinel site health centre, dotted lines are main roads.

Table 1 Age-standardised provincial and regional parasite prevalence

Province/Region	N	Age-standardised prevalence (%)			
		All	*P.f.*	*P.v.*	*P.m.*
1 Western	244	0.0	0.0	0.0	0.0
2 Gulf	517	1.3	1.1	0.0	0.0
3 Central	613	1.4	0.5	0.7	0.0
4 National Capital District	0				
5 Milne Bay	305	9.2	6.2	1.5	0.0
6 Oro	775	20.7	15.5	2.9	0.7
Overall Southern region	2454	8.3	6.0	1.3	0.2
7 Southern Highlands	0				
8 Enga	0				
9 Western Highlands	143	4.9	3.4	1.5	0.0
10 Chimbu	0				
11 Eastern Highlands	457	3.1	1.1	0.9	0.2
Overall Highlands region	600	3.5	1.6	1.1	0.1
12 Morobe	489	4.4	3.8	0.2	0.0
13 Madang	264	18.4	7.8	2.5	1.5
14 East Sepik	254	2.0	0.5	0.6	0.0
15 Sandaun	167	4.8	4.8	0.5	0.0
Overall Momase region	1174	7.3	4.1	0.9	0.3
16 Manus	303	9.0	6.0	2.7	0.0
17 New Ireland	439	16.2	11.2	3.6	1.3
18 East New Britain	443	11.4	6.8	3.2	0.8
19 West New Britain	722	30.8	16.7	12.6	0.3
20 Bougainville	511	7.6	2.1	3.8	0.1
Overall Islands region	2418	16.7	9.2	6.2	0.4

Table 2 Age-standardised parasite prevalence in sentinel sites

Province	Sentinel site	*N*	Age-standardised prevalence (%)			
			All	*P.f.*	*P.v.*	*P.m.*
Western	Wipim	368	0.8	0.5	0.3	0.0
Western Highlands	Tabibuga	324	9.3	5.2	2.4	1.2
Morobe	Finschhafen	451	8.2	4.3	2.9	0.4
Morobe	Mumeng	279	10.8	7.6	3.1	0.0
Madang	Sausi	334	10.8	5.8	3.4	0.0
Sandaun	Yapsie	534	34.9	16.8	9.2	1.7

(Table 3) and with *P. malariae* infection in particular (OR = 2.23, 95% CI 1.11, 4.50, $P = 0.02$). *P. malariae* infections were not associated with altitude or LLIN use.

Morbidity associated with infection

Of all survey participants, 12.5% reported a recent episode of fever (last 2 days), while 3.4% had axillary body temperature of >37.5 °C at the time of the survey (Table 4). Acute measured fever was more strongly associated with malaria infection than a self-reported recent fever episode (OR = 4.8 *vs.* OR = 2.1) and more cases of measured fever than of reported episodes in the population could be attributed to malaria infections. At higher altitudes (1400–1599 m), reported fever episodes were more likely associated with malaria infection than in lowland areas (<1000 m) (OR = 5.7, *vs.* OR = 2.0, $\chi^2_{3df} = 7.84$, $P = 0.049$).

The mean Hb (±SD) was 11.7 ± 2.0 in males and 11.0 ± 1.7 in females (Wilcoxon rank-sum $z = -15.49$, $P < 0.001$) and differed significantly between pregnant and non-pregnant women aged 15–49 years (10.1 ± 1.6 *vs.* 11.4 ± 1.7, $t = 8.56$, $P < 0.001$). Of all participants, 67.9% were anaemic (56.1% in males, 69.7% in females, $\chi^2_{1df} = 12.5$, $P < 0.001$) and 6.1% (95% CI 5.6, 6.6) had moderate-to-severe anaemia (Hb < 80 g/l; Hb < 70 g/l in children <5 years and pregnant women). Anaemia was significantly associated with malaria infection (OR = 2.6, severe anaemia OR = 1.8) but in the context of high overall prevalence, only 10% of anaemia cases in the community could be attributed to a concurrent *Plasmodium* spp. infection (Table 4).

In children 2–9 years, 18.8% had a palpable spleen (3.4% had moderate to massive splenomegaly, Hackett score 3–5) with no differences between sexes, but strong association with malarial infection (OR = 5.6; Table 4). In the Islands region, this correlation was weaker (OR = 2.9, 95% CI 2.1, 4.0) with more uninfected individuals having an enlarged spleen (19.0% in Islands *vs.* 8.6% in other regions, $\chi^2_{1df} = 48.1$, $P < 0.001$). In one village in New Ireland Province (Islands region), 55.2% of all children had an enlarged spleen. Most enlarged spleens in children infected with malarial parasites could be attributed to this infection (MAF = 82.0%) and 43% of all cases of splenomegaly in the population could be attributed to malaria. The association was stronger in children 1–4 years (OR = 13.1, 95% CI 8.7, 19.9) than in the other age groups (test of homogeneity, $\chi^2_{6df} = 46.45$, $P < 0.001$).

Discussion

The wealth of historical malaria data from PNG, some of it dating back to Robert Koch's expedition to German New Guinea in 1900 [40], calls for an assessment of trends over time. Considering small-area variations in transmission, direct comparisons are somewhat constrained by the different geographical ranges and uncertainties with regard to the sampling methodology in previous 'mass blood surveys'. This survey confirms

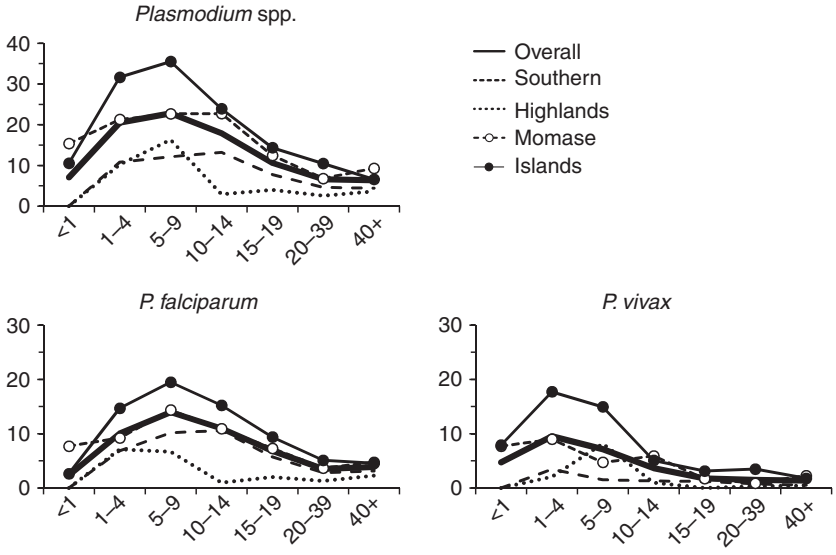

Figure 2 Age-specific parasite prevalence by geographical region.

Table 3 Multivariable analysis of predictors of malaria infection

	Any infection			*P. falciparum*			*P. vivax*		
Predictors	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Altitude									
>1000 m	0.36	(0.27, 0.47)	<0.001	0.37	(0.25, 0.53)	<0.001	0.36	(0.22, 0.58)	<0.001
Age (years)									
<1	1.10	(0.47, 2.58)		0.54	(0.13, 2.26)		3.61	(1.21, 10.72)	
1–4	3.98	(3.12, 5.06)		2.76	(2.03, 3.76)		7.84	(4.98, 12.35)	
5–9	4.52	(3.58, 5.69)		4.03	(3.02, 5.38)		5.66	(3.58, 8.93)	
10–14	3.24	(2.52, 4.16)		3.02	(2.21, 4.14)		2.77	(1.65, 4.66)	
15–19	1.70	(1.24, 2.32)		1.75	(1.19, 2.58)		1.29	(0.64, 2.60)	
20–39	1.07	(0.83, 1.37)		0.91	(0.66, 1.26)		1.11	(0.66, 1.87)	
40+	1		<0.001	1		<0.001	1		<0.001
LLIN use	0.64	(0.54, 0.76)	<0.001	0.76	(0.62, 0.93)	0.02	0.54	(0.40, 0.72)	<0.001
High LLIN use in village*	0.07	(0.03, 0.19)	<0.001	0.09	(0.03, 0.27)	<0.001	0.39	(0.25, 0.61)	<0.001
Antimalarial 2 days				2.02	(1.03, 3.94)	0.04			
Sentinel site survey	1.23	(1.06, 1.42)	<0.001						

*80% for any infection and *P. falciparum*, 50% for *P. vivax*. No *P. vivax*-infected individuals were found in villages with 80% use.

substantial heterogeneity in the prevalence of malaria across PNG with marked differences even between nearby villages (Figure 1 and Table S3). Previously, these had been attributed to entomological factors, the use of antimalarials and mosquito nets, and the abundance of alternative hosts [3, 41]. With raising coverage under the current control programme [42], the use of mosquito nets may play an increasingly important role in determining heterogeneity.

Prior to intensified control in the 1960s, malaria was endemic throughout the lowlands provinces of PNG with high prevalence of infection and splenomegaly [7]. The 2008/2009 survey found major differences between parts of Southern region (Southern parts of Western Province to Central), where prevalence was very low, and the remaining lowlands provinces, where clusters of higher prevalence (>20%) were found. In the South and Middle Fly of Western Province, in Gulf and Central, spleen rates of 100% and parasite prevalence of >50% in children younger than 5 years were common in the pre-control era [41]. A reduction to <5% overall parasite prevalence was documented in Central Province in the early 1970s

Table 4 Morbidity associated with malarial infection

Morbidity	*N*	% with symptom (95% CI)	% with symptom in infected (95% CI)	OR (95% CI)	*P*	MAF % (95% CI)	PMAF %
Fever 37.5 °C	8732	3.4 (3.1, 3.8)	10.4 (8.6, 12.3)	4.8 (3.7, 6.1)	<0.001	79.0 (73.1, 83.6)	31.4
Reported recent fever*	8771	12.5 (11.8, 13.2)	21.0 (18.7, 23.5)	2.1 (1.8, 2.5)	<0.001	52.7 (44.3, 59.7)	11.6
Anaemia	8185	67.9 (66.9, 68.9)	83.2 (81.0, 85.4)	2.6 (2.2, 3.1)	<0.001	61.7 (54.8, 67.6)	10.4
Severe anaemia	8185	6.1 (5.6, 6.6)	9.5 (7.8, 11.2)	1.8 (1.4, 2.2)	<0.001	43.9 (29.2, 55.3)	9.4
Splenomegaly (age 2–9 years)	2825	18.8 (17.4, 20.3)	42.4 (38.6, 46.3)	5.6 (4.5, 6.9)	<0.001	82.0 (77.9, 85.4)	42.8

*Previous 2 days; MAF, malaria attributable fraction; PMAF, malaria attributable fraction in population.

following scaling up of IRS [7]. Samples collected from these provinces in 2008/2009 revealed once again similar levels of prevalence: In Western province, none of the 244 blood samples collected in post-LLIN campaign villages was positive, and in the Wipim sentinel site, pre-LLIN prevalence was only 0.8%. Low endemicity in this area was also confirmed in clinical cases [43]. In neighbouring Gulf and Central provinces, the majority of villages had <1% overall prevalence. In the same region, average parasite prevalence remained higher in Milne Bay (12.9%) and in Oro (31.1%), yet still below pre-control levels; in Milne Bay, prevalence was comparable to the 1970s. A direct comparison of Kiriwina (Trobriand Islands, Milne Bay) illustrates how individual locations may have followed different trends: overall prevalence 1.6% in 1966 and 10.6% in 2008/2009. In Oro, 10% prevalence was initially achieved with IRS in 1970, but the gains could not be sustained [7].

Morobe, Madang, East Sepik and West Sepik (Sandaun) Provinces (Momase region) were areas of high endemicity, documented as early as 1900 [40]. In Madang, pre-control prevalence of 24.9% (1960) was recorded by Parkinson [7], while for some places, >40% and >70% in children (e.g. Utu) were documented [41]. In the Maprik area (East Sepik), prevalence reached nearly 90% in children, triggering a pilot IRS campaign in 1957 [7]. Major gains were recorded in the 1960s reducing prevalence to <8% in Madang and Morobe and from 75% to <17% in East Sepik [7]. In a study conducted near Madang in the early 1980s, prevalence varied from 35% to 43% [22], and in the Maprik area, it reached 60% in the early 1990s [23] but subsequently dropped [44]. Compared to the 1970s, 2008/2009 prevalence was again higher in the Madang province (up to 34.1%) and largely unchanged in Morobe. Results for East Sepik should be interpreted with caution as the Maprik area was excluded from this survey due to other ongoing field research activities [45]. In villages of the Yapsie(i) sentinel site in West Sepik (Sandaun), prevalence ranged from 27.1% to 42.2% (spleen rates reached >80%), illustrating high malaria endemicity and confirming earlier reports (1986) from this remote part of PNG [46].

High malaria prevalence was also found in villages of the Islands region, particularly in the province of West New Britain (up to 49.7%). Documented average pre-control prevalence in the Islands region ranged from 10.3% in New Ireland to 44.9% in West New Britain. Intensive control in the 1960s resulted in reductions in all Islands provinces to <10% in the early 1970s, except in West New Britain, where it remained between 20% and 30% [7, 41]. The 2008/2009 prevalence in these provinces is lower than before the start of intensive control but has increased again since the 1970s, most markedly in West New Britain Province.

Highlands provinces were not extensively surveyed (Tables S1, S2) because of data available from previous surveys. Betuela *et al.* [4] reported that malaria endemicity in the early 2000s was comparable to the situation in the 1950s–1960s and the highlands point estimates in the 2008/2009 survey confirm this observation. Certain results for this region, such as prevalence by age (Figure 2) should be interpreted with caution due to the limited number of survey points. Across PNG, peak prevalence of infection with *P. falciparum* was found in the age group 5–9 years and with *P. vivax* in the age group 1–4 years. In the Highlands region, this situation was reversed, while in Southern region, where overall prevalence was lowest, the peak prevalence of *P. falciparum* was shifted even further to 10- to 14-year-olds. It was not possible in this survey to identify an increase in *P. vivax* dominance with increasing altitude. Much rather were *P. falciparum* and *P. vivax* infections present throughout the country with no clear geographical preference. The most marked *P. vivax* dominance was found in villages of New Britain and Bougainville in the Islands

region. *P. malariae* was most frequently found in the Islands region, while no *P. ovale* infection was identified in the survey samples, confirming previous findings that this species was rare [22, 41, 47].

Age, altitude, individual use of an LLIN as well as high community LLIN coverage were independently and strongly associated with reduced odds of malaria infection. Interestingly, this association of LLIN use was observed with infections of both *P. falciparum* and *P. vivax*, while previous surveys in the highlands suggested an association exclusively with *P. falciparum* infection [4]. The finding was further supported by the observation that pre-LLIN distribution surveys in sentinel sites found, with few exceptions, higher prevalence than in surrounding areas of the same provinces, where LLIN had already been distributed. Recent intake of an antimalarial medicine may be expected to be a predictor of less parasitaemia (assuming drugs were effective), but the opposite was the case for *P. falciparum*, suggesting that the recent treatment was in fact a result of a malarial infection and most likely a clinical episode.

The most important pattern of morbidity found during this survey was the high level of anaemia in the population (61.7% when applying WHO thresholds [38]). Even though severe anaemia was less common, the prevalence of anaemia in the general population would be considered a severe public health problem [38]. According to this survey, only 12% of anaemia cases in the community can be attributed to malarial infection and while recently cleared infections may contribute a further portion, significant additional risk factors for anaemia must be present in the population [48, 49]. Palpable spleen (Hackett score 1–5) was found in 19% of children age 2–9 years, and most of these cases could be attributed to a malarial infection. Other factors affecting the spleen were not investigated.

Methodological considerations

This survey included sites from across PNG yet excluding many highlands areas and villages in East Sepik province (where other studies were carried out at the time of the survey). The national level data were derived from villages in districts previously covered with an LLIN distribution campaign, which should be taken into consideration in the interpretation of the results. The strong association of parasite prevalence with age and the differences in age distributions between survey locations demand for an age standardisation of prevalence results to allow unbiased comparisons between sites. Age standardisation has not commonly been performed in reports from other (historical and contemporary) surveys, which should be considered in the direct comparison of results. Double-read microscopy with a confirmatory third read in case of discordant pairs has not resulted in conclusive species identifications in 192 cases. PCR data were available to confirm the species of 44 of these samples, while the remaining 148 discordant results could not be confirmed. This approach provided more accurate results than the single-read diagnostic which has been applied in many historical studies or in those relying on routinely collected diagnostic data.

Seasonal weather patterns influence malaria transmission, incidence of clinical episodes, but to a lesser extent prevalence of infection [50]. While including this factor in the analysis of predictors of infection would have been desirable, no sufficiently reliable weather data were available. As prevalence has been shown to vary less over the year [50], a major bias of the results would not be expected.

Conclusions

Following the first large-scale distribution of LLIN, malaria prevalence across PNG is comparable to the levels achieved during the 1970s with DDT-spraying campaigns. The strong association of LLIN use and reduced parasitaemia supports efforts to achieve high LLIN coverage across PNG. A further reduction in population parasite prevalence can be expected if usage levels increase. The presence of *P. vivax* across the country will require special attention and targeted approaches, particularly with regards to the treatment of infections. Continuous monitoring of prevalence and incidence rates at a national level will provide useful evidence of the impact of the intensified malaria control efforts in PNG.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. National survey sample by age group and geographical region.

Table S2. Sentinel site survey sample by age group and site (province).

Table S3. Age-standardized *Plasmodium* spp. prevalence by individual survey location.

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