A HIGH MALARIA REINFECTION RATE IN CHILDREN AND YOUNG ADULTS LIVING UNDER A LOW ENTOMOLOGICAL INOCULATION RATE IN A PERIURBAN AREA OF BAMAKO, MALI

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Abstract.
In areas of intense malaria parasite transmission, preliminary studies of the rate of reinfection after curative therapy suggest that small sample size studies of vaccine efficacy are feasible. However, the effect of transmission rate, which may vary considerably between transmission seasons, on reinfection rate has not been assessed in areas of mesoendemicity with seasonal transmission. To address this question, the Plasmodium falciparum reinfection rate after curative therapy was measured in Sotuba, a Malian village with historically low transmission rates, as estimated by the entomological inoculation rate (EIR). The reinfection rate after curative Fansidar® (sulfadoxine-pyrimethamine) treatment was 80.7% (88/109). The EIR during the 13-week study period (seasonal transmission) varied between 1 and 4.5 infected bites/person/month. The finding that reinfection rates were high despite low EIRs suggests that a low EIR may be sufficient to support small sample size vaccine efficacy trials in mesoendemic areas.

INTRODUCTION

At the pediatric hospital in Bamako, malaria is the most common cause of febrile convulsions in children between the ages of 16 months and 10 years (Diarwara FM, personal communication) and ranks high among causes of childhood mortality. Consequently, the development of an effective malaria vaccine strategy remains a priority in Mali and throughout much of sub-Saharan Africa. In the absence of clinically validated in vitro surrogates of protective efficacy, the initial evaluation of most malaria vaccines, and particularly asexual blood stage vaccines designed to reduce parasitemia and the severity of disease, requires human clinical testing. These early human clinical trials will also be essential in evaluating both in vitro and in vivo surrogates of efficacy. For example, several Aotus blood stage challenge models have been used to determine the immunogenicity and efficacy of lead vaccine candidates; however, in the absence of parallel clinical trials in humans, these model systems cannot be validated as accurate predictors of human responses to vaccination and subsequent infection.1

Because preexistent antimalarial immunity and the boosting effect of frequent exposure to infected vectors may affect the host immune responses to asexual blood stage vaccines, the assessment of vaccine efficacy in areas of differing endemicity is essential. Regions of less intense and seasonal transmission will be particularly important in view of the inverse correlation between malaria morbidity and the intensity of transmission that has been reported in some regions.2,3

Entomological inoculation rate (EIR), the product of the vector biting rate times the proportion of sporozoite-infected mosquitoes, is commonly used as a measure of malaria transmission intensity. In a study conducted in an area of intense malaria transmission (daily EIRs averaging 0.75 infective bites/person/night) in Kenya, the Plasmodium falciparum reinfection rate after Fansidar® treatment was correlated with fluctuations in EIR,4 suggesting that EIR might be a good predictor of attack rate and consequently the suitability of a given site for small-scale (Phase IIb) malaria vaccine trials. Several studies suggest, however, that EIR may underesti-

mate the attack rates in areas of seasonal transmission. First, whereas the EIR appears to be well correlated with the prevalence of P. falciparum infection in most instances, some regions with extremely low EIRs have P. falciparum prevalence rates exceeding 40%.5 Second, a lack of boosting of antimalarial immunity during the low transmission season could lead to an increased efficiency of infection during the high transmission season. Such an increased efficiency in the setting of decreased immunity has been demonstrated in a region of malaria reemergence in Madagascar.6

In view of these concerns, this study was designed to determine the malaria parasite reinfection rate during the high transmission season in a region with seasonal malaria transmission and a low entomological inoculation rate and assess the ability of such a site to support a small sample size Phase IIIb malaria vaccine trial.

MATERIALS AND METHODS

Study site. Sotuba was chosen on the basis of malaritimetric collected over several transmission seasons and actual EIRs collected within the past 3 years. It is a village of approximately 2,500 people located in periurban region of Bamako, Mali, West Africa. Malaria is mesoendemic in this region, and transmission is markedly seasonal (peak EIR is approximately 2.0 infected bites/person/month in October and near 0 in June; Toure Y, unpublished data). P. falciparum is the predominant species, accounting for > 95% of malaria cases. The primary vector responsible for the transmission of P. falciparum in Sotuba is Anopheles gambiae s.l. Consistent with previous years, A. funestus accounted for a small proportion (0.3%) of the vector population in Sotuba and none of the EIR during the study period.

Study participants. The study protocol and informed consent and assent forms were reviewed and approved by OPRR-approved ethical review boards in the United States and in Mali. Apparently healthy nonpregnant persons aged 7 to 20 years were considered for the study. After informed consent/assent was obtained, a brief clinical examination was performed, and blood was obtained for thick blood smear for

310
malaria parasites, hemoglobin level, hemoglobin electrophoresis, and glucose-6-phosphate dehydrogenase (G6PD) levels. Individuals with documented *P. falciparum* parasitemia were selected for the study if they did not have a history of allergy to sulfadoxine-pyrimethamine, abnormal hemoglobin electrophoresis, G6PD deficiency, hemoglobin ≤ 8 g/dl, or severe malaria. Severe malaria was defined in accordance with World Health Organization protocols as the presence of asexual *P. falciparum* parasitemia plus one or more of the following: hemoglobin < 5 g/dl, parasitemia ≥ 100,000/mm³, prostration, respiratory distress, bleeding, recent seizures, coma or obtundation, inability to drink, or persistent vomiting. Pregnant women, as documented by history, clinical examination, or positive urine pregnancy test, were also excluded. Because of the variability in malaria parasite transmission in different regions of Mali, individuals who traveled outside of the study area overnight were withdrawn from the study and their post-travel data excluded from the analysis. In all, 114 individuals were enrolled in the study. Five were excluded from the analysis because of noncompliance with follow-up (n = 3) or travel outside of the study area (n = 2). The resulting 109 study individuals consisted of 94 children (ages 7–15 years) and 15 young adults (ages 16–20 years); 67 were male and 42 female. Geometric mean parasitemia in this group was 864 parasites/μl (range 25–82,200) at the start of the study.

**Study design.** Study participants were treated with sulfadoxine-pyrimethamine (1 tablet/20 kg body weight; Fansidar®, F. Hoffmann-La Roche, Basel, Switzerland) on September 24, 1998 and evaluated weekly until December 19, 1998, with clinical and laboratory examinations, including thick blood smears for malaria parasites and hemoglobin levels. Additional clinical and laboratory evaluations were performed for axillary temperature ≥ 37.5°C or symptoms occurring between scheduled visits. Antimalarial treatment was given to asymptomatic participants with ≥ 5,000 parasites/μl blood and to all participants with parasitemia and symptoms or signs of malaria (history of fever/chills, headache, > 1 episode of vomiting, > 3 episodes of diarrhea, any abnormal level of consciousness, seizure, axillary temperature ≥ 37.5°C, evidence of dehydration, icterus, rales/rhonchi, or palpably enlarged spleen [Hackett stage ≥ 1]).

**Entomological methods.** Entomological studies were conducted during the first 12 days of each month during the entire transmission season. Human biting rates were assessed by two different methods: insecticide spray catch and human landing catch. For pyrethrum spray catches, mosquitoes were collected after pyrethrum spraying in 180 randomly selected houses in the village. Human landing catches were conducted from 6 PM to 6 AM, indoors and outdoors of human dwellings at three designated collection sites, and mosquitoes were collected with a mouth aspirator. Mosquito infection rates were calculated from results of mosquito infectivity determined by using an enzyme-linked immunosorbent assay for *P. falciparum* circumsporozoite protein (CSP).

**Data analysis.** Epi-Info (version 6; Centers for Disease Control and Prevention, Atlanta, GA) and SPSS were used for data analyses. The reinfection rate was calculated as the number of persons reinfected at least once with *P. falciparum* divided by the total number of the participants (n = 109). Entomological inoculation rates were obtained by multiplying the man biting rates by the proportion of mosquitoes infected with sporozoite-stage malaria parasites.

**RESULTS**

Monthly EIRs were estimated in the study village over the course of the entire transmission season (June 1 to December 31, 1998) using two different methods of mosquito collection: human landing catch and pyrethrum spray catch. Although the rates obtained using data from human landing catches were somewhat higher than those calculated from spray catches, EIRs calculated using the two methods showed a comparable pattern with peak EIRs measured in October and November (Table 1). Regardless of the method used, the monthly EIR remained low throughout the transmission season, consistent with data from prior years.

Despite these low transmission rates, 80.7% (95% CI 72.1–87.7%) of the study participants became reinfected with *P. falciparum* during the course of the study, as defined by the presence of *P. falciparum* on thick blood smear after documented clearance of parasitemia at 21 days after Fansidar® treatment. The cumulative reinfection rates in the study population as a function of time are summarized in Table 2. As would be expected in the absence of drug resistance, all participants had negative blood smears during the 3 weeks after Fansidar® treatment. Maximal reinfection rates were observed between 7 and 9 weeks posttreatment (November 14–28); 60 of the 88 reinflections occurred during this time period. The mean age in the reinfected group was 11.8 years versus 12.5 years in the nonreinfected group (P = 0.38, Stud-
dent’s t-test), and the reinfeaction rate in the 7- to 15-year age group was 81.9% versus 73.3% in the 16- to 20-year age group ($P = 0.48$, Fisher’s exact test). Thus, there was no correlation between reinfection rate and age, although the age range of the study population was narrow (7–20 years).

Because smears were performed on a weekly basis regardless of symptoms, reinfection was asymptomatic in 66% of cases (58/88). Signs or symptoms were apparent at the time of the first positive blood smear in 20 of 88 individuals (23%), and an additional 10 developed symptoms in the period between first detection of parasitemia by blood smear and their next weekly visit. In those patients in whom clinical manifestations of reinfection developed, fever, headache, and respiratory symptoms were the most frequent (Table 3). Geometric mean parasitemia was significantly greater in symptomatic patients versus asymptomatic patients (geometric mean parasitemia 469 [95% CI 216–1022] vs. 258 [95% CI 212–313] parasites/μl, respectively, $P < 0.05$, Student’s t-test; Figure 1). Furthermore, 5 of the symptomatic patients and none of the asymptomatic patients had parasitemia levels $\geq 5000/\mu l$ ($P = 0.003$, Fisher’s exact test).

**DISCUSSION**

Despite a low daily EIR (0.003–0.01 infected bites/person/night), the *P. falciparum* reinfection rate in Sotuba after curative therapy with Fansidar® was 80.7%. This reinfection rate is similar to that reported by Beier and others (77% [n = 809]) in a study of Kenyan children aged from 6 months to 6 years old living in an area of year-round high transmission rates who were monitored for 12 weeks after Fansidar® treatment (average daily EIR = 0.75 infective bites/person/night)$^{10}$ and to the rate observed in Doneguebougou (75.8%; n = 120), a rural Malian village with seasonal high transmission rates (Sagara I and others, unpublished data). Although recrudescence cannot be absolutely excluded as a cause of recurrent parasitemia, there were no cases of reinfection observed in the first 3 weeks after Fansidar® therapy, and the peak incidence of reinfection was not observed until 7 to 9 weeks posttreatment (November 14–28), a delay consistent with the absence of significant drug resistance to Fansidar® at the time of the study.$^9$

A study of *P. falciparum* prevalence in relation to transmission, as assessed by EIR, demonstrated a correlation between the prevalence of parasitemia and EIR in regions of intense transmission, although some sites with EIRs < 5 infective bites per year had prevalence rates as high as 40%.$^5$ Increased efficiency of transmission in the setting of lower levels of host immunity to malaria has been proposed to explain these findings. This hypothesis is supported by the fact that immigrants to a malaria endemic region in Indonesia had a significantly higher attack rate during the low transmission season ($EIR = 0.018$ infective bites/person/night) than long-term residents of the region.$^10$

Unexpectedly, high rates of severe disease have been reported from areas of low and seasonal transmission.$^2,11$ Although data suggest that clinical immunity against severe disease may be acquired rapidly,$^{12}$ such immunity is likely to wane rapidly in the absence of reexposure to parasite antigens. Symptomatic infections occurred in 44% of reinfected individuals in our study; fever, chills, and headache were the most common symptoms. Severe malaria did not occur in any of the participants, although the study population was small, and individuals were monitored closely and treated promptly for symptomatic infection. As has been reported by others, parasitemia levels were higher in symptomatic patients.$^{13}$

**CONCLUSION**

Despite low EIRs throughout the transmission season, we found a high reinfection rate among children and young adults in Sotuba after curative antimalarial treatment with Fansidar®. These data suggest that a low EIR need not preclude small sample size Phase Ib trials of asexual blood stage malaria vaccine candidates in regions of seasonal malaria transmission. The increased efficiency of malaria infection (infections/bite) observed in Sotuba may reflect a lower level of immunity to malaria in this region.

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**Table 3**

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>No patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>12 (14%)</td>
</tr>
<tr>
<td>Headache</td>
<td>12 (14%)</td>
</tr>
<tr>
<td>Fever</td>
<td>12 (14%)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Anemia (hemoglobin ≤ 8)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>58 (66%)</td>
</tr>
</tbody>
</table>

**FIGURE 1.** Parasitemia levels in symptomatic and asymptomatic patients at reinfection. Closed diamonds represent the *P. falciparum* parasitemia levels for each patient at reinfection. Open diamonds indicate parasitemia levels in individuals who were asymptomatic on the day of reinfection but developed symptoms before their next scheduled clinic visit (< 7 days after reinfection). Horizontal lines indicate geometric means for each group.
REFERENCES


