Force of infection is key to understanding the epidemiology of *Plasmodium falciparum* malaria in Papua New Guinean children

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Genotyping *Plasmodium falciparum* parasites in longitudinal studies provides a robust approach to estimating force of infection (FOI) in the presence of superinfections. The molecular parameter molFOI , defined as the number of new *P. falciparum* clones acquired over time, describes basic malaria epidemiology and is suitable for measuring outcomes of interventions. This study was designed to test whether molFOI influenced the risk of clinical malaria episodes and how far molFOI reflected environmental determinants of transmission, such as seasonality and small-scale geographical variation or effects of insecticide-treated nets (ITNs). Two hundred sixty-four children 1–3 y of age from Papua New Guinea were followed over 16 mo. Individual parasite clones were tracked longitudinally by genotyping. On average, children acquired 5.9 (SD 9.6) new *P. falciparum* infections per child per y. molFOI showed a pronounced seasonality, was strongly reduced in children using ITNs (incidence rate ratio, 0.49; 95% confidence interval, [0.38, 0.61]), increased with age, and significantly varied within villages ($P = 0.001$). The acquisition of new parasite clones was the major factor determining the risk of clinical illness (incidence rate ratio, 2.12; 95% confidence interval, [1.93, 2.31]). Adjusting for individual differences in molFOI completely explained spatial variation, age trends, and the effect of ITN use. This study highlights the suitability of molFOI as a measure of individual exposure and its central role in malaria epidemiology. It has substantial advantages over entomological measures in studies of transmission patterns, and could be used in analyses of host variation in susceptibility, in field efficacy trials of novel interventions or vaccines, and for evaluating intervention effects.

infection dynamics | cohort studies | molecular monitoring

Understanding the relationships between transmission intensity, prevalence of infection, and the incidence of clinical malaria is at the heart of malaria epidemiology. Transmission intensity is traditionally quantified through entomological measures of exposure, in particular the entomological inoculation rate (EIR) (1). However, accurately measuring EIRs is notoriously difficult and labor-intensive (2). Furthermore, prevalence (3, 4) and clinical incidence (5) depend on susceptibility as well as exposure to infectious stages of the parasite, and so these quantities may be more closely related to measures of transmission intensity determined by parasitological parameters within the human host.

The force of infection (FOI), that is, the number of *Plasmodium* infections acquired over time, has long been proposed as an alternative measure of transmission, and different approaches to measuring FOI have been proposed. For example, infant conversion rates (6) (i.e., time to a patent infection in uninfected children less than 1 y of age) were widely used in the 1950s–1970s. Although being an excellent marker for transmission intensity, it requires monitoring of a large number of very young children over long periods. An alternative way of estimating the FOI of *Plasmodium* parasites is light microscopic detection of parasites following

antimalarial treatment, and this has been applied in field trials of vector control and of vaccines (7, 8).

With the wide acceptance of molecular approaches to malaria epidemiology, more precise measures of molecularly determined FOI (molFOI) can now be generated by genotyping individual parasite infections (9–12). When multiple coinfecting parasite clones within one host can be distinguished, a more realistic measurement of FOI is generated, because natural superinfections can be monitored in hosts who already harbor asymptomatic *P. falciparum* infections (13, 14). PCR-based techniques have the added advantage of higher sensitivity by detecting infections at an earlier time point, when parasite density is still under the limit of detection of microscopy. Because of superinfection, the true FOI is considerably higher than estimates from blood-slide positivity. A molecular approach to estimating FOI therefore provides greater sensitivity.

Whereas the relationship between transmission intensity and the burden of malaria illness is increasingly well described and understood on global (Malaria Atlas Project populations) or continental (African) scales (15, 16), it has been much more difficult to relate local variation in malaria burden to transmission. On the Kenyan coast, locally estimated EIRs were found to be only weakly or not associated with prevalence of infection (17, 18) or incidence of severe disease (17). Except for areas with very low EIR, saturation in the infection rate with increasing transmission was reported from Tanzania and Senegal (19, 20). Such transmission studies at the local level have been hampered by the technical difficulty of accurately quantifying exposure at the population level, and even more at the individual level. Improving on this shortfall, molFOI makes it possible to measure exposure of susceptible hosts and provides data on individuals as well as the study population. We therefore propose to use precise estimates of molFOI to index transmission intensity and to assess effectiveness of malaria control measures. The present study aimed to validate this novel approach in a cohort of young Papua New Guinean children.

Besides molFOI , a second molecular parameter, multiplicity of infection (MOI), is generated by genotyping. MOI is defined as the number of concurrent parasite clones per *P. falciparum*-positive host. In endemic areas, multiple infections of haploid *P. falciparum* clones are the rule, whereby MOI varies according to the degree of malaria endemicity (21). A mean of five concurrent infections was

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found in children from highly endemic settings in Africa (22, 23), whereas in Papua New Guinea (PNG), the reported MOI ranged from 1.7 to 1.9 (24, 25). In children less than 3 y old, each additional infecting clone was associated with a higher prospective risk, whereas for older children it appeared that multiple infections protect against clinical attacks (22, 26–28).

This study was designed to explore patterns in both classical (prevalence of infection and incidence of clinical disease) and molecular measures ($_{\text{mol}}\text{FOI}$ and MOI) of *P. falciparum* burden in children 1–4 y of age and to test for association of $_{\text{mol}}\text{FOI}$ with risk of clinical malaria episodes. A further objective was to determine to what extent variation in $_{\text{mol}}\text{FOI}$ reflects environmental factors, such as seasonality, small-scale geographical variation, effects of insecticide-treated nets (ITNs), and their association with *P. falciparum* episodes.

Results

Between March and September 2006, a total of 264 children aged 0.9–3.2 y (median, 1.7; interquartile range, [1.3, 2.4]) were enrolled and followed for up to 69 wk. Of the children, 93.9% were retained until the end of the study, with between 96.0 and 100.0% of children seen at each scheduled 2-mo blood sampling. A more detailed description of the cohort has been reported previously (29).

Prevalence by PCR and Multiplicity of *P. falciparum* Infections. Over the entire active follow-up period, 87% of children had *msp2* PCR-detectable *P. falciparum* infections at least once during the study period. Prevalence of infection was significantly associated with age (at start of interval) (Table 1), ranging from an average of 25% for children aged 12 mo to 49% for children aged 24–30 mo (Fig. 1A). Thereafter, the likelihood of being infected started to level off/decrease, as indicated by the significant negative quadratic coefficient in the final model (Table 1). As in earlier analyses (29), recent antimalarial treatment and the presence of a febrile illness were both associated with increased risk of *P. falciparum* infections (Table 1). Despite adjusting for these factors, prevalence of infection still varied significantly between villages and among children within villages (Table 1, random effects). Prevalence also showed strong seasonal variation (Fig. 1B and Table 1), ranging from a low average of 31% in early August to a high of 52% in early February. Personal ITN use

(i.e., proportion of nights slept under an ITN) was associated with a very strong reduction in *P. falciparum* prevalence (Fig. 1C and Table 1), dropping from an average of 56% with no ITN use to 22% when ITNs were always used.

Unlike prevalence of infection, MOI continued to increase significantly throughout the age range (Fig. 1A and Table 1), with mean MOI increasing from about 1.3 at 1 y of age to 2.2 at 4 y of age. Average ITN use was also associated with a reduction in MOI (Fig. 1C and Table 1). Average MOI showed only limited, statistically nonsignificant variation over time (Fig. 1B and Table 1), and there was no significant variation in MOI between and within villages (Table 1, random effects). Additional parameter estimates are summarized in Table 1.

Force of Infection in 2-mo Intervals. Excluding any period with residual drug levels from the time at risk, the average $_{\text{mol}}\text{FOI}$ was 5.9 (SD 9.6) new *P. falciparum* infections per child per year. The distribution of raw data is shown in Fig. S1. $_{\text{mol}}\text{FOI}$ showed a very pronounced seasonality (Table 1), ranging from a peak of 9.3 in early February to a low of 2.8 at the beginning of August (Fig. 1B). Regular ITN use was associated with a significant reduction (Table 1) in acquisition of new clones, from a high of 7.5 with no ITN use to a low of 3.0 (Fig. 1C). $_{\text{mol}}\text{FOI}$ appeared to increase linearly between 1 and 3 y of age, with little difference thereafter (Fig. 1A). However, there was no significant quadratic term (Table 1 and Fig. S2), despite the apparent leveling off after 3 y of age (Fig. 1A). There was highly significant variation in $_{\text{mol}}\text{FOI}$ both between villages and between children living in the same village (Table 1, random effects).

Predictors of Clinical *P. falciparum* Illness. Over the 69 wk of follow-up, a total of 1,134 febrile episodes [incidence rate (IR), 4.60 child per y] with parasitemia (by light microscopy) had been observed (29). *P. falciparum* (Pf) was the most common cause of malarial illness [any density, 630 (IR, 2.56); Pf >2,500/ μL , 472 (IR, 1.92)], followed by *P. vivax* (Pv) [any density, 605 (IR, 2.46); Pv >500/ μL , 391 (IR, 1.59)]. *P. malariae* and *P. ovale* episodes were rare. All further analyses were done using only the more specific definition of *P. falciparum* malaria of febrile illness plus Pf parasitemia >2,500/ μL . Age (at interval), season, ITN use, and recent antimalarial treatment were all significant predictors of clinical episodes of *P. falciparum* malaria (Table 2). The incidence of *P. falciparum* malaria peaked at 3.4 clinical episodes

Table 1. Parameter estimates from GLMMs for prevalence by *msp2* PCR, multiplicity of infection, and force of *P. falciparum* infections

	Prevalence			MOI			$_{\text{mol}}\text{FOI}$		
	OR	CI ₉₅	P value	IRR	CI ₉₅	P value	IRR	CI ₉₅	P value
Age (at interval)	5.50	[2.10, 14.4]	0.0005	1.17	[1.08, 1.27]	<0.0001	1.38	[1.23, 1.56]	<0.0001
Age ²	0.77	[0.64, 0.93]	0.006						
Season									
Sin (week)	1.37	[1.17, 1.59]	<0.0001*				1.53	[1.37, 1.70]	<0.0001*
Cos (week)	1.42	[1.21, 1.67]					1.58	[1.46, 1.71]	
Year (2007)							0.49	[0.42, 0.59]	<0.0001
ITN use [†]	0.36	[0.24, 0.54]	<0.0001	0.77	[0.67, 0.89]	0.0003	0.49	[0.38, 0.61]	<0.0001
Treated 28 d [‡]	2.17	[1.70, 2.76]	<0.0001						
Fever	1.62	[1.22, 2.16]	0.001				1.22	[1.06, 1.39]	0.004
Random effects									
Village	0.38		<0.0001	~0		0.5	0.05		0.001
Child within village	0.43		<0.0001	~0		0.5	0.22		<0.0001
Seasonal effects									
Amplitude	0.47						0.62		
Peak (week)	6						6		
Trough (week)	32						32		

CI₉₅, 95% confidence interval; IRR, incidence rate ratio; OR, odds ratio.

*Joint P value for sin and cos.

[†]Insecticide-treated net use: 0% vs. 100% use.

[‡]Antimalarial treatment within 28 d before the start of the interval.

A Age effects

B Seasonality

C Insecticide treated net (ITN) usage

Fig. 1. Patterns of prevalence, MOI, $_{\text{molFOI}}$, and incidence of *P. falciparum* malaria in relation to age, season, and ITN use. Estimated curves from generalized additive models and 95% confidence intervals are shown.

early in the rainy season (early January), and was lowest at 1.0 in the middle of the dry season (early July) (Fig. 1B). The incidence of *P. falciparum* malaria increased nonlinearly with age (Fig. 1A). Children 3 y of age had an incidence of 2.3, compared with an incidence of 1.0 for 1 y olds (Fig. 1A). ITN use also decreased the incidence from 2.4 with no ITN use to 1.3 with constant use (Fig. 1C and Table 2). Interestingly, even after adjusting for all of the above factors, the incidence of *P. falciparum* malaria still varied significantly between villages and between children within villages (Table 2, random effects).

When $_{\text{molFOI}}$ was added to the model (fitted as the cube root of the rate of new clones acquired per year-at-risk), it was highly significantly associated with an increase in the incidence of *P. falciparum* malaria (Table 2). The incidence of clinical episodes increased rapidly between zero and five new clones per year-at-risk and then steadily increased at a rate of approximately one

clinical episode per five newly acquired clones per year-at-risk (Fig. S3). Adjusting for $_{\text{molFOI}}$ reduced the influence of all other factors previously associated with incidence of *P. falciparum* malaria (Table 2). The effects of ITN use, age, and recent anti-malarial treatment were no longer significantly associated with incidence of *P. falciparum* malaria once $_{\text{molFOI}}$ was added to the model. Similarly, the variability between villages and among children within a village no longer contributed significantly after inclusion of $_{\text{molFOI}}$ (Table 2). Although significant seasonal variation in incidence remained (Table 2), adjustment for $_{\text{molFOI}}$ halved the range of the seasonal variation and shifted the peak of incidence by 6 wk (Fig. 2). Consequently, after taking into account the number of new clones acquired during an interval, the risk of experiencing clinical *P. falciparum* illness was highest at the beginning of the rainy season (i.e., week 48) and lowest at the start of the dry season (week 22). $_{\text{molFOI}}$ was therefore the most

Longitudinal random-effects models or generalized linear mixed models (GLMMs) were used for data analysis (for details, see *SI Methods*). We fit a Poisson-Gaussian random-effects model with a log-link function to relate the fixed and random effects to the number of clinical episodes experienced during a 2-mo interval (defined as febrile illness plus any *P. falciparum* density or *P. falciparum* >2,500 parasites/ μ L). Similarly, for the prevalence of *P. falciparum* infection by *m*sp2 PCR, we fit a binomial-Gaussian random-effects model with a logit-link function. Covariates were selected based on earlier analyses of the same data (29). Exploratory data analysis guided the specification of the model when the relationship between the covariates and the outcome did not appear to be linear, such as with age, FOI rate, and season.

Seasonality was characterized by two parameters, amplitude and phase, which define a nonlinear regression term. For computational convenience, they are replaced by fixed-phase sine and cosine terms according to ref. 48 (*SI Methods*). For all outcomes except prevalence, an offset was fit to adjust for years at risk. Estimation of these models was done using the lme4 package (49) in R version 2.12 (50).

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