INCIDENCE OF PLASMODIUM FALCIPARUM INFECTION IN INFANTS IN RELATION TO EXPOSURE TO SPOROZOITE-INFECTED ANOPHELINEs

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Abstract. The relationship of the incidence of Plasmodium falciparum infection to entomologic inoculation rates (EIRs) was studied in 163 children less than one year of age in a Tanzanian village to determine likely effects of transmission-reducing interventions on infection incidence. A total of 66,727 Anopheles gambiae s.l. and 17,620 An. funestus mosquitoes were caught in 1,056 light trap collections from 139 houses over a period of more than two years. Time period–specific human biting rates were estimated for 11 village neighborhoods. Sporozoites were detected by ELISA in 4.4% of the An. funestus and 2.5% of the An. gambiae s.l. Eight hundred seventeen pairs of blood slides with approximately two-week intervals between slides were used to estimate incidence of parasitemia by fitting reversible catalytic models to parasite positivity data. Estimated EIRs during the four weeks preceding each intersurvey interval averaged 1.6 (SD = 2.1) per adult per night. Parasites were present at the end of 31% of the 443 intervals that commenced with a parasite-negative slide. Attack rates were comparable with those in western Kenya, and the proportion of bites resulting in human infections was strongly dependent on mosquito density. Incidence of infection increased with the EIR up to approximately one bite from a sporozoite-carrying mosquito per adult per night. However, higher levels of transmission observed locally in the wet season did not result in a correspondingly higher incidence. These data suggest that transmission-reducing measures cannot be expected to reduce incidence of infection at the highest levels of EIR.

Successful trials of insecticide-impregnated bed nets against malaria1 and the prospect of field testing of transmission-blocking malaria vaccines2 have drawn attention to the paucity of data from areas of very high transmission on the epidemiologic consequences of variations in exposure of the human host to sporozoites of Plasmodium falciparum.3 Understanding of the relationships between malariologic outcomes and exposure is essential if the impact of these transmission-reducing measures is to be predicted.

Exposure to malaria is normally estimated by counting the number of mosquitoes biting an average person per night (ma) and determining the proportion of these mosquitoes with sporozoites (s). The product of these estimates, mas, is the entomologic inoculation rate (EIR). Since mosquito densities can vary widely in time and space,3–6 only a considerable sampling effort over extended periods and large areas will provide reliable estimates of ma. Most studies have consequently reported mas estimates for whole villages7,8 or for extended periods9 and have therefore not been able to estimate relationships between exposure and malariologic outcomes.

An alternative method of measuring malaria exposure is to estimate the incidence of infection.10–12 Incidence rates and EIRs are not strictly interchangeable because not all feeds by sporozoite-carrying mosquitoes result in a blood-stage infection.13–15 This is because infected mosquitoes do not always inject sporozoites when feeding,16 and if they do they may inject an insufficient number.17 Moreover, the injected sporozoites may not be viable. These factors reduce the proportion of bites resulting in blood stage infection independently of transmission intensity.

Other potential mechanisms such as pre-erythrocytic immunity,18,19 which may also ameliorate incidence are likely to depend upon previous exposure. In addition, at high levels of endemicity, many hosts are already parasitemic and it is difficult to determine whether additional bites result in superinfections. To minimize the effects of immunity, incidence rates are often measured in relatively naive groups, usually infants or young children who have been cleared of parasites with an effective short-acting antimalarial.

Few studies have quantified the relationship between incidence rates and EIRs.6–20 We have recently used longitudinal parasitologic data collected over a one-year period to estimate the incidence and recovery rates from parasitemia of infants living in a large Tanzanian village with a high endemicity of P. falciparum.21 In the same village, and both at the same time and for a preceding year, mosquito densities were determined using light traps, and sporozoite rates were determined by ELISA. We have used these data to obtain different estimates of mas for subdivisions of the village and for different time periods and relate these estimates to the incidence of parasitemia in the infants.

MATERIALS AND METHODS

Study site. The study took place in the village of Idete,22 20 km west of the town of Ifakara in southern Tanzania, in conjunction with a malaria vaccine trial in children 1–5 years of age.22 The general area was already known to have a high endemicity of P. falciparum malaria,23 and this particular study site was selected because of the availability of a functional dispensary in a previously unstudied village within the area. The Great Uhuru Railway (Tazara) crosses the village (Figure 1). Areas to the south of the railway line lie in the plain of the Kilombero Valley, which is seasonally flooded. Houses to the north are built in the foothills of the Udzungwa Mountains. Rainfall and temperature data were obtained from the agricultural research station near Ifakara, which is also in the foothills of these mountains.

A total of 4,758 inhabitants and 868 houses were enumerated in a census conducted between October 1992 and February 1993; 45% of the residents were less than 15 years of age, and 5% were less than one year of age.

House positions were determined using a portable Global Positioning System (Trimble Navigation, Sunnyvale, CA) receiver. The centroid of several independent determinations
was taken as the best estimate of the location of each house. Maps were prepared using Mapinfo 7 (Mapinfo Corp., Troy, NY) software. The village is divided by the local population into 14 neighborhoods or hamlets (Figure 1). These are generally bounded by natural features, the railway, or roads. Each neighborhood, however, contains a few outlying houses that cross these boundaries. House densities are greatest in the middle of the village, where neighborhoods are also smaller and where the dispensary is located (center of inset in Figure 1).

In conjunction with the malaria vaccine trial, the village dispensary was strengthened so that services and drugs, which were supplied free of charge to local residents, were assured. The dispensary was then the predominant source of anti-malarial drugs and the prescription of drugs to children in the study was recorded by project personnel. In agreement with national guidelines for rural dispensaries, treatment was given on the basis of clinical diagnoses before confirmation of infection. Microscopy cannot be routinely carried out at dispensaries such as this because there is no continuous power supply. Malaria and fevers of unknown origin were usually treated with chloroquine, which remains the standard first-line drug in Tanzania despite chloroquine resistance.24

Ethical clearance for the study was provided by the Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR) and research clearance was obtained from the Tanzanian Commission of Science and Technology as per ref. NSR/RCA90. Written informed consent was obtained from every mother or guardian before an infant was enrolled in the study.

**Sampling of mosquitoes.** Houses were chosen for entomologic sampling with the objective of providing samples of mosquitoes representative of each area of the village. The 139 chosen houses were dispersed around the inhabited parts of each section of the village. A regular sampling grid could not be used because houses were selected before the digitized maps of the village were available. The choice of houses was independent of the locations of study children and of features of the houses (e.g., construction types) or of the occupants, which might influence mosquito densities. Mosquito numbers were monitored using Centers for Disease Control and Prevention (Atlanta, GA) miniature light traps,25 operated for more than two years (including the period of the epidemiologic study) approximately once every two weeks, each time in a different subsample (averaging 15%) of these houses. On each sampling occasion, intact mosquito nets were suspended over all sleeping locations. The number of trap-nights are given in Tables 1 and 2.

**Sporozoite ELISA.** The heads and thoraces of *Anopheles gambiae* s.l. and *An. funestus* captured in light traps were stored on silica gel at −20°C, and examined (either in pools of 10 or individually) for the presence of the repetitive sequence (NANP)n of the *P. falciparum* circumsporozoite protein using an established sandwich ELISA technique.26

**Entomologic inoculation rate estimation.** Estimates of the population of each vector species, for each neighborhood of the village in each two-week period, were made using a Poisson regression model (Appendix 1). Separate estimates of the spatial pattern were made for wet (January to July) and dry (August to December) seasons. The model allowed for variation in mosquito densities between each two-week period.

We assumed proportionality between light trap catches and human biting rates27 for both *An. gambiae* s.l. and *An. funestus*. For comparability with other studies, expected light trap catches estimated from the model were multiplied by 1.5 to converted them to equivalent human biting rates (ma)27 as described previously for studies in Kilombero.23 The EIR estimates were made by multiplying this number by the species-specific sporozoite rates.

**Infection rate estimates.** Force of infection estimates based on microscopic examination of blood slides collected in the community were obtained for children less than one year of age. The children enrolled in this part of the study were a random sample of those in the village, stratified ac-
acording to one month age group. Each child was followed for a four-month period (or until his or her first birthday) and blood slides were obtained at intervals of approximately two weeks. At any one time, approximately 60 infants were being monitored. Further details of this monitoring are provided elsewhere.\textsuperscript{21} Of the 304 infants included in this surveillance, 163 were in the areas of the village covered by the entomologic sampling. These provided a total of 817 pairs of samples that could be included in the analysis of incidence of infection. The numbers of pairs of samples for each neighborhood of the village are given in Tables 1 and 2.

Blood slides (thick and thin films) were air-dried and read after application of the standard Giemsa staining method, using a light microscope (Leica, Zurich, Switzerland) with a 50× oil immersion lens and 10× eyepieces. Each slide was assessed twice independently by counting the number of asexual stage parasites against 200 leukocytes. Slides were read a third time if there was a discrepancy in positivity or as a result of slide positivity was based on the majority verdict.\textsuperscript{22}

Infections of malaria species other than \textit{P. falciparum} were not considered in the present analyses.

Initial estimates of incidence of infection were made by dividing the number of intervals that began with a negative slide and ended with a positive slide by the total duration of intervals commencing with negative slides. This procedure underestimates the true force of infection because it does not allow for infections that are cleared during the interval. To allow for this, age-standardized, exposure-specific estimates of the force of infection (\( h \) = the number of new infections per child per night), not considering superinfections, were obtained using a reversible catalytic model\textsuperscript{28} as described in Appendix 2.

RESULTS

Mosquito densities. A total of 139 different houses were sampled for mosquitoes on 220 different days (a total of 1,056 collections) between March 1992 and October 1994. Altogether, 66,727 \textit{An. gambiae} s.l. and 17,620 \textit{An. funestus} were collected (maximum numbers in single light traps =

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<th>Neighborhood</th>
<th>Parasitology</th>
<th>Entomology</th>
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<td></td>
<td>Initial negative</td>
<td>Initial positive</td>
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<tr>
<td></td>
<td>N</td>
<td>%</td>
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<tr>
<td>Godauni mlimani</td>
<td>14</td>
<td>29</td>
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<tr>
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<td>75</td>
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<td>Msumbiji B</td>
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<tr>
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<tr>
<td>Msumbiji chini</td>
<td>13</td>
<td>54</td>
</tr>
<tr>
<td>Doko</td>
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<td>100</td>
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<tr>
<td>Miwangani</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>Kihogosi</td>
<td>2</td>
<td>0</td>
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</tbody>
</table>

\* For definitions of abbreviations and other information, see Table 1. \( \pm \) = not defined. Although entomologic data were available for Kihogosi only for the dry season, four intervals at the start of the wet season are included because their exposure was predominantly during the dry season.
temperature. Anopheles gambiae s.l. in the dry season. Peak populations of the latter species occurred shortly after the peak period of rainfall, reflecting the presence of transient breeding sites for this species, which was concentrated in low-lying parts of the village adjacent to the Kilombero plain where small transient water bodies are more common. These pools disappear in the dry season and so does An. gambiae s.l. Anopheles funestus populations were more stable over the period of study. The estimates of ma are rather higher than the mean light trap catches because of the constant of 1.5 used in their determination. There are also differences because the two-week periods with the highest densities contribute a large proportion of the estimated average human biting rate (ma). The biting rate estimates plotted on the map (Figure 3) were computed as the arithmetic mean of the estimate of ma for a neighborhood for each of the two-week periods.

Because of difficulties of access, there were imbalances in the data collected. Thus, the neighborhood of Miwangani was not sampled during the periods of peak densities in the rest of the village but at the time when it was sampled densities there were higher than those elsewhere. Therefore, although a relatively small number of An. gambiae s.l. were caught in Miwangani, this area was nevertheless estimated to have among the highest wet season An. gambiae population density, and the unadjusted average numbers of mosquitoes caught (Table 1) show a different ranking from the exposure estimates.

There were permanent breeding sites for An. funestus along the Tazara railway; thus, this species was present in the central part of Idete throughout the year. Consequently, densities are less seasonally variable than those of An. gambiae s.l. This is different from the neighboring village of Namawala where An. funestus breeds in flooded rice fields. In Namawala, there are no permanent breeding sites of this species and it occurs predominantly in the wet season at the edge of the village.6

Parasitology. Of the total of 949 pairs of malaria slides studied previously, 799 (taken from 163 different children) were in subdivisions of the village for which there were entomologic data. These were included in the present analyses (Tables 1 and 2). In 443 of these pairs, the slide at the start of the interval was negative by microscopy for asexual stages of P. falciparum and in 134 (30.7%) the slide was positive at the end of the interval. Table 1 shows these numbers of pairs and the percentages ending in positive slides broken down by areas of the village for the wet season and Table 2 gives the corresponding dry season data. Transitions from slide negativity to slide positivity clearly occur in both seasons and throughout the village. The mean duration of the intervals between these slides was 14.5 days (SD = 2.8 days). Anti-malarials were prescribed at the dispensary during 41 (31%) of these 134 intervals compared with 39 (13%) of 309 intervals ending with a negative slide ($\chi^2 = 20.4, P = 0.001$).

A total of 356 intervals, with a mean duration of 14.6 days (SD = 2.6 days) began with a slide that was positive. In 296 of these (83.1%), the slide at the end of the interval was also positive (Tables 1 and 2). Transitions from slide positivity to slide negativity also occurred throughout the village and in both seasons. Among the initially positive intervals, positivity at the end of the interval was not related to prescription of anti-malarials at the dispensary.

Entomologic inoculation rates. Overall, 367 (2.5%) of 14,604 An. gambiae s.l. and 272 (4.4%) of 6,184 An. funestus tested positive for circumsporozoite protein in the ELISA. A preponderance of An. arabiensis in Idete may be one of the reasons why sporozoite rates were lower in An. gambiae s.l. than in An. funestus since An. arabiensis is not usually as efficient a vector of malaria as An. gambiae s.s. However, these sporozoite rates are rather higher than those...
in Namawala\textsuperscript{5} where the highest densities of \textit{An. gambiae} s.l. also consist of \textit{An. arabienensis}.\textsuperscript{6}

These sporozoite rates were combined with the estimates of mosquito population densities to give neighborhood- and two-week period–specific estimates of \( m \). Despite the fact that the highest mosquito densities were reached by \textit{An. gambiae} s.l., for much of the year \textit{An. funestus} was responsible for most of the malaria transmission.

The EIR for the four weeks preceding the start of each interval between blood slides was computed by taking the arithmetic mean of the estimates for the two two-week periods. This amounts to assuming an average prepatent period of two weeks following inoculation. The distribution of \( m \) in Tables 1 and 2 is shown separately for the intervals with negative initial slides and for those starting with a positive slide (Tables 1 and 2). Among intervals with a negative initial slide, the overall mean infectious bites per adult per night was 1.6 (SD = 2.1). The mean of \( m \) was 1.8 where anti-malarials were prescribed compared with 1.5 in those with no anti-malarial prescription (\( Z = 1.9, P = 0.06 \), by Wilcoxon test).

**Exposure-response relationships.** Figure 4 gives the monthly means of the estimated \( m \) for the 450 intervals with negative initial slides. The corresponding infection rates were computed by dividing the number of infants becoming
infected each month by the total days at risk for infants with an initial negative slide. The EIRs are much higher than the corresponding infection rates (note that both rates are plotted on a logarithmic scale), but except at the peak of the wet season the seasonal pattern is similar, with the decrease in infection rate during the dry season closely tracking the decrease in the EIR (Figure 4). During the wet season, the average EIR increased considerably, but the infection rate peaked early in February, long before the maximum rates of mosquito biting were reached.

The EIR can be analyzed into distinct components attributable to An. funestus and to An. gambiae (Figure 5). A much wider range of exposures were observed for An. gambiae s.l. than for An. funestus (Table 1 and Table 2). Over the range of exposures for which there were data for both vectors, there were similar clear increases in infection rates with EIRs (lower curves in Figure 5). Logistic regression analysis, adjusting for age effects and fitted to the data for initially negative intervals, indicated highly statistically significant relationships between the probability of becoming infected and the logarithm of the estimated exposure for both An. funestus ($\chi^2 = 7.5, P = 0.0061$, odds ratio [OR] associated with a 10-fold increase in biting rate $= 2.4$, 95% confidence limit [CL] = 1.3–4.7) and for An. gambiae s.l. ($\chi^2 = 9.0, P = 0.0026$, OR = 1.5, 95% CL = 1.2–2.0). However, the increase for An. gambiae s.l. applied only to EIRs less than approximately one per night, and was not proportional to the increase in EIR. Above this level of exposure, there was little or no relationship between infection rate and EIR. A logistic regression model fitted only to the data for EIR less than one per night gave an OR of 3.9 (95% CL = 2.0–7.6) associated with a 10-fold increase in EIR, while for an EIR greater than one per night the OR was 1.1 (95% CL = 0.7–1.9). Exploratory analyses using time period–specific sporozoite rates gave even weaker relationships between EIR and force of infection. The rate at which initially positive slides lost infections did not show any clear pattern in relation to exposure to either vector (Table 3 and Figure 5).

The saturation in the infection rate is also illustrated by Figure 6 in which the infection rate is shown relative to the aggregated EIR calculated from the data for both vectors. We carried out both analyses that include all intervals (Figure 6), and also analyses excluding all intervals during which anti-malarials were prescribed at the dispensary. These two different analyses gave very similar pictures of the relationship between EIR and infection rate.

Above a $mas$ of approximately one per adult per night there was no further increase in the infection rate with increasing EIR. Even when the estimated EIR was more than five per person per night, 30 of 75 microscopy slides at the ends of the intervals were negative. Thus, there must be a considerable decrease in the proportion of bites that result in patent infections at high EIRs. To estimate this proportion, the crude infection rates were first converted to estimates of the equivalent force of infection (instantaneous infection rate, $h$), which allows for the possibility that a child both gained an infection and recovered from it during the same interval (Appendix 2).

The estimates of $h$ are higher than the crude infection rates at all values of $mas$ (Figure 6). However, the proportion of sporozoite inoculations resulting in an infection, obtained as the ratio $hil(mas)$, is much less than unity at all values of $mas$. This ratio decreases steeply from a value of almost 0.07 at the lowest exposures to 0.006 at an EIR of four or more per persons per night.

### Table 3

<table>
<thead>
<tr>
<th>Bites/person/night</th>
<th>Slide initially negative for $P.$ falciparum</th>
<th>Slide initially positive for $P.$ falciparum</th>
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<tbody>
<tr>
<td>N</td>
<td>% (95% CL)</td>
<td>% (95% CL)</td>
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<tr>
<td>0.0–&lt;0.3</td>
<td>64   15.6 (7.8–26.9)</td>
<td>38   81.6 (65.7–92.3)</td>
</tr>
<tr>
<td>0.3–&lt;0.4</td>
<td>42   11.9 (4.0–25.6)</td>
<td>18   94.4 (72.7–99.9)</td>
</tr>
<tr>
<td>0.4–&lt;0.5</td>
<td>32   25.0 (11.5–43.4)</td>
<td>22   95.5 (77.2–99.9)</td>
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<tr>
<td>0.5–&lt;0.6</td>
<td>53   37.7 (24.8–52.1)</td>
<td>42   73.8 (56.0–86.1)</td>
</tr>
<tr>
<td>0.6–&lt;0.8</td>
<td>60   43.3 (30.6–56.8)</td>
<td>56   78.6 (65.6–88.4)</td>
</tr>
<tr>
<td>0.8–&lt;1.0</td>
<td>25   36.0 (18.0–57.5)</td>
<td>21   76.2 (52.8–91.8)</td>
</tr>
<tr>
<td>1.0–&lt;2.0</td>
<td>77   35.1 (24.5–46.8)</td>
<td>83   84.3 (74.7–91.4)</td>
</tr>
<tr>
<td>2.0–&lt;4.0</td>
<td>43   30.2 (17.2–46.1)</td>
<td>36   88.9 (73.9–96.9)</td>
</tr>
<tr>
<td>≥4.0</td>
<td>47   34.0 (20.9–49.3)</td>
<td>40   85.0 (70.2–94.3)</td>
</tr>
<tr>
<td>Overall</td>
<td>443  30.2 (26.0–34.8)</td>
<td>356  83.1 (78.4–86.9)</td>
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*N is the total number of pairs of slides (intervals); % is the percentage of intervals for which the slide at the end of the interval was positive for blood stages of Plasmodium falciparum; CL = exact binomial confidence limits.

![Figure 5](image1.png)

**Figure 5.** Transition rates in relation to vector-specific entomologic inoculation rates (number of intervals with infections at the end/total duration of intervals). — = intervals commencing with negative slides; —— = intervals commencing with positive slides. Error bars indicate 95% confidence intervals.

![Figure 6](image2.png)

**Figure 6.** Infection rates and force of infection by overall entomologic inoculation rate. —— = infection rate (transitions/total days at risk); —— = estimated force of infection (infections/total days at risk); — = ratio of force of infection to infectious bites per night. Error bars indicate 95% confidence intervals.
As expected, at low inoculation rates the incidence of patent malaria infection increased with EIR. However, when the EIR was estimated to be more than one bite per person per night, there was no increase with EIR in the incidence of parasitemia in infants. The proportion of bites from sporozoite-infected mosquitoes that resulted in patent blood stage infections consequently showed a very marked decrease as the EIR increased. This saturation in the force of infection is evident in the seasonal pattern, which indicates that there is no increase in force of infection during the main part of the wet season, when anophelines are much more abundant.

Our confidence in the level of exposure at which this saturation occurs depends on the validity of the EIR estimates, derived from light trap collections of mosquitoes. The standard method of determining the EIR is to count sporozoite-positive mosquitoes landing on sentinel adult individuals. Such collections provide a standard against which other trapping techniques can be assessed. However, collectors vary in attractiveness to mosquitoes and are also likely to differ in their efficiency at catching the insects. Moreover, they are usually young men, and are therefore not representative of the whole village population.

Light traps, as used in the present study, sample the human-biting fraction of the mosquito population in a more standardized way, are less labor-intensive, and more ethically acceptable than biting catches. This allows the collection of a greater number of samples from more sites.

Irrespective of collection method, estimates of EIR are subject to uncertainties and a corollary of the use of estimates of mosquito population densities for short-time periods and neighborhoods within the village is that our EIRs are imprecise. We initially hoped to analyze variation in mosquito population densities within neighborhoods in the same way as was done for the nearby, much smaller, village of Namawala. However, although we were able to sample from 139 houses in Idete, the sampled houses were generally more than 400 m apart, which may well be further than typical flight ranges of the vectors, and this precluded useful spatial analysis within neighborhoods. The aggregation of data for whole neighborhoods ignores heterogeneity in exposure within them. Similarly, sporozoite rates vary over space and time, but we found that breaking the sporozoite rate into shorter time periods did not help to explain the variation in the infection rates; therefore, we used only a single sporozoite rate for each vector species.

The small body size of infants is likely to be the most important factor accounting for the difference between incidence and estimated exposure at the lowest levels of EIR since the biting rate estimates are for adults and biting rates of An. gambiae s.l. (and probably other mosquitoes) are related to the surface area of the host. The true inoculation rate in infants in our study is therefore likely to be an order of magnitude lower than our estimates of adult EIRs, and decoupling between incidence and exposure must occur at a lower true EIR than our nominal estimate. However, the ratio between child and adult biting rates is presumably independent of the mosquito population density, and thus this effects should not affect the shape of the exposure response relationship. Other factors such as inoculation of inadequate numbers of sporozoites and classification of mosquitoes with only immature sporozoites in the thorax as sporozoite positive will also have contributed in a density-independent manner to overestimation of the EIR in infants.

Most epidemiologic exposure response relationships show saturation, but the possibility that this applies to malaria transmission has not been widely discussed. In a previous investigation of untreated adults and children in Kilombero, we could find little or no relationship between infection prevalence, parasite density, and EIR. The study of Beier and others in western Kenya differed from ours in that the children were treated with sulfadoxine-pyrimethamine and attack rates could therefore be assessed directly rather than via a statistical model. At comparable EIRs, attack rates were similar to those we estimated. There were strong relationships between exposure and both infection and parasite densities up to an EIR of approximately one bite per night. The proportion of bites resulting in infection also decreased with increased EIR, but since few children in that study were exposed to very high EIRs comparable to the highest observed in Idete, the saturation that we observed was not as evident.

The measurement of exposure for groups rather than individuals generally leads to underestimation of the magnitude of exposure-response relationships, but because this underestimation applies at all levels of transmission, it does not explain why a relationship between EIR and patent infection was observed at low but not at high exposure levels.

The lack of a relationship between EIR and infection at high EIRs is unlikely to be due to increased defensive responses of the human population at high mosquito densities. The feeding success of anophelines in Kilombero is not related to their population densities, probably because they bite while people are asleep and unable to defend themselves. Infants are particularly helpless in this respect. Other studies have suggested mosquito net use may increase in response to exposure, but in Idete net use was minimal. The small increase in the use of anti-malarial drugs with exposure cannot explain the very striking decrease in the proportion of mosquito bites that result in patent infections as EIRs Chloroquine was generally ineffective in preventing infections: a high level of parasitologic resistance has previously been documented in Idete. It is also unlikely that the saturation is a consequence of unrecorded anti-malarial treatment. The private market for anti-malarials in the village was small because the dispensary supplies were available free of charge at the time of the study. Antimalarial drugs other than chloroquine were not generally available and/or affordable. Recrudescences of previously subpatent infections could bias estimates of the force of infection, but it is difficult to believe that this is an important phenomenon in older infants, who normally have high parasite densities when they are infected, or that it could cause the observed relationships with EIR.

The observed saturation in incidence implies that distinct inoculations of the same host interfere with each other. We do not know what is the explanation for this interference. The high recovery rates observed are consistent with many infections being cleared before they become patent, but we did not observe an increase in recovery rate with EIR. In
rodent models, protection against infection has been associated with the persistence of intrahepatic parasites, suggesting that liver stage parasites interfere with subsequent infections. If this occurs in humans, it could account for the saturation, but we have no direct evidence for such a phenomenon in these children.

This saturation is of practical importance because it implies that substantial reductions in transmission at high levels of exposure will not necessarily reduce either prevalence or incidence. However, these are not the only malarialogic indices that might be related to EIR. For example, malaria morbidity and mortality rates depend not only on the incidence of infection but also on qualitative differences between inocula. At very high exposures the relationships of morbidity and mortality risks to EIR remain unclear. In western Kenya, parasite densities in small children showed an increase with the EIR. We have not yet analyzed this relationship in our dataset.

The saturation also has implications for the quantification of malaria transmissibility. It has been suggested that this is best quantified using parasitologic or serologic age profiles of conversion rates. However, since infection rates are not sensitive to EIR over the whole range of exposure, comparisons both within and between study sites of entomologic measures of transmission still have a role to play in high-transmission areas.

Acknowledgments: This study would not have been possible without the hard work of the entomology, parasitology, and data processing staff of the Ifakara Centre, and the cooperation and assistance of the people of Ifate, especially the staff of the dispensary. We thank Drs. Peter Billingsley and Bob Snow for helpful comments on previous versions of the manuscript and Professor Wen Kilama (Director General, NIMR) for support.

Financial support: This study was supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR), the Swiss Agency for Development and Cooperation, and the Swiss National Science Foundation (grant no: 32-43527.95). A. Y. Kitua is a TDR Research Training Fellow and P. L. Alonso is partly supported by grants PNG (PTR92-0089) and FIS (93/0269). M. Booth is in receipt of a Wellcome Travelling Research Fellowship. 

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### Appendix 1. Entomologic Inoculation Rate Estimation

Estimates of mosquito population densities, for each neighborhood, for each two-week period and for each vector species were made using a spatio-temporal regression model as described previously. The number of mosquitoes (Y) caught in a light trap in neighborhood i during two-week period j and of species k was treated as a Poisson variate with expectation E_{i,j,k}. Algebraically, Y_{i,j,k} \sim \text{Poisson}(E_{i,j,k}).

Within seasons (wet and dry) variation between neighborhoods was assumed to be independent, on the logarithm scale, from variation between a two-week period. The natural logarithm of E_{i,j,k} was therefore analyzed as a linear function \log E_{i,j,k} = \Psi_{i,k} + \theta_{i,k}, where \Psi_{i,k} is the effect associated with neighborhood i and mosquito species k and \theta_{i,k} is the random effect associated with two-week period j and mosquito species k. Separate values of \Psi_{i,k} were fitted for each season.

To prevent overestimation of the temporal variation due to sampling variation, the two-week period-specific parameters for each species, \theta_{i,k}, were assumed to be drawn from a normal distribution, with mean 0 and variance \Phi_i. \Phi_i \sim \text{Normal}(0, \Phi_i). This statistical model made it possible to estimate mosquito densities for each sampled neighborhood and for each two-week period during which any neighborhood was sampled.

The EIR for neighborhood i, two-week period j, was then estimated as

\[
EIR_{i,j} = 1.5 \sum_k s_k E_{i,j,k}
\]

where the factor of 1.5 is a standard constant of proportionality used to convert light trap results to approximate equivalent biting densities originally obtained by Lines and others and s_k is the species-specific sporozoite rate.

### Appendix 2. Exposure specific estimates of the force of infection

Estimates of the force of infection, h, and of the recovery rate, r, (the number of infections cleared per child per night) were obtained by fitting reversible catalytic models. Previous analyses indicated that h is only weakly age dependent, and the crude recovery probabilities evident from Tables 1 and 2 indicated that r has little dependence on the EIR. We therefore estimated EIR-specific estimates of h, (h_s) and age specific estimates of r (r_s).

Among intervals which commenced with a negative slide, the catalytic model gives a probability of patent infection at the end of an interval of duration t days as

\[
p_{(\alpha, 0)} = \frac{h_s}{h_s + r_s}\left[1 - \exp(-(h_s + r_s)t)\right]
\]

and among intervals that commenced with a positive slide it is given by

\[
p_{(\alpha, 1)} = \frac{h_s}{h_s + r_s + \frac{r_s}{h_s + r_s}\exp(-(h_s + r_s)t)}
\]

The model was fitted by maximum likelihood assuming a binomial error distribution.