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RELATIONSHIP BETWEEN THE ENTOMOLOGIC INOCULATION RATE AND THE FORCE OF INFECTION FOR *PLASMODIUM FALCIPARUM* MALARIA

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Abstract. We propose a stochastic model for the relationship between the entomologic inoculation rate (EIR) for *Plasmodium falciparum* malaria and the force of infection in endemic areas. The model incorporates effects of increased exposure to mosquito bites as a result of the growth in body surface area with the age of the host, naturally acquired pre-erythrocytic immunity, and the reduction in the proportion of entomologically assessed inoculations leading to infection, as the EIR increases. It is fitted to multiple datasets from field studies of the relationship between malaria infection and the EIR. We propose that this model can account for non-monotonic relationships between the age of the host and the parasite prevalence and incidence of disease. It provides a parsimonious explanation for the faster acquisition of natural immunity in adults than in children exposed to high EIRs. This forms one component of a new stochastic model for the entire transmission cycle of *P. falciparum* that we have derived to estimate the potential epidemiologic impact of malaria vaccines and other malaria control interventions.

INTRODUCTION

The prevention of new infections by stimulating immunity against sporozoites or liver stages of *Plasmodium falciparum* parasites is a major strategy being pursued for the development of malaria vaccines.[1] There is substantial immunologic evidence that immune responses to infected hepatocytes occur in nature, and the effectiveness of inoculation with irradiated sporozoites demonstrates the feasibility of stimulating effective protective responses,[2] as do the effects demonstrated in recent challenge and field trials of the RTS,S/AS02A vaccine against pre-erythrocytic stages of the parasite.[3–5]

However, there is little evidence from field studies that naturally acquired pre-erythrocytic immunity is of epidemiologic importance. The most obvious effect of acquired immunity in malaria is to reduce asexual parasite densities without preventing infection, and people exposed repeatedly to *P. falciparum* infections over a lifetime do not become completely refractory to infection. The incidence of new infections in adults in settings of high transmission is surprisingly similar to that in their children. In Dielmo, Senegal, reinfection rates were lower in adults than children;[6] in Saradidi in western Kenya, daily attack rates in adults[7] were approximately half those observed in children;[8] while in Navrongo in northern Ghana, incidence of infection in a cohort of adults[9] was similar to that of young children.[10] The proportion of inoculations that result in infection decreases as the intensity of transmission increases.[8,11]

The most accepted integrated models of malaria transmission dynamics and immunity to date remain those of the Garki project[12] and variants of it.[13,14] These are complex mass action models based on calculation of transition rates between compartments within the host population with allowance for latent periods. These models assume that the relationship between the entomologic and epidemiologic inoculation rates is independent of prior exposure. This is equivalent to assuming that there is effectively no immunologic memory of natural immunity against pre-erythrocytic stages of the parasite.

To estimate the effects of such immunity, and how rapidly this is acquired, data are needed from epidemiologic settings where incidence of infection has been compared across a range of age-groups or exposure histories. This requirement is fulfilled by data from the village of Matsari in northern Nigeria, which was studied as part of the Garki project,[15] where mass treatment was administered every 10 weeks for a total of 80 weeks during 1972–1973, and reinfection was recorded in the whole village population before each round of treatment.

Anopheline mosquitoes bite larger people more often than smaller ones.[16,17] When this is allowed for, it is apparent that the success rate of inoculations in young children in endemic areas must be much greater than that in adults in the same communities. Otherwise, the high incidence of clinical infections in the youngest children cannot be explained,[18] and incidence rates in adults in studies such as those in Saradidi and Navrongo would be much greater than those in children. Therefore, naturally acquired immunity that prevents blood stage infection should not be ignored in malaria models, although epidemiologic data alone cannot strictly distinguish whether this is directed against pre-erythrocytic stages or whether it represents an infection-blocking response to some asexual blood stages. These effects of host size in malaria transmission have been considered in mathematical models of malaria epidemiology.[12–14,19]

With the objective of making realistic predictions of the potential impact of malaria vaccines and other malaria control interventions, we are developing a new dynamic model for the epidemiology of *P. falciparum*.[20] This model considers (among other phenomena) the effects of host size on the rate at which the host is bitten by anophelines, the relationship between the entomologic inoculation rate (EIR) and the infection rate, and decreasing survival of the inoculations as the hosts acquire pre-erythrocytic immunity. We now report the statistical fitting of this model to the re-infection data from both Saradidi and Matsari and use this to make inferences about the importance of immunity that prevents the establishment of infections.

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MATERIALS AND METHODS

Description of the model. *Differential feeding by mosquitoes depending on body surface area.* To estimate the age-specific EIR assuming proportionality between mosquito bites received and the body surface area of the host, we estimated the mean body surface area by age for a typical African rural population (from Tanzania)[21] using the formula of Mosteller.[22] Defining $A(a(i,t))$ as the mean body surface area for individuals in the same age group as individual i, at time t, age group a, we obtained $E_a(i,t)$, the age-adjusted EIR, from

$$E_a(i,t) = E_{\max}(t)\frac{A(a(i,t))}{A_{\max}} \quad (1)$$

where, $A(a(i,t))$ is the average body surface area estimated for an individual of age ($a(i,t)$ and $A_{\max}$ is the average surface area of people $\geq$ 20 years of age in the same population. $E_{\max}(t)$ refers to the usual measure of the EIR computed from human bait collections made by fully grown mosquito collectors.

Control of pre-erythrocytic stages. We use equation 1 to estimate for each time and for each individual i, the number of infective bites received per unit time, adjusted for age, $E_a(i,t)$. We then define a survival function $S_p(i,t)$ denoting the probability that the progeny of each inoculation survive to give rise to a patent blood stage infection, and model the force of infection as

$$\lambda(i,t) = S_p(i,t)E_a(i,t) \quad (2)$$

We then assume that the number of infections introduced in unit time in each individual follows a Poisson distribution with mean $\lambda(i,t)$, i.e.,

$$h(i,t) \sim \text{Poisson}(\lambda(i,t)) \quad (3)$$

The probability $S_p(i,t)$ is formed as the product of two terms,

$$S_p(i,t) = S_1(i,t)S_2(i,t) \quad (4)$$

Respectively:

$S_1(i,t)$ captures innate density dependent effects that ensure that as $E_a(i,t)$ increases, the proportion of inoculations that result in infections decreases. We assume that at any time all individuals are susceptible. At very low $E_a(i,t)$ in the naive host this corresponds to the proportion of infectious bites that lead to an infection, i.e., $\lambda(i,t) = E_a(i,t)$ and $S_1(i,t) = 1$. As $E_a(i,t)$ increases, the proportion of inoculations that are successful decreases. A functional form that satisfies these requirements for the relationship between entomologic and epidemiologic inoculations in naive individuals is

$$S_1(i,t) = S_\infty + \frac{1 - S_\infty}{1 + \frac{E_a(i,t)}{E^*}} \quad (5)$$

where S_∞ represents the lower limit attained by $S_1(i,t)$ as the inoculation rate is large, and E^* is the value of $E_a(i,t)$ at which half the reduction in $S_1(i,t)$ is achieved. These parameters are estimated with the constraints $0 < S_\infty < 1$ and $E^* > 0$.

$S_2(i,t)$ measures the effects of acquired pre-erythrocytic immunity, which leads to reduced take in hosts who have been previously exposed and who have accrued some level of exposure to pre-erythrocytic challenge, $X_p(i,t)$, where

$$X_p(i,t) = \int_{t-a(i,t)}^{t} E_a(i,\tau)d\tau \quad (6)$$

and $a(i,t)$ is the age at time t.

Exploratory analyses of the data indicate that a good fit is obtained by assuming that at high $X_p(i,t)$ there is a lower limit to the susceptibility, and that the relationship of susceptibility to $X_p(i,t)$ can be captured using a Hill function as follows:

$$S_2(i,t) = \left(S_{imm} + \frac{(1 - S_{imm})}{1 + \left(\frac{X_p(i,t)}{X_p^*}\right)^{\gamma_p}} \right) \quad (7)$$

where S_{imm} is the maximal survival of the inoculum in the most immune individuals, and X_p^* is a critical value of the cumulative exposure. Assuming independence of the effects of transmission intensity and acquired immunity, we combine equations 1–7 to obtain

$$\lambda(i,t) = E_a(i,t)\left(S_\infty + \frac{1 - S_\infty}{1 + \frac{E_a(i,t)}{E^*}} \right)\left(S_{imm} + \frac{1 - S_{imm}}{1 + \left(\frac{X_p(i,t)}{X_p^*}\right)^{\gamma_p}} \right) \quad (8)$$

Immunologic evidence suggests that the liver stages of the parasite are also controlled by innate mechanisms related to the density of erythrocytic stages. In particular, infected hepatocytes are highly sensitive to interferon-γ, the production of which is stimulated by asexual forms of *P. falciparum*. We propose that a further control is applied to liver stage infections that have survived the effect of acquired pre-erythrocytic immunity five days after inoculation. At this point, only a random sample, $S_h(i,t)$, of the infections proceed where

$$S_h(i,t) = \frac{1}{1 + \frac{Y(i,t)}{Y_h^*}} \quad (9)$$

and Y_h^* is a parameter determining the strength of this innate immune effect and $Y(i,t)$ is the current density of asexual blood stage parasites.

Data sources. *Biting rate in relation to human weight, The Gambia.* Port and others[16] reported a series of experiments in The Gambia in which fed mosquitoes were collected from the mosquito nets of 35 groups of people who normally slept together. The blood meals were assigned to hosts using haptoglobin or ABO typing, and the proportion of mosquitoes that had fed on each host was analyzed in relation to the host's contribution to the total biomass and surface area of the people sleeping in the mosquito net. Similar relationships were found between the biting rate and both weight and the surface area of the host (Figure 1), but the investigators could not determine which of these was the key determinant of how many mosquitoes bite an exposed host.

Infection rates by EIR, Saradidi, western Kenya. Beier and others[8] studied the reinfection of 21 cohorts of between 43 and 50 children between 6 months and 6 years of age, each followed-up during four successive two-week periods after initial clearance of *P. falciparum* parasites (Figure 2). They reported entomologically assessed exposures in terms of the numbers of sporozoite-positive mosquitoes biting during each two-week period.

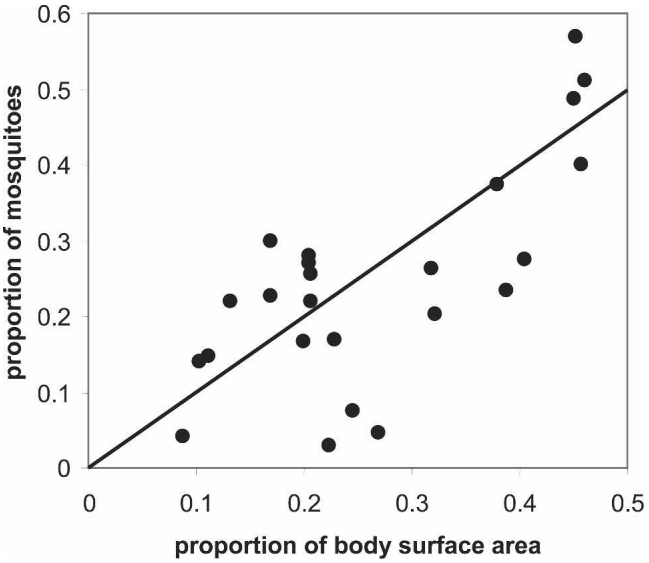

FIGURE 1. Effect of body surface area on mosquito bites received The proportion of bites from *Anopheles gambiae* s.s mosquitoes received by the child is plotted against the proportion of the surface area contributed by the child. The diagonal line corresponds to proportionality.

Infection rates by age and EIR, Matsari village, Nigeria. Matsari village was monitored entomologically for four years (November 1970–November 1973) during the Garki project.[23] The first two years corresponded to the pre-intervention phase, during which eight cross-sectional malariologic surveys of the whole village population and intensive entomological surveillance were carried out. The latter provided estimates of the EIR computed from human bait collections of mosquitoes and dissections of the mosquito salivary glands for sporozoites (Figure 3) (for details of the methods see Molineaux and Gramiccia[23]).

The intervention phase began in mid 1972, and an additional eight surveys were carried out at 10-week intervals

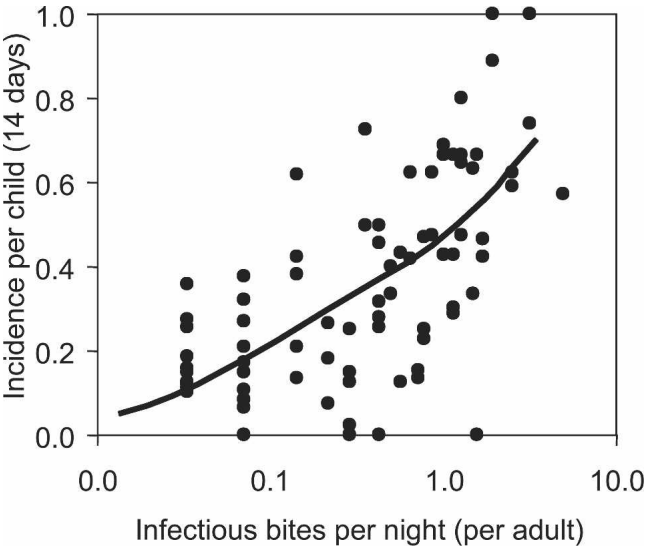

FIGURE 2. Incidence by entomologic inoculation rate in Saradidi, Kenya. The filled circles indicate observed proportions of children 0.5–6 years of age who became infected in a two-week period. The line gives the fit of the model.

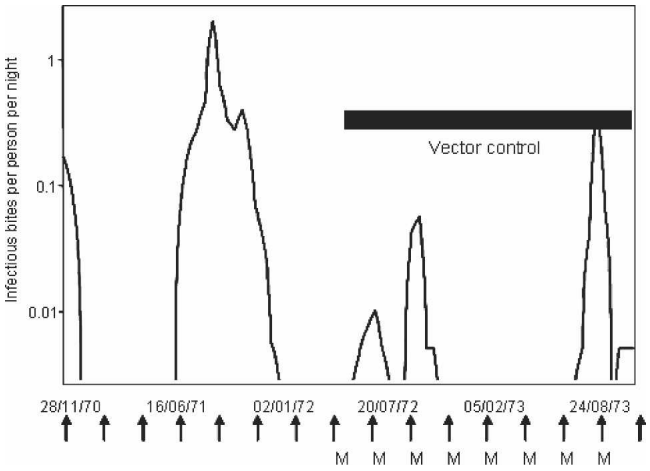

FIGURE 3. Inoculation rates and intervention history in Matsari, Nigeria. The vertical arrows indicate the time points of cross-sectional surveys of the village population. The letter M indicates that mass treatment of the population was carried out at the same time as the survey.

(surveys 9–16). During the intervention phase, indoor residual spraying with Propoxur was carried out comprehensively in the village, as was mass treatment of the population, again at 10-week intervals, immediately after assessment of their parasitologic status. We analyze the data from cross-sectional surveys 9–16, which provide an assessment of infection during the preceding 10-week period.

Model implementation and fitting. Estimation of the model parameters required fitting of γ_p, S_∞, S_{imm}, E^*, X_p^* to both the data from Matsari and those from Saradidi. Because the incidence estimates in these studies were made by following-up hosts in whom the parasites are cleared, they do not provide any data with which to estimate the parameter Y_h^*. Thus, we report here models that ignored this control on liver stages of the parasite.

Exploratory analysis of the Matsari data indicated that the effect of acquired immunity, $1 - S_2(i,t)$, must be small in children even in highly endemic areas. To estimate the parameters E^* and S_∞, we therefore fitted the model of equations 1–8 to the data from Saradidi conditional on a value of 1 for $S_2(i,t)$. To obtain the correct effect of host size, we assumed the children in Saradidi to have a uniform age distribution, and the proportion that became infected in each two-week period to be binomially distributed about the proportion predicted by the model. We fitted the model using a Bayesian Markov Chain Monte Carlo algorithm in the software Winbugs.[24]

To estimate S_{imm}, X_p^* and γ_p, we fitted the full model of equations 1–8 to the data from Matsari conditioning on the estimates of E^* and S_∞ obtained from Saradidi. We simulated infections for a human population exposed since birth to the pre-intervention seasonal pattern of EIR in Matsari using a proposal for the parameter vector and equations 1–8 to determine the infection probability and immunologic status of each individual at each time point. We then continued the simulation to include the intervention period, assuming each mass-treatment to clear all the parasites, and using the measured EIR for Matsari during the intervention phase. In a first model (A) we predicted the age-prevalence pattern at each

survey during the intervention period by making deterministic estimates for each individual of $\lambda(i,t)$ and $X_p(i,t)$, and thus of the probability that they became infected during the preceding 10-week (70-day) interval, i.e.,

$$\Pr(\text{infected}) = \int_{\tau=t-70}^{\tau=t} \lambda(i,\tau)d\tau. \quad (10)$$

Taking the mean of this for all individuals in each age group as our prediction of the age-specific prevalence, we computed the likelihood for the proposal assuming binomial errors.

In a second model (B), we used a model for parasite densities to allow for sub-patent infection in semi-immune individuals. The model for the infection process was the same, but the parasite densities were generated via a stochastic simulation model fitted jointly to the Matsari data and to data from other epidemiologic settings.[25] This simulation provided predictions of the parasite density at each time point and each simulated individual that were compared with the limit of detection of the slide-reading actually used in Matsari to make predictions of the age-specific prevalence of patent parasitemia at each survey.

We used a simulated annealing algorithm[26,27] to maximize the likelihood for Matsari conditional on the estimates of E^* and S_∞ obtained for Saradidi, and thus to obtain estimates of S_{imm}, X_p^* and γ_p.

RESULTS

We explored the fit of a wide range of models to the Matsari and Saradidi datasets, but report detailed results only for those that conformed to what we know of the biology of malaria and simultaneously gave a good fit to the data. As well as considering the fit to the Matsari and Saradidi data, we also examined whether the models were consistent with age-prevalence patterns in untreated individuals from a range of epidemiologic settings. This consideration led us to prefer models assuming the bites received by the host to be proportional to $A(a(i,t))$, rather than those that we presented previously[18] that relied on host weight. Whereas surface area increases with the square of the linear dimensions of the host, weight increases with the cube of the linear dimensions, and hence shows a steeper relationship with age (Figure 4a). When body weight was used we could not easily reconcile the very steep age-inoculation relationship with observed age patterns of parasitemia in untreated individuals even by introducing a model for acquired immunity.

One aim was to obtain a parsimonious model, and we were able to obtain a good fit using only the five parameters γ_p, S_∞, S_{imm}, E^*, and X_p^*. To model the relationship between EIR and the survival of the inoculum we required a sigmoidal function with the properties that at EIR = 0, the force of infection $\lambda(i,t) = 0$, and that as the EIR increases, $\lambda(i,t)$ must increase, although $S_1(i,t)$ should decrease monotonically. Although there is considerable scatter in the data from Saradidi, we were able to obtain a good fit that satisfies these constraints (Figures 2 and 4b). There is no evidence from the data that the proportion of hosts becoming infected saturates at high EIR, although this is a feature both of those malaria models based on that of Garki,[12] and of the statistical model that we fitted previously to the Saradidi data.[18] We could not improve the fit of our model by allowing for inoculations that do not result in blood stage infection at the lowest inoculation rates, i.e., our best fitting model constrains $S_1(i,t) = 1$ when $E(i,t) = 0$.

The EIR in Matsari for the 1971 transmission season was approximately 67 inoculations per person per year (Figure 3).[23] We estimated that vector control reduced this during the 1972–1973 seasons to an average of 5.5 infectious bites per year. This estimate is approximate because only 5 of 613 *An. gambiae s.s.* analyzed from this period were sporozoite positive, and our estimate of the variations in EIR therefore depends mainly on the variation in the measured human biting rates.

Even at this relatively low inoculation rate, many individuals became infected, and 226 (8%) of 2,663 follow-up blood slides were positive for *P. falciparum*. The average proportion of slides positive was maximal in the 10–15-year-old age group. Younger children showed a gradual increase in slide

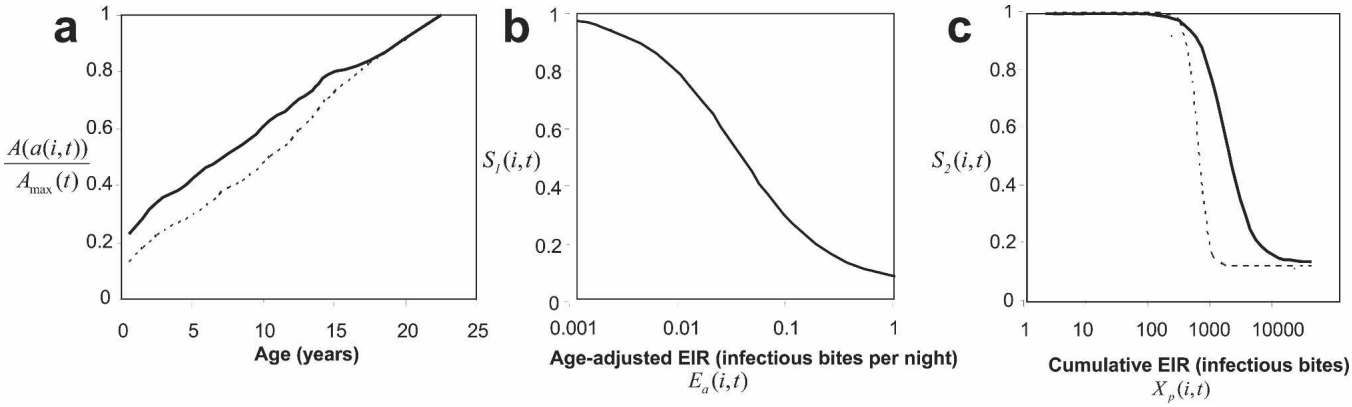

FIGURE 4. Proposed functions relating infection incidence to exposure. **a**, Effect of age on number of mosquito bites received. Shown is the ratio of bites received to those received by an adult ($A(a(i,t))/A_{max}(t)$) by age ($a(i,t)$). The continuous line corresponds to proportionality between bites received and expected body surface area (the model that we implemented); the dotted line assumes proportionality between bites received and body weight. **b**, Effect of entomologic inoculation rate (EIR) on survival of the inoculum. Shown is the proportion of inocula surviving in malaria-naive individuals, ($S_1(i,t)$), by age-adjusted EIR ($E_a(i,t)$). **c**, Effect of cumulative exposure on survival of the inoculum. Shown is the proportion of inocula surviving (in the limit when ($S_1(i,t)\rightarrow 1$), ($S_2(i,t)$), by cumulative EIR, ($X_p(i,t)$. Dashed line = model A; continuous line = model B.

positivity with age, while the infection rate in adults averaged only approximately 5% (Figure 5).

The Saradidi database contains much more information about incidence in the 0.5–6-year-old age group than does the Matsari database because of the large number of observations of non-zero prevalence that could be compared with the model predictions. The parameters that gave the best fit to the Saradidi database (Figure 2) were thus very similar to those of the jointly best-fitting model (Table 1). The model giving the best fit to Matsari conditional on the values of S_∞, E^* fitted to Saradidi (model A) gave an excellent fit to the data for adolescents and adults (Figure 5), with the shift from the non-immune state to the state of pre-erythrocytic immunity appearing to occur extremely abruptly when the host has been exposed to approximately 420 infectious bites (Figure 4c). Since the transition appeared to be so abrupt, this model estimated that young children had no acquired immunity and consequently could not give a good fit to the observed incidence rates in children in Matsari (Figure 5), for which it predicted higher values than those observed.

A major simplification made when fitting the model initially (model A) was to assume that all new infections are patent at the time of sampling. This is not necessarily the case, and biases the estimates especially for adults (in whom many infections are sub-patent) and in children less than six months of age who still have some maternally derived protection leading to very low parasite densities. For this reason we also present the results (model B) of fitting the model for infection jointly with a model for the control of parasite densities to the Matsari data (allowing for sub-patent parasitemia in Matsari) as well as to an additional six datasets comprising repeated cross-sectional survey data. Parameter estimates from model B are given in Table 1, the fitted curves are again shown in Figure 5, and the functions estimated are shown in Figure 4c.

As a validation exercise we compared the relationship between the proportions of infants converting and the EIR ($E_{\max}(t)$) predicted by model B with the data from the study of Charlwood and others[11] for the village of Idete in south-central Tanzania (Figure 6). We used our model (including functions representing blood stage immunity[25]) to simulate this study including the survey frequency and parasitologic procedures. There was a remarkably good congruence between the two sets of proportions, although the field data were lower than the predictions made using model B at very high transmission levels. At these high levels of transmission saturation was observed in Idete, but it was not predicted by our model.

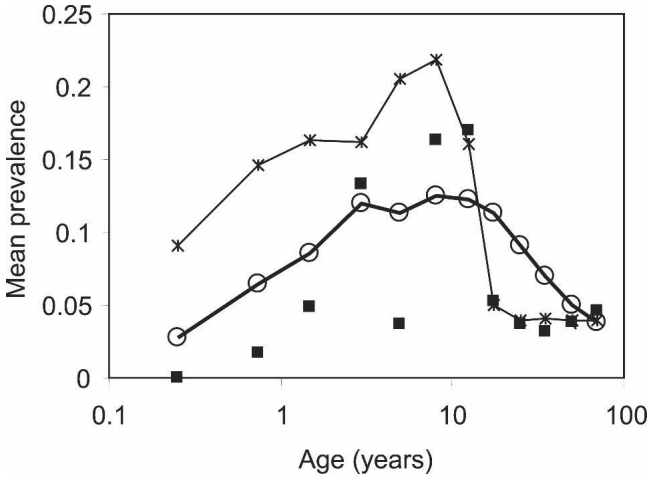

FIGURE 5. Age-prevalence pattern in the Matsari, Nigeria intervention phase. ■ = mean of observed prevalence; *—* = mean of predictions from model A; ○—○ = mean of predictions from model B.

DISCUSSION

The challenge of fitting a model to predict the force of infection for malaria from the EIR has led us to several insights about the factors that limit the frequency of infection, including host size and both acquired and innate immunity. Our strategy allowed us to consider these factors separately both from those determining the levels of immunity that control asexual blood stage densities[25] and from those modifying the clinical response to specific parasite loads.[28]

Lower exposure of small children to bites from anophelines has substantial epidemiologic implications that remain to be fully explored. In areas of high transmission, prevalence, multiplicity, and in many situations the incidence of clinical malaria, all show non-monotonic relationships with age,[29,30] implying that some risk factor increases with age at the same time as immunity decreases the risk. We propose that this risk factor is the increase in exposure to anopheline biting as children grow older. The higher exposure of adults can also perhaps explain why they appear to acquire immunity more quickly than children when exposed to endemic malaria transmission for the first time.[31]

An upper limit to the rate of increase in the infection rate with age is imposed by the assumption that this increase results entirely from the growth in body size. Given this constraint, we found that only models that assumed a rather abrupt onset of control of infection can provide a good fit to the much lower force of infection in adults than in older children in Matsari. With only the Matsari data we are not able to identify whether this control is caused by acquired immunity or to an innate age-dependent mechanism. However, the high susceptibility to *P. falciparum* infection of immunologically naive malariatherapy patients[32] and volunteers in challenge trials[33] suggests that acquired immunity is the more likely explanation.

When we treated all simulated infections as patent, the best fitting model for Matsari (model A) estimated a very abrupt onset of pre-erythrocytic immunity, with $X_p^* = 523$ inoculations corresponding to a good fit for the data for adults (Figure 5). The model (model B), which was fitted jointly with a model for asexual blood stage immunity[25] and allowed for sub-patent infections, explained much of the decrease in prevalence with age as a result of acquired immunity to asexual blood stages. Model B predicted sub-patency in a high proportion of the infections in adults and more gradual acquisition of pre-erythrocytic immunity. Although its fit to age-prevalence curves in adults in Matsari was inferior to that of model A, it gave better predictions of the force of infection in the Matsari children and of age-prevalence patterns in untreated populations in the Garki project.[23,25] This model thus appears more plausible than model A and we have adopted it in our further simulations.

An important feature of all our models is the reduction of

TABLE 1
Estimated parameter values

	Parameters determined from Saradidi data	Units/dimensions	Point estimate (95% confidence limits)
S_∞	Lower limit of success probability of inoculations at high $E_a(i,t)$	Proportion	0.049 (0.026, 0.073)
E^*	Critical value of $E_a(i,t)$	Inoculations/person-night	0.032 (0.025, 0.041)
	Log likelihood (Saradidi)		–1,159.3

	Parameters determined from Matsari data		Model A (assuming no sub-patent infections)	Model B (allowing for sub-patent infections in Matsari data)*
S_{imm}	Lower limit of success probability of inoculations in immune individuals	Proportion	0.12	0.14 (0.13, 0.15)
γ_p	Steepness of relationship between $S_3(i,t)$ and $X_p(i,t)$	Dimensionless constant	5.1	2.04 (1.45, 2.89)
X_p^*	Critical value of cumulative number of entomologic inoculations	Inoculations	523.0	1,514.4 (1,441.3, 1,591.3)
	Log likelihood (Matsari)		–778.2	–779.7

* Confidence intervals for Model B estimated as described by Maire and others.[25]

the survival probability of the inoculum with an increase in the inoculation rate. It has long been known that conversion rates in infants are much less than the EIR,[34,35] and it was apparent during the development of the Garki model[12,23] that some pre-erythrocytic density-dependent regulation of the inoculation rate is needed for realistic simulations of patterns of transmission. We have constrained the success probability of the inoculations to decrease as $E_a(i,t)$ increases (Figure 4b), but based on the pattern evident in Saradidi, and in distinction from the Garki and related models,[14,36] saturation is never reached. One likely factor contributing to this pattern is host heterogeneity in susceptibility. The only host heterogeneity that our model explicitly includes is the effect of host age, but humans are known to be heterogeneous in their attractiveness to mosquitoes.[37,38] It is likely that an improved model could be formulated by incorporating effects of additional sources of variation, such as variation between hosts in innate susceptibility to infection, and explicit modeling of heterogeneity in exposure to mosquitoes. The latter would lead to extra-Poisson variation in the infection process. However, in the absence of appropriate field data on which to base such extensions, we consider that model B represents an appropriate representation of the various factors determining the force of infection for *P. falciparum* for use in predictive models.

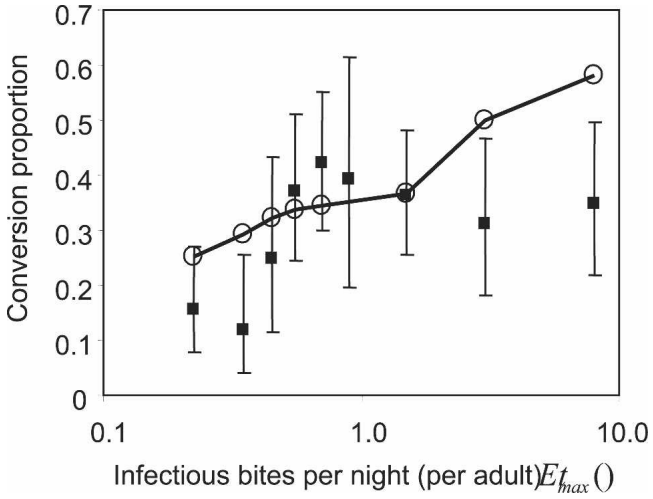

FIGURE 6. Proportion of infants converting in Idete, Tanzania. ○–○ = predictions from model B; ■ = observed conversion proportion[11] (from slide negative to slide positive) with 95% confidence intervals.

An additional factor that contributes to the apparent reduction in the survival of the inoculum with increased EIR is likely to be blocking of super-infection by innate immunity to hepatic stages. This could be stimulated by either hepatic or erythrocytic stages of *P. falciparum*. Infected hepatocytes are highly sensitive to interferon-γ, the production of which is stimulated by asexual forms of *P. falciparum*[39] and which in turn stimulates hepatic anti-malaria activity by natural killer T cells.[40] Although we were able to propose a model for feedback from asexual parasitemia on pre-erythrocytic stages, all asexual parasites were cleared at the start of the studies we used to assess infection rates; thus, we could not fit this model to these data. We attempted to estimate this feedback as part of our model for blood stage immunity, but incorporation of this effect did not improve the fit. However, equations 5 and 9 are competing for simulating innate density-dependent regulation, and available data may be inadequate to dissociate their respective effects.

At very low transmission levels distinct inoculations of the same host are widely separated in time. Thus, it is unlikely that there is any real competition between them. However, even under controlled conditions for therapeutic or experimental trials with very high sporozoite loads, there is less than 100% take in inoculations of *P. falciparum.*[32,41,42] Our models predict that at the very lowest exposure in naive individuals all inoculations will be successful (Figure 4b). However, this is an extrapolation outside the range of EIRs that can be estimated in field studies, and the success probability at the lower limit of measurable EIRs (of the order 0.003 inoculations per night) should be taken as our best estimate of the upper limit of the survival of inocula.

The validation of model B using the data of Charlwood and others[11] confirms that it can give estimates of conversion proportions similar to those from field studies other than those to which the model was fitted. At very high transmission intensities these data, in contrast to those of Beier and others,[8] suggest that saturation occurs. Infections are inapparent in

some of the youngest children studied in Idete, who included newborns protected by maternal immunity.[43] There is a need for further studies to determine whether such saturation occurs in other settings.

We conclude that the epidemiologic evidence suggests that there is naturally acquired immunity that prevents establishment of some blood stage infections of *P. falciparum*. However, this is acquired even more slowly than immunity that controls asexual blood stage densities and plays an important role only in individuals who have already been heavily exposed to the parasite. Pre-erythrocytic vaccines that aim to prevent infections in the most vulnerable group (very young children) will need to stimulate responses that are stronger or qualitatively different from those observed in nature.

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