

Quantifying Heterogeneous Malaria Exposure and Clinical Protection in a Cohort of Ugandan Children

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Background. *Plasmodium falciparum* malaria remains a leading cause of childhood morbidity and mortality. There are important gaps in our understanding of the factors driving the development of antimalaria immunity as a function of age and exposure.

Methods. We used data from a cohort of 93 children participating in a clinical trial in Tororo, Uganda, an area of very high exposure to *P. falciparum*. We jointly quantified individual heterogeneity in the risk of infection and the development of immunity against infection and clinical disease.

Results. Results showed significant heterogeneity in the hazard of infection and independent effects of age and cumulative number of infections on the risk of infection and disease. The risk of developing clinical malaria upon infection decreased on average by 6% (95% confidence interval [CI], 0%–12%) for each additional year of age and by 2% (95% CI, 1%–3%) for each additional prior infection. Children randomly assigned to receive dihydroartemisinin-piperazine for treatment appeared to develop immunity more slowly than those receiving artemether-lumefantrine.

Conclusions. Heterogeneity in *P. falciparum* exposure and immunity can be independently evaluated using detailed longitudinal studies. Improved understanding of the factors driving immunity will provide key information to anticipate the impact of malaria-control interventions and to understand the mechanisms of clinical immunity.

Keywords. malaria; epidemiology; immunity; heterogeneity in transmission.

Despite progress in expanding the coverage of malaria control interventions [1], malaria remains a major cause of morbidity and mortality in Africa [1, 2]. An important feature of malaria epidemiology is the enormous range in transmission intensities, from 1 infectious bite per person per year to 1 per day [3–5]. Across the transmission spectrum, immunity to malaria develops gradually and provides only partial protection. This protection manifests as a decline in clinical malaria with increasing age and repeated infection, such that after a few years of repeated infections, clinical malaria is much less common [6–11]. An important challenge is to understand the total malaria burden accumulated over a lifetime for individuals in relation to transmission intensity, age, and the development of immunity. This is particularly important for planning and implementing interventions with the goal of reducing morbidity.

Epidemiologic data consistently suggest that clinical immunity to *P. falciparum* develops gradually in children who are

highly exposed to infection, reducing the likelihood of disease and severity of symptoms arising from each infection. Immunity against infection, on the other hand, is slower to develop and at best weakly protective such that older individuals may continue to be reinfected (or superinfected) hundreds of times despite the absence of clinical malaria [12]. In high-transmission settings, the age-specific incidence of clinical malaria is generally seen to peak in the first few years of life and then decline, while the prevalence of parasitemia reaches a plateau later [13]. However, study of the underlying biology of these distinct epidemiologic patterns is limited by a lack of understanding of how the risk of infection per se and the risk of disease once infected are influenced by age and repeated infection. Interpretation of observed epidemiologic patterns is further complicated by the large, usually unmeasured, heterogeneity in individual exposure that exists across the transmission spectrum at all spatial scales [14–18].

Better quantification of individuals' exposure (ie, the rate at which an individual is reinfected) and immunity would be particularly useful for studies of the manifestations and underlying mechanisms of immunity to malaria. Studies aiming to identify immunologic mechanisms or biomarkers of protection have been hampered by the strong but poorly quantified association between exposure and protection. Individuals who get exposed and reinfected more frequently are likely to have higher

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immunologic responses, and this can lead to false-positive associations with protection [19] or seemingly paradoxical findings, such as associations between anti-malarial antibodies and a higher risk of disease [20]. Individual-level quantitative biomarkers of exposure would help with this identifiability but are not yet established [21]. Making matters more challenging, different manifestations of immunity (eg, immunity against disease, which limits clinical manifestations despite infection, vs immunity against parasite infection, which limits parasite replication and biomass) may develop at different rates, depending on exposure and age, such that associations between immunological markers of protection and measures of both infection and clinical disease incidence change throughout life.

Prior studies have used mathematical models to try and distinguish between multiple plausible immune mechanisms that could be driving the observed age-specific incidence curves [22–24]. Results of these studies are consistent with the idea that immunity against infection and clinical disease result from multiple processes and develop at different rates [25]. However, while most of these studies were calibrated to extensive epidemiological (aggregated age-associated prevalence and age-associated incidence) data, they failed to explicitly take into account the heterogeneity in individual exposure to parasite-infected mosquitoes that is characteristic in these settings.

Here, we used data from a detailed cohort study to jointly quantify individual heterogeneity in the risk of infection and development of immunity against clinical disease in Tororo, a district in southeastern Uganda where malaria transmission is holoendemic. By following children longitudinally over their first 5 years of life, we were able to quantify both exposure and protection and to identify important factors associated with each.

METHODS

Data

We used data from the Tororo Child Cohort study conducted in Tororo District, Uganda, where the annual entomological inoculation rate was estimated by human landing catches in separate studies performed a decade apart to be 562 infectious bites per person-year (during 2001–2002) and 125 infectious bites per person-year (during 2011–2012) [3, 5]. The population and methods of this study have been described extensively elsewhere [26–28]. In particular, we used data on recurrent *P. falciparum* infection and fever from children who were born to human immunodeficiency virus (HIV)–negative mothers and enrolled between 6 weeks and 10 months of age and followed up to 5 years of age.

Participants were followed for all medical problems at a dedicated study clinic open 7 days a week. Participants with a documented elevated temperature ($\geq 38^\circ\text{C}$ [tympanic]) or a history of fever in the previous 24 hours had blood obtained by finger prick for a thick blood smear. If the smear was positive for asexual parasites, the patient received a diagnosis of malaria, regardless of parasite density. At the time of their first uncomplicated

malaria episode, children were randomly assigned to receive open-label artemether-lumefantrine (AL) or dihydroartemisinin-piperaquine (DP) for the current and all subsequent uncomplicated malaria episodes. Children were followed-up on days 1, 2, 3, 7, 14, 21, and 28 after diagnosis, and blood smears were performed at all follow-up visits. In addition, all participants had routine blood smears performed on a monthly basis. Individuals with positive blood smears in the absence of fever were classified as having asymptomatic parasitemia and were not treated.

For the purpose of this analysis, new clinical malaria episodes were defined as febrile episodes with parasite-positive blood smears occurring at least 14 days since the last treatment. Asymptomatic infections were defined as instances of parasitemia that were not followed by symptomatic malaria (ie, fever) in the next 7 days. Based on previous publications, we assumed all new episodes of malaria to be reinfections (rather than recrudescence) [28].

Ethical Approval

The study protocol was reviewed and approved by the Uganda National Council of Science and Technology and the institutional review boards of the University of California–San Francisco, Makerere University, and the Centers for Disease Control and Prevention. Informed consent was obtained from the parent or guardian of all participating children.

Characterizing Reinfection and Development of Immunity Against Clinical Malaria

To explore factors associated with recurrent *P. falciparum* infections and the risk of clinical malaria, we specified a simple probabilistic (recurrent event) model. Following any parasite negative visit, individuals may (1) remain uninfected (parasite negative), (2) get infected and develop clinical malaria, or (3) get infected and develop asymptomatic parasitemia. These outcomes can be expressed as products of probabilities, where the probability of remaining uninfected is equal to $e^{-\lambda_i t}$, the probability of getting infected and developing malaria is equal to $[1 - e^{-\lambda_i t}] \times \phi$, and the probability of getting infected and developing asymptomatic parasitemia is equal to $[1 - e^{-\lambda_i t}] \times [1 - \phi]$, where λ_i is the hazard of infection (our metric of exposure) for individual i , $1 - e^{-\lambda_i t}$ is the probability of being infected during time t , and ϕ is the probability of developing malaria, conditional on having been infected. Thus, a higher λ_i implies that a child is at higher risk of infection, while a ϕ of < 1 can be interpreted as indicative of some level of immunity against clinical disease. The probability of a new malaria episode is the product of the probabilities of being infected and developing malaria, given infection.

The hazard of infection of participant i at time t can be expressed as $\lambda_i[t|z_i] = \lambda_0 z_i$, where λ_0 is the basal hazard, and z_i is the individual frailty (relative hazard), which is assumed to follow a gamma distribution [$z \sim \Gamma(1/\theta, 1/\theta)$], with a mean of 1 and a variance equal to θ . Thus, our models assume that recurrent

events are independent, conditional on the individual frailty parameter.

While the outcomes that follow a parasite-negative visit are generally well defined, outcomes following an asymptomatic infection are not. In particular, we do not know whether an asymptomatic infection that is followed by an episode of malaria represents disease progression from the same infection or a new infection. Similarly, we do not know whether 2 consecutive visits in which an asymptomatic infection is detected represent the same infection that has not cleared or a new infection. For the purpose of this analysis, we assumed that episodes of malaria occurring >7 days after the detection of an asymptomatic infection, as well as consecutive episodes of asymptomatic parasitemia, represented new infections. While these undefined events accounted for <3% of the data set, we performed extensive sensitivity analyses to assess the impact of these assumptions.

To explore the effects of several individual-level covariates (eg, age, body surface area, treatment arm, and prior number of infections) on the probabilities of infection and clinical disease, we expressed the hazard (λ_i) and the probability of malaria, given infection (ϕ), in terms of these covariates of interest.

All models were fitted using Bayesian Markov-chain Monte Carlo methods with the RStan package [29,30]. We ran 4 chains of 1000 iterations each and used the last half of each chain to determine the posterior distributions. We used noninformative priors for all fixed effects and estimated variance components. Convergence was assessed using visual examination of chains and the Gelman and Rubin \hat{R} statistic [31]. We compared the fit of the models to the data, using the deviance information criterion (DIC) [32]; a smaller DIC indicates a better model. Further details on the assumptions and specific models explored are provided in the [Supplementary Materials](#).

RESULTS

Between August 2007 and January 2008, 100 children born to HIV-negative mothers were enrolled in the study. Analyses below were limited to the 93 children who experienced ≥ 2 malaria episodes during follow-up. Of note, 4 of 7 excluded participants were followed for <1 year. A total of 2005 incident clinical malaria episodes and 221 episodes of asymptomatic parasitemia were recorded throughout the study period (Table 1). The median yearly incidence of clinical malaria was 5.9 episodes per person-year but varied significantly between participants, ranging between 0.63 and 11.3 episodes per person-year. Figure 1A illustrates the experiences of a subsample of children in the study.

Consistent with previous analyses of this data set [27], we found significant heterogeneity between study participants in the times to reinfection (ie, days between a treated malaria infection and the next detected infection; Figure 1B). The median time to reinfection ranged between 21 and 642 days (overall median, 44 days). The median time was significantly longer for children treated with DP (50 days; 95% confidence interval

Table 1. Basic Characteristics of Study Participants

Characteristic	Value (n = 93)
Female sex	39 (42)
Rural residence	75 (81)
Age, mo	
At enrollment	5.3 (1.5–9.9)
At randomization	9.6 (3.8–29.5)
At last follow-up	60.0 (12–60.0)
Total person-time of follow-up, person-years	376.1
Randomization group	
DP	51 (55)
AL	42 (45)
Malaria episodes, no.	2005
Asymptomatic parasitemia episodes, no.	221

Data are no. (%) of participants or median value (range), unless otherwise indicated. Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperazine.

[CI], 48–53 days) as compared to those treated with AP (28 days; 95% CI, 28–36 days), which was expected, given the longer posttreatment prophylaxis effect of piperazine versus lumefantrine [28,33].

Factors Associated With the Hazard of Infection

To explore factors driving the large variation in times to reinfection, we fit a series of models in which the hazard of infection was expressed as a function of covariates (Table 2). Details of the specific models that were explored are provided in the [Supplementary Materials and Supplementary Table 1](#).

In unadjusted analyses, the most important predictor of the hazard of infection was living in a rural residence. Children residing in rural houses experienced a hazard that was 3.2 times (95% CI, 2.7–3.7 times) that experienced by children living in an urban setting. In agreement with previous studies [34], increasing body surface area was also associated with shorter times to infection (hazard ratio [HR], 3.7 per m² increase in surface area; 95% CI, 2.5–5.3 per m² increase in surface area). Given the large correlation between body surface area and age, increasing age was also associated with a higher hazard (HR, 1.15; 95% CI, 1.11–1.18). Allowing for a nonlinear relationship between body surface area (or age) and hazard improved the fit of the models.

In this cohort, another key predictor of the hazard of infection was the antimalarial therapy to which children were randomly to receive for all malaria treatments. On average, treatment with AL was associated with a hazard of infection that was 1.3 times (95% CI, 1.2–1.4 times) that of treatment with DP. To characterize this relationship further, we fit models that allowed the hazard to vary as a function of time since the last treatment (Figure 2). As expected, the hazard of infection peaked earlier for children receiving AL as compared to those receiving DP (60 vs 110 days). However, the hazard peaked higher for children receiving DP (hazard, 0.079 [95% CI, .059–.098] vs 0.036 [95% CI, .027–.042]).

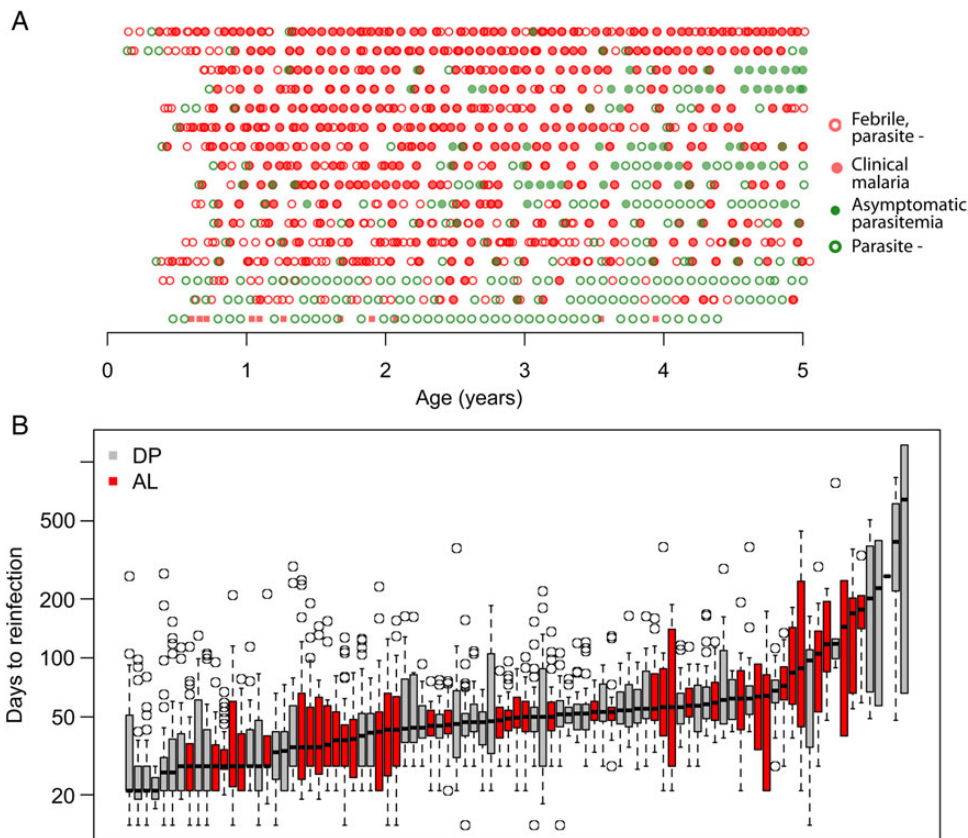


Figure 1. A, Figure showing the infection history of a subset of participants throughout the 5-year follow-up. Each row represents the experience of a specific participant. Children are sorted on the basis of their estimated individual frailty, from low exposure to high exposure. B, Times between subsequent infections for children in the data set. Each box plot represents the distribution of gap times (times to reinfection) for a particular child. Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine.

Models that included a multiplicative random effect (frailty) for the individual hazard of infection fit the data significantly better, consistent with a large heterogeneity in the hazards (relative risks of infection) experienced by individuals. In models that were adjusted for body surface area and place of residence, individual frailties ranged between 0.17 and 3.4 (Figure 3A), implying that the individual at highest risk of infection experience hazards that are approximately 20 times higher than that of the individual at lowest risk. Differences are even larger when comparing individuals who live in urban vs rural settings (Figure 3B).

Overall, the best adjusted model of the time to infection included body surface area and location of residence (rural vs urban) and is shown in Figure 4A. Treatment arm did not improve the fit in models that allowed for an individual random effect, which was expected because treatment was randomly assigned at the individual level.

Factors Associated With the Probability of Clinical Malaria, Given Infection

To explore factors associated with the probability of developing clinical malaria upon infection, we then fit a series of models in which this probability was expressed as a function of covariates.

Since we were particularly interested in quantifying the effects of age and prior number of infections on the probability of clinical disease, we fit several models evaluating these associations (Supplementary Text 1 and Supplementary Table 2).

Both age and the cumulative number of prior infections (symptomatic and asymptomatic) were negatively associated with the probability of clinical malaria upon infection (Table 2). In unadjusted analyses, the risk of developing clinical malaria upon infection decreased on average by 20% (95% CI, 7%–42%) for each additional year of age and by 3% (95% CI, 2%–3%) for each additional prior infection. Both associations remained significant when included simultaneously in the model. The risk of developing clinical malaria upon infection decreased on average by 6% (95% CI, 0%–12%) for each additional year of age and by 2% (95% CI, 1%–3%) for each additional prior infection.

Interestingly, our results suggest that immunity against clinical malaria may develop faster in individuals receiving AL for treatment of malaria (relative risk [RR], 0.76; 95% CI, .72–.8) than in those receiving DP (RR, 0.84; 95% CI, .81–.88; Supplementary Figure 1). This interaction between age and treatment allocation remained significant even after adjustment for the

Table 2. Factors Associated With the Hazard of Infection

Covariate	Univariate HR (95% CI)	Adjusted HR (95% CI)
Factors associated with the hazard of infection		
Body surface area (per m ²)	3.7 (2.48–5.34)	... ^a
Age (per additional year)	1.15 (1.11–1.18)	...
Residence		
Urban	Reference	...
Rural	3.16 (2.73–3.66)	3.18 (2.20–4.42)
Treatment arm		
DP	Reference	...
AL	1.30 (1.19–1.41)	...
Prior infection (per additional no.)	1.04 (1.04–1.05)	...
Variance of the individual random effect	0.48 (.35–.65)	0.36 (.26–.50)
Factors associated with the probability of clinical malaria, given infection		
Age (per additional year)		
Overall	0.80 (.77–.84)	
Among patients receiving DP	0.84 (.81–.88)	0.92 (.85–.99)
Among patients receiving AL	0.76 (.72–.80)	0.83 (.77–.9)
Prior infection (per additional no.)	0.97 (.97–.98)	0.99 (.98–1.00)
Malaria treatment in past mo		
DP in past mo	0.49 (.39–.60)	0.44 (.35–.55)
AL in past mo	0.61 (.54–.69)	0.79 (.67–.91)

Abbreviations: AL, artemether-lumefantrine; CI, confidence interval; DP, dihydroartemisinin-piperaquine; HR, hazard ratio.

^a Adjusted for a nonlinear function of the body surface area and as such, coefficient is not interpretable.

cumulative number of prior infections and in all sensitivity analyses (Supplementary Table 3). As expected, given the post-treatment prophylaxis of antimalarials used, malaria treatment in the last month decreased the risk of clinical malaria upon infection for the next infection following treatment both in

participants treated with AL (RR, 0.61; 95% CI, .54–.69) and in those treated with DP (RR, 0.49, 95% CI, .39–.6).

The best multivariate model of the probability of clinical malaria upon infection included age, cumulative number of prior infections, and antimalarial treatment in the last month. The model also included the interaction between age and treatment arm and between recent treatment and treatment arm. Figure 4 illustrates the fit of the model to the data.

Overall, our analyses revealed great heterogeneity in the development of immunity against clinical malaria among children in this cohort and how it is related to an individual's level of exposure (Figure 4B). While individuals under higher infection hazards (ie, individuals with higher frailties) experienced a larger number of infections, they also tended to develop immunity faster and experienced a more rapid decline in the probability of presenting clinical malaria upon infection.

Quantifying Changes in the Probability of Malaria With age and Different Levels of Exposure

To further quantify the joint effects of exposure and age on malaria parasite infection and disease, we used the best-fitting model to predict the expected monthly probabilities of infection; probabilities of malaria, given infection; and resulting monthly probabilities of malaria across a range of hazards and ages represented in the data. Figure 5 shows the predictions for children in this cohort and illustrates how changes in the observed risk of malaria are the product of nonlinear changes in the risk of infection and changes in the probability of clinical disease.

DISCUSSION

Although it is well known that immunity to clinical malaria develops gradually in individuals living in malaria-endemic

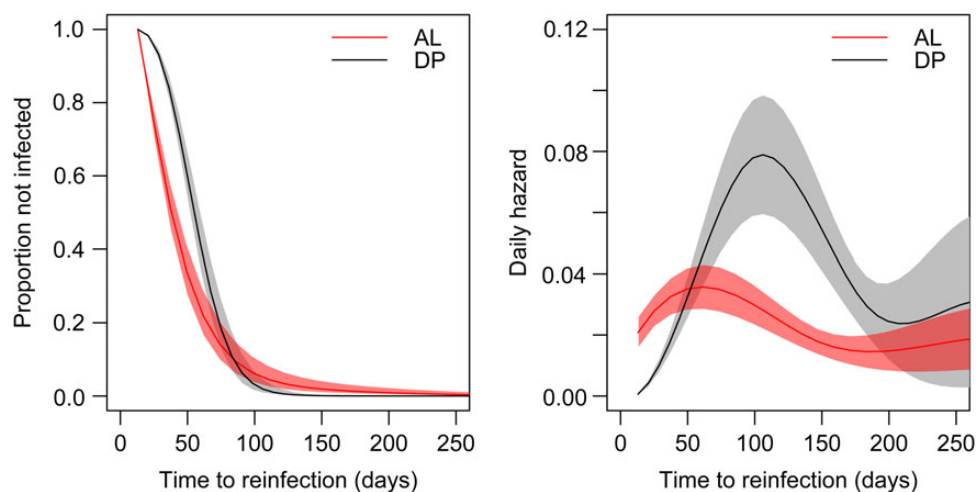


Figure 2. Figure showing survival curves and daily hazards for children in the dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine (AL) groups. The left panel shows the survival curves of the times to reinfections (in days) for both treatment arms. The right panel shows the average estimated daily hazards (average daily rates of infection) obtained by fitting models that allowed the hazard to vary as a function of time since the last treatment.

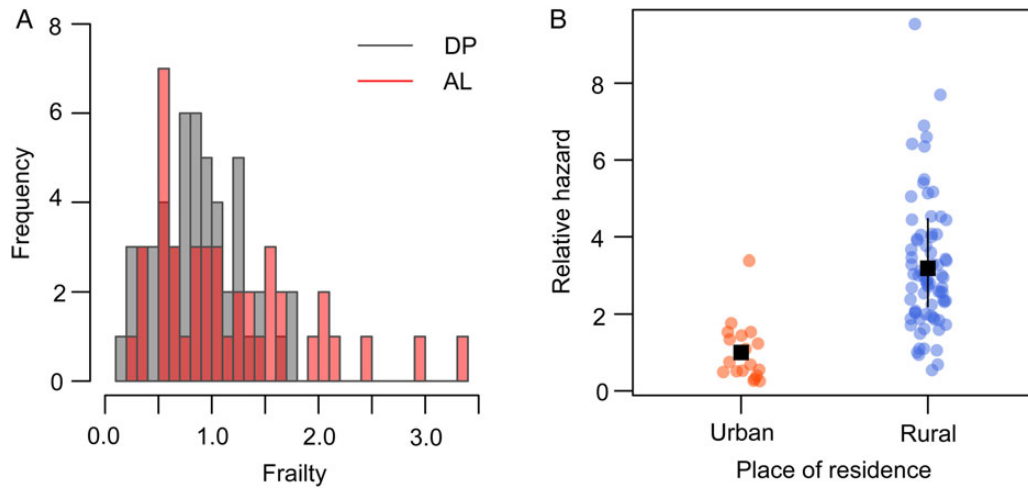


Figure 3. A, Histogram showing distribution of estimated individual frailties (relative hazards of infection as compared to the population average) after adjustment for body surface area and location of residence. B, Relative hazards of individuals living in urban versus rural households. The average relative hazard of individuals living in rural households versus urban households (reference group) is shown in black. Abbreviations: AL, artemether-lumefantrine; DP, dihydroartemisinin-piperaquine.

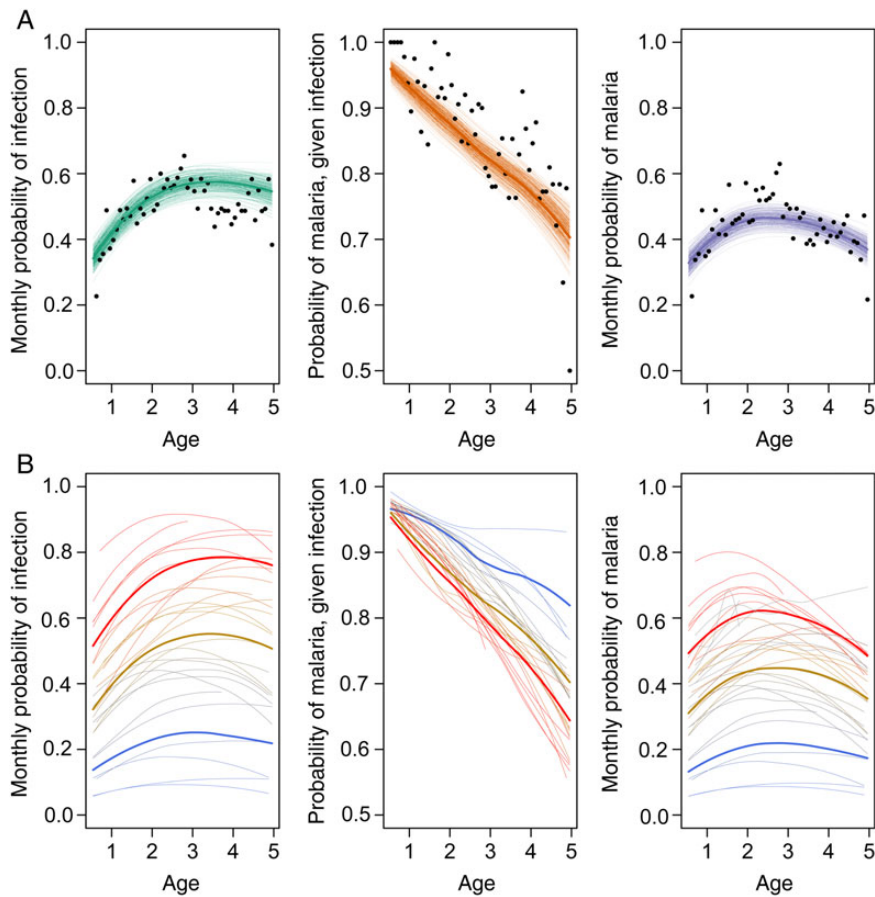


Figure 4. Data and fit of model to the mean monthly probability of infection (A), probability of malaria, given infection (B), and resulting monthly probability of malaria (C) as a function of age. Data are shown as dots. Thick lines represent mean fitted values, and thin lines represent draws from the posterior distribution (uncertainty around mean values). Whereas panel A shows the fit of the model to mean probabilities, panel B illustrates fitted individual probabilities across 3 tertiles of exposure (tertiles of estimated frailties). Thin lines represent specific trajectories predicted for a subset of children within each tertile and thus illustrate the large heterogeneity that exists in the sample. Thick lines represent the mean expected trajectory for each tertile of frailty values.

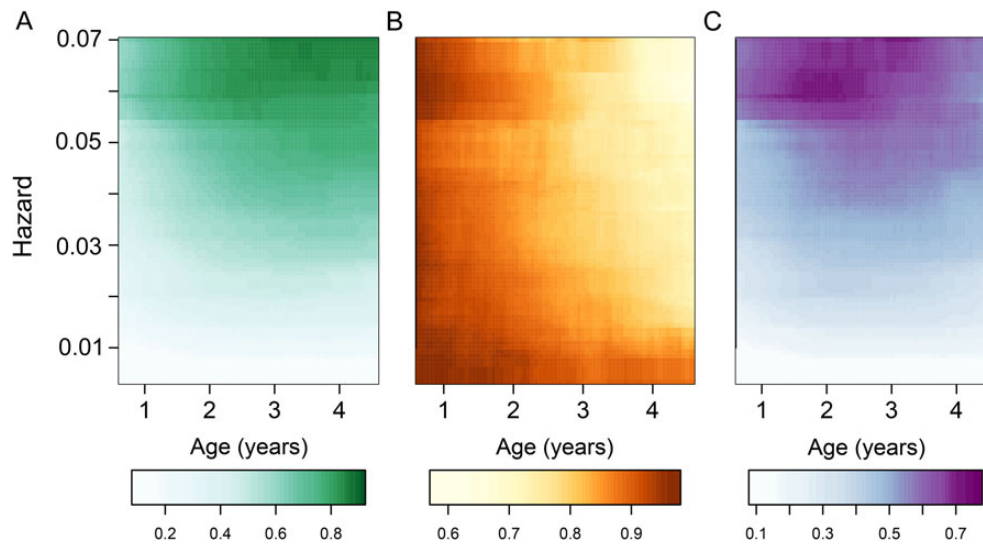


Figure 5. Predicted mean probabilities of infection and clinical malaria for different ages and exposures. *A*, Monthly probability of infection. *B*, Probability of malaria, given infection. *C*, Monthly probability of malaria.

settings, factors driving its development are not fully understood [6–9, 11]. Here, we used very detailed longitudinal data from 93 children living in an area where the level of malaria transmission is known to be high to quantify the contributions of individual-level factors, including age and exposure, on the risk of infection and of clinical disease during early life. Our results are consistent with the idea that age-associated incidence patterns observed in malaria-endemic settings are shaped by multiple factors. While repeated infection leads to reductions in the probability of clinical disease upon reinfection, age appears to act independently in both directions, increasing the probability of infection (primarily through increases in the body surface area and the likelihood of being bitten) and reducing the probability of clinical disease upon infection. Independent effects of age and exposure have been extensively discussed before [22, 35–38], but their quantification is often elusive due to the inevitable collinearity that exists between the two in malaria-endemic settings (age is a proxy for cumulative exposure). Longitudinal data sets like this one, with detailed follow-up and sufficient heterogeneity in the exposed population, are required to isolate the two effects.

In the absence of reliable biomarkers of immunity, numerous studies have modeled epidemiological data in an attempt to characterize the development of immunity [22, 39–41]. These studies generally model age-associated incidence of clinical malaria or age-associated prevalence of parasitemia and often ignore the large heterogeneity in exposure between individuals living in most malaria-endemic settings. A major strength of this study is that it involved longitudinal data, allowing us to model heterogeneity in transmission explicitly. In addition, the study design included regular active and passive surveillance

of infection and disease, therefore capturing both asymptomatic and symptomatic infections. This enabled us to separate the processes of infection and disease, modeling independently the factors driving both.

Surprisingly, our findings suggest that immunity against clinical malaria may develop faster in children regularly treated with AL as compared to those treated with DP. This might be explained in part by the fact that DP has been shown to protect against infection for longer periods and thus reduces the total exposure of the immune system to the parasite during that time [28, 42]. Our finding that recent treatment with DP is more protective against subsequent clinical malaria than treatment with AL is also consistent with this idea. While reducing the number of infections is certainly a desirable outcome, potential interference with development of clinical immunity needs to be considered when evaluating the overall impacts of such interventions.

Our findings also underscore the great heterogeneity that exists in the risk of infection between individuals and the resulting heterogeneity in the rate of development of immunity against clinical disease. Properly accounting for this heterogeneity is fundamental when modeling data on the age-associated incidence of malaria, to avoid misinterpretation of observed epidemiologic patterns. This is particularly true when comparing data from different sites where the extent of heterogeneity may also differ.

While the analysis presented here was based on a rich, longitudinal data set, a limitation is that parasitemia measured by microscopy was the only available metric of *P. falciparum* infection. Poor sensitivity for the detection of low-density parasitemia and consequent underestimation of the number of times

children were infected by *P. falciparum* could explain some of the patterns in the monthly probabilities of infection in this data set. Further, since microscopy does not reveal whether infection comprises parasite clones from one or more infective mosquito bites, it may further underestimate the number of times a person might have been infected, thus leading to an underestimate of the true hazard of infection. Subsequently, our estimate of the impact of each additional episode on the probability of clinical malaria may be an overestimate. In any case, these potential biases should not invalidate our finding of independent effects of age and exposure on the development of immunity against clinical disease. Studies that include independent measures of transmission intensity or exposure (eg, household-level entomological inoculation rates and molecular forces of infection) will be needed to properly quantify the effects of exposure on the development of immunity against malaria. Finally, while our approach provides strong statistical evidence of the independent effects of age and exposure on the development of immunity against clinical malaria, it does not provide any mechanistic insight or distinguish between types of immunity (eg, immunity against parasite infection vs immunity against disease). Similarly, since severe malaria was extremely rare in this study, our model is not informative about the role of age and exposure in the development of immunity against severe disease.

Few studies have been able to quantify the independent effects of individual-level factors, including age and exposure, in the development of immunity against malaria, while accounting for heterogeneity in transmission. Careful analyses of longitudinal data sets, like this one, will be key in filling these gaps. Improved understanding of the factors driving antimalaria immunity will provide much needed information to properly evaluate malaria-control strategies, anticipate the impact of malaria-control interventions, and guide policy decisions.

Supplementary Data

Supplementary materials are available at <http://jid.oxfordjournals.org>. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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