RELATIONSHIPS BETWEEN HOST INFECTIVITY TO MOSQUITOES AND ASEXUAL PARASITE DENSITY IN PLASMODIUM FALCIPARUM

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Abstract. We describe a statistical model for the relationship between asexual parasite densities of Plasmodium falciparum and the infectivity of the host to mosquitoes. The model takes into account the delay between asexual parasitemia and infectivity resulting from the time course of gametocytogenesis. It also allows for the need for the blood meal to contain gametocytes of both sexes if infection is to take place. We show that by fitting this model to data from malarial therapy patients it can explain observed patterns of infectiousness of the human host and is consistent with distributions of gametocyte densities in malarial therapy patients. By integrating this model into an individual-based simulation of human populations exposed to endemic P. falciparum transmission, we are able to predict the contributions of different host age groups to the infectious reservoir. Comparison of model predictions with published estimates of this quantity confirms that infected adults hosts are likely to make a significant contribution to the reservoir of transmission, and points to the need for improved population-based estimates of this age-dependence in infectivity of humans in endemic areas.

INTRODUCTION

Transmission of malaria from humans to the vector occurs when a female anopheline imbibes the sexual stages of the parasite (gametocytes) that arise as a result of developmental switching of a small proportion of the population of erythrocytic parasites. However, not all anophelines feeding on gametocyte-infected hosts become infected and cryptic gametocytogenesis can result in mosquito infections.1,2 Oocyst rates in mosquitoes in endemic areas can be similar regardless of whether gametocytes or trophozoites are detected in the human host. Some studies have found little or no relationship of infectivity with gametocyte densities3,4 while others found these to be related.5,6 In artificially induced infections, on average, infectivity to vectors rapidly reaches a persistent low but non-zero level as the infection proceeds.7 However, the decrease in infectivity is not as steep as that in measured gametocyte densities. There is a general decrease with age in infectiousness but even highly immune hosts contribute to the infectious reservoir.8,9

Community effects of vaccines or of other interventions targeting the human host (herd immunity) result either directly or indirectly from changes in infectivity. To understand these, mathematical models are needed. Infectivity of the human host is a key determinant of the intensity of malaria transmission, but in contrast to field data, models of malaria transmission dynamics have generally assumed that the probability of transmission to a vector is equal across all infected humans and throughout the course of infections,10 or that complete transmission-blocking immunity is acquired as a result of repeated exposure.11

As one component of a project to develop simulation models for the potential impact of a malaria vaccine, we now develop a new model for infectivity. We avoid directly incorporating the poorly understood processes of gametocytogenesis and the subsequent fate of gametocytes. Instead, we implicitly model gametocytogenesis by estimating infectivity from analyses of the association between lagged asexual blood stage densities and infectivity. We use data from delib-

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erated infection of human subjects with Plasmodium falciparum between 1940 and 1963 as a treatment of tertiary syphilis7,12 and fit a statistical model to these data to estimate the relationship between recent asexual parasitemia and infectivity.

MATERIALS AND METHODS

Model for infectivity as a function of parasite density. We define Y(i,t) as the density of asexual parasites in patient i that gave rise to the gametocytes present at time t. Our stochastic simulation model of malaria epidemiology13,14 uses five-day time steps. To incorporate transmission to the vector into this model, we propose that Y(i,t) should be estimated as a weighted sum of the asexual parasite densities measured 10, 15, and 20 days prior to the feeding experiment. Thus if β1, β2 and β3 are constants, then:

\[ Y(i,t) = \beta_1 Y(i,t-10) + \beta_2 Y(i,t-15) + \beta_3 Y(i,t-20) \]  

(1)

This quantity is constrained to be zero when there is no history of patent infection in the last 20 days, or when patent infection first arose in the last 5-day period. We justify the choice of the lag periods of 10, 15, and 20 days by observing that previous analyses of gametocyte dynamics in the same malariatherapy patients suggested that gametocytes spend an average of 7.4 days sequestered before appearing in the peripheral circulation and have a subsequent mean circulation time of 6.4 days.15

We considered a number of models for infectivity. In our preferred model, the ratio of functional female gametocytes per blood meal to Y(i,t) is log normally distributed, with some geometric mean, \( \rho \), so that the density of functional female gametocytes in the host blood, \( y_g(i,t) \), is related to \( Y(i,t) \) by

\[ \ln(y_g(i,t)) \sim \text{Normal}(\ln(\rho Y(i,t)), \sigma_g^2) \]  

(2)

For a mosquito to become infected with P. falciparum, it must imbibe at least one female and one male gametocyte in the same blood meal. We assume that functional gametocytes are those able to give rise to sporozoites. We define \( y^*_g \) to be the density of female gametocytes necessary for infection of the mosquito, corresponding to 1 functional gametocyte per blood meal. The probability that at least one
functional female gamocyte is taken up is then \( \Pr(y^*_g(i,t) > y^*_g) \) and it follows that:

\[
\Pr(y^*_g(i,t) > y^*_g) = \Phi \left[ \frac{\ln(pY(i,t)) - \ln(y^*_g)}{a_y} \right] = \Phi \left[ \frac{\ln(Y(i,t))}{a_y} + a_y \right]
\]  

where \( \Phi \) is the percentile of the cumulative standard normal distribution and \( p^* = (\ln(p) - \ln(y^*_g))/a_y \) is a constant which depends on the volume of the blood meal, the viability of the gametocytes, and the variability in the system. If the volume of the blood meal is assumed to be \( 3 \mu L \) (and there is assumed to be no concentration of erythrocytes in these species), then the ratio of the functional female gamocyte density to \( Y(i,t) \) is given by \( p/\beta \).

If the sex ratio of functional male to female gametocytes is 1:1, then the probability that at least one functional male gametocyte is taken up is also \( \Pr(y^*_g(i,t) > y^*_g) \), and the probability that a mosquito is infected with both male and female gametocytes is \( \Pr(y^*_g(i,t) > y^*_g)^2 \). The number of mosquitoes out of a batch of \( n_{inf}(i,t) \) mosquitoes fed on patient \( i \), at time \( t \), that are infected follows a Binomial distribution

\[
n_{inf}(i,t) \sim \text{Binomial}(n_{fed}(i,t), \Pr(y^*_g(i,t) > y^*_g)^2)
\]  

Our estimate of the proportion of mosquitoes feeding on individual \( i \) at time point \( t \) that would become infected, \( I_m(i,t) \), is then the expected proportion from this binomial

\[
I_m(i,t) = E \left[ \frac{n_{inf}(i,t)}{n_{fed}(i,t)} \right].
\]  

Data and model fitting. We fitted the model given by equations 1–5 to archive data provided by Dr. W. Collins (Centers for Disease Control and Prevention, Atlanta, GA) collected by the United States Public Health Service in South Carolina and Georgia between 1940 and 1963. At that time, malaria therapy was a recommended treatment of neurosyphillis. We obtained archive data for 392 patients inoculated with varying species, strains, and parasite stages of Plasmodium. In each case, densities of asexual parasitemia and of gametocytemia were recorded on a daily basis. Batches of between 5 and 60 caged Anopheles quadrimaculatus or An. albimanus mosquitoes were allowed to feed on gametocytogenic patients and then kept to allow oocysts to develop in the midgut before dissection to record infection status. Details of the methods have been previously published.

We excluded patients inoculated or co-inoculated with Plasmodium species other than \( P. falciparum \) and those who were inoculated for a second time or who were treated with any of the antimalarial drugs used and those with insufficient data. We initially explored various possible predictors of the proportion of the mosquitoes that became infected. These comprised day since first parasitemia, fever, the concurrent asexual parasite density, and the history of parasitemia for the preceding 40 days. To maintain compatibility with our population model of asexual parasitemia, we simplified the history by considering parasite density only at five-day intervals.

The model described by equations 1–5 was selected as the most appropriate on the basis of these exploratory analyses. We treated it as a hierarchical Bayesian model, and fitted it using the Metropolis-Hastings algorithm in the program WinBUGS version 1.4. \( \beta_1 \) was fixed at a value of 1 and we used imprecise log normal prior distributions for \( \beta_2 \) and \( \beta_3 \), and a gamma prior for \( 1/\sigma^2 \).

Age-pattern of infectivity. Field estimates of the relative contribution to the infectious reservoir by different age groups have been made for settings in Liberia, The Gambia, Tanzania, western Kenya, Papua New Guinea, and Cameroon. The estimates from Africa are based on feeding of insectary An. gambiae, while those from Papua New Guinea used a colony of An. farauti.

To validate our model against these data, we simulated the epidemiology of malaria in field settings with entomologic data using our stochastic simulation model. This model makes predictions of parasite densities using a five-day time step assuming an annually recurring stable pattern of transmission as input. We used this model to implement an individual-based simulation of a population of at least 10,000 simulated individuals exposed from birth to the local transmission patterns. For each individual and time point during the year we use equations 1–5 to make predictions of \( I_m(i,t) \).

There is evidence that the risk of being bitten by an anopheline is approximately proportional to body size. To allow for this, we make an estimate of the contribution to the infectious reservoir for age group \( j \), weighted proportionately to \( A(a(i,t)) \), the estimated body surface area of the host, where \( a(i,t) \) is the age of individual \( i \) at time \( t \). The proportion of infectiousness contributed by group \( j \) is then

\[
\sum_{i,t}(A(a(i,t))I_m(i,t)J(j,i,t)) / \sum_{i,t}(A(a(i,t))I_m(i,t))
\]  

where \( J(j,i,t) = 1 \) if individual \( i \) is in age group \( j \) at time \( t \), and \( J(j,i,t) = 0 \) otherwise, and where the summations are over the whole simulated population and a whole year of follow-up time.

RESULTS

Data description. Seventy one patients with 730 feeding experiments (median of 4 experiments per individual (90% central range = 1–29) were included in the analyses. A total of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Point estimate</th>
<th>95% credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta_1 )</td>
<td>Effect of asexual density (lag 10 day) on expected gametocytemia</td>
<td>1</td>
</tr>
<tr>
<td>( \beta_2 )</td>
<td>Effect of asexual density (lag 15 days) on expected gametocytemia</td>
<td>0.46</td>
</tr>
<tr>
<td>( \beta_3 )</td>
<td>Effect of asexual density (lag 20 days) on expected gametocytemia</td>
<td>0.17</td>
</tr>
<tr>
<td>( \rho )</td>
<td>Location parameter for the distribution of the ratio of gametocytes to asexual parasites</td>
<td>0.00031</td>
</tr>
<tr>
<td>( \sigma_g )</td>
<td>Standard deviation of the distribution of the ratio of gametocytes to asexual parasites</td>
<td>3.91</td>
</tr>
</tbody>
</table>

* To ensure identifiability.
22,431 mosquitoes were analyzed (median = 28 mosquitoes per experiment, 90% central range = 5–60).

A total of 562 (77%) blood slides were positive for *P. falciparum* 10 days prior to the feeding experiments, with median density of 1,792 parasites/μL (range = 30–25,990). Parasite prevalence and densities on the other days analyzed were comparable. A total of 565 (77%) of the 730 samples on the day of the feeding experiment were gametocytemic, with median gametocyte densities of 190 (range = 10–3,330).

Overall, 5,420 (24%) of 22,431 mosquitoes became infected. However, there was considerable variation in the observed proportions of mosquitoes infected in any one experiment (Figure 1). For the simulation model, we aimed to describe the average relationship between asexual parasite density and infectivity as accurately as possible for all values of the parasitemia history.

In analyses that considered only single predictors of the proportion of mosquitoes infected, asexual parasitemia 20, 15, 10, and 5 days previously and the reciprocal of days since first parasitemia were found to be most closely related to the proportion of mosquitoes that are infected. In multivariable models, the effects of parasite density five days previously and the reciprocal of time since first asexual parasitemia did not improve the fit.

**Model for infectivity.** We rejected a number of models for infectivity before selecting the model given by equations 1–5. Fixed effects models that ignored variation between patients or samples in the relationship predicted very high proportions of infected mosquitoes for hosts with a history of high parasite densities. There is substantial variation in infectivity in the data even at high values of the weighted average of asexual parasitemia $\Upsilon(i,t)$ (Figure 1), and models with fixed effects of the weighted sum of asexual parasitemia gave a better fit only if there was allowance for overdispersion in infectivity. However, allowing for overdispersion in the response led to problems of convergence, with the estimates of random effects highly correlated with those of $\Upsilon(i,t)$.

Our preferred model (equations 1–5) introduces random variation into the explanatory variable, $\Upsilon(i,t)$, intended to simulate random variation in the process of gametocytogenesis. The fit was good (Figure 2 and Table 1) except at very high values of the weighted sum of asexual density $\Upsilon(i,t)$. There is little data at these high values, and although proportions of 100% infected mosquitoes were observed in almost one-third of the experiments with the highest 5% of values of $\Upsilon(i,t)$, there was also considerable variation, with some proportions as low as 17%. This variation coupled with the relatively small amount of data makes it difficult to specify a model that fits well in this range.

**Gametocyte densities.** From equation 2 it follows that

$$\ln \left( \frac{y_{f(i,t)}}{\Upsilon(i,t)} \right) \sim \text{Normal}(\ln(p), \sigma^2_p) \quad (6)$$

To test this assumption of log-normality, we plotted the logarithm of the ratio of the observed density of female gametocytes $y_{f(i,t)}$ to the weighted sum of the asexual parasite densities (Figure 3a). The observed distribution of this ratio is somewhat truncated because the limit of detection in the malaria therapy dataset was 10 gametocytes/μL. However, the normal quantile plot (Figure 3b) suggests that the log-normal approximation is roughly valid, with a mean of approximately –5 for $\ln(y_{f(i,t)}/\Upsilon(i,t))$.

Thus, on average $y_{f(i,t)} = \Upsilon(i,t) \exp(–5)$, and the expectation of the normal distribution in equation 6 is

$$E \left[ \ln \left( \frac{y_{f(i,t)} \exp(5)}{y_{f(i,t)}} \right) \right] = \ln(p). \quad (7)$$

Using our best estimate of $p$ (Table 2), this gives an approximation for the proportion of gametocytes that are functional of $y_{f(i,t)}/y_{f(i,t)} = \exp(5) = 0.5$.

**Age pattern of infectivity.** The field estimates of the relative contribution of different age groups to the infectious reservoir are shown in Table 2. Using the transmission patterns for Ifakara and Farafenni, we estimated the relative contributions from our model predictions. The field studies make the assumption that people of different ages are equally frequently bitten by mosquitoes. Thus, the most comparable

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**Figure 1.** Box plot of the proportion of mosquitoes infected in the experiments for each category of asexual parasitemia. **a.** 10 days previously. **b.** 15 days previously. **c.** 20 days previously. The box indicates the 25th and 75th percentiles and the central line is the median. The upper whisker extends to the largest value below the 75th percentile plus the box height multiplied by 1.5. Similarly for the lower whisker. Values outside the whiskers are plotted individually.
estimates are those not weighted by body surface area. The unweighted contributions that we predicted are shown for Ifakara, and these are similar to those from the field study. Weighting the contributions of different ages of hosts to the infectious reservoir proportionately to the estimated surface area of the host as well as to the representation of the age group in the population led to higher predicted contributions from adults.

The studies of Drakeley and others investigated infectivity using feeds only on blood from demonstrable gametocyte carriers, and this makes it difficult to be confident in comparing the results with our population-based estimates. The assumption that individuals without detectable gametocytes do not transmit to mosquitoes is questionable. However, the estimated contributions of different age-groups were similar to those for Ifakara in settings where population-based assessments have been carried out. The general pattern in our simulations was in agreement with the data: at higher transmission (as in Kano, Kenya) the contribution of the younger age groups was increased, and at lower transmission (as in Farafenni, or in Papua New Guinea) the older age groups are predicted to be relatively more important.

**DISCUSSION**

We have fitted a statistical model to describe the relationship between *P. falciparum* asexual parasite densities and infectivity. The model makes use of the requirement for infectivity, that both male and female gametocytes must occur in the same blood meal, to provide an explanation for the non-linear relationship between infectivity and gametocyte densities in the malarial therapy dataset. We developed this model specifically for inclusion in our mathematical model of populations exposed to endemic *P. falciparum* transmission, and this dictated some simplifications. To correspond to our simulations we used a five-day time step, which makes less use of the available data than would a model based on shorter time intervals. We also avoided directly fitting a model to gametocyte densities because we required a model for infectivity as a function of asexual parasite densities.

The latter simplification allowed us to avoid any strong assumptions about the problematical relationship between observed and functional gametocytemia or about the poorly understood factors affecting gametocytogenesis. Our examination of the distribution of gametocyte densities confirmed that our model for gametocyte densities is a reasonable approximation. Our estimate for the percentage of gametocytes that are functional is low, in agreement with estimates from life tables, which indicate that many parasites are killed between the macrogamete and oocyst stages.

We assumed a 50:50 ratio of functional male:female gametocytes. The median proportion of male gametocytes recorded in the malarial therapy dataset was 0.43 with a 90% central range of 0–1, while other studies have found lower proportions (approximately 0.25) of male gametocytes. However, male gametocytes produce more gametes than female gametocytes, and thus perhaps *y* for male gametocytes should be lower than for female gametocytes to compensate for any imbalance in the sex ratio. We considered the sensitivity of *I*(t), the proportion of mosquitoes feeding on individual *i* at time *t* who became infected, to variations in the

![Figure 2](image2.png)

**FIGURE 2.** Relationship of infectivity to weighted sum of asexual densities (*Y*(*i*,*t*)). The thin line represents the estimated probability that the mosquito is infected with a female gametocyte *Pr*(*y*(*i*,*t*) > *y*), and the thick line is the estimated probability of infection with both male and female gametocytes, *Pr*(*y*(*i*,*t*) > *y*)

![Figure 3](image3.png)

**FIGURE 3.** Distribution of the observed density of female gametocytes. **a.** Histogram of ln(*y*(*i*,*t*)/*Y*(*i*,*t*)), the logarithm of the observed ratio of the density of female gametocytes to the weighted sum of asexual parasite densities. **b.** Normal quantile plot of ln(*y*(*i*,*t*)/*Y*(*i*,*t*)). For each data point (*x*,*y*), *y* is the observed value and *x* is the value expected for the same quantile of the corresponding normal distribution. Points lying along the straight line indicate that the data fits a normal distribution.
ratio of functional male:female gametocytes. $I_m(i,t)$ showed little sensitivity to variations in the assumed sex-ratio of gametocytes over a wide range. This supports the claim that the sex ratio of gametocytes is unlikely to be of major importance in determining infectivity, and studies to date have shown little or no effect of sex ratio on infectivity.

We do not consider heterogeneity between individuals or parasite clones in their innate propensity to produce gametocytes because our interest focuses on overall infectivity. Moreover, we ignore any direct effects of fever on infectivity and of drug treatment on gametocytemia. These effects are likely to be of epidemiologic importance mainly in low transmission areas.

To apply our model to endemic areas in Africa, we must make several further simplifications. The malarialtherapy experiments used *An. quadrimaculatus* and *An. albimanus* in controlled conditions, which differ from African field conditions where the main vectors are *An. funestus* and the *An. gambiae* complex. We consider the extent to which our approach leads to an overall bias in the estimates of the proportion of mosquito bites resulting in infections of the vector in a separate paper.

We also assume that the relationship between asexual parasitemia and infectivity in adults infected for the first time can be generalized to those with varying levels of acquired immunity, which entails assuming that there is no acquisition of transmission-blocking immunity and no effect of antibodies to gametocytes. This is supported by our finding that the model predicts age patterns that concur with those from field data. Although children have often been thought to contribute most of the malaria transmission to vectors because of their higher parasite densities, those studies that have estimated the contributions of different ages of hosts to the infectious reservoir have found that adults make a substantial contribution (Table 2).

Our model supports the conclusions of these field studies and indeed suggests that the contribution of adults has been underestimated because of the usual assumption that each potential host is bitten equally frequently. This corroborates the assumption that transmission-blocking immunity does not markedly increase with age, and that natural acquired immune responses to gametocytes are also not of epidemiologic importance. However, these conclusions rest on a limited evidence base. There is a need for improved population-based estimates of age- and exposure- dependence in the infectivity of humans to mosquitoes in endemic areas.

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