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RAPID REAPPEARANCE OF *PLASMODIUM FALCIPARUM* AFTER DRUG TREATMENT AMONG SENEGALESE ADULTS EXPOSED TO MODERATE SEASONAL TRANSMISSION

CHEIKH-SADIBOU SOKHNA, FARBA B. K. FAYE, ANDRÉ SPIEGEL, HAMADY DIENG, AND JEAN-FRANÇOIS TRAPE
Laboratoire de Paludologie, Institut de Recherche pour le Développement (IRD, formerly ORSTOM), Dakar, Senegal; Unité d'Epidémiologie, Institut Pasteur, Dakar, Senegal

Abstract. To investigate the relationship between the entomologic inoculation rate (EIR) and time to reappearance of malaria parasites after radical treatment under moderate seasonal transmission conditions, a study was undertaken in a mesoendemic area of Senegal where malaria transmission is concentrated over an annual three-month period and averages 12 infective bites per person per year. A three-day course of quinine was administered to 48 asymptomatic adults between 19 and 66 years of age. Malaria transmission and parasitemia were monitored every week for two months and cases of fever or symptoms were investigated as part of a daily clinical surveillance. The proportion of persons reinfected at Days 28, 35, and 56 was 25%, 38%, and 54%, respectively. Adults less than 40 years of age had a shorter time to reinfection. In this age group, the median *Plasmodium falciparum* reappearance time was 28 days, and it was estimated that only one infected mosquito bite was able to induce a patent infection among half of the subjects. Only 8% (2 of 26) of the reinfections caused a clinical attack. These data are discussed in the light of previous studies conducted among adults naturally exposed to intense perennial transmission or among naive volunteers receiving artificial challenges. Rapid reinfection occurs at very low EIRs and dramatic differences in actual and cumulated exposure to infected mosquito bites poorly affect the median time to reappearance of malaria parasites in endemic populations.

INTRODUCTION

The acquisition of premunition by persons frequently reinfected by *Plasmodium falciparum* enables them to reduce the level of parasitemia during reinfections and to decrease the incidence, duration, and potential severity of clinical attacks. This control of reinfections by semi-immune persons may be first exerted at the pre-erythrocytic level, limiting the proportion of parasite populations inoculated by the vectors that will go beyond the hepatic stage.[1,2] If parasites emerge from the liver, the rapid control of the level and duration of the peaks of parasitemia following the first cycles of erythocytic schizogony in semi-immune persons will reduce the incidence of clinical attacks and enable the spontaneous clearance of symptoms within a few hours.[3,4]

To investigate this control of reinfections, a simple method consists of measuring the time to reappearance of *P. falciparum* in the blood of asymptomatic persons after treatment and analyzing the clinical and parasitologic patterns of the recurrent infections. In a study conducted in a population exposed to intense perennial transmission, we have previously shown that age was a major factor affecting time to reappearance of malaria parasites after treatment and that adults successfully resisted a large number of natural challenges before presenting a new infection.[5] Here we report the results of a study conducted among semi-immune adults exposed to moderate seasonal transmission. In contrast with our previous study, a high proportion of infected mosquito bites were able to induce a patent infection in this cohort of adults. We suggest that for a wide range of transmission levels in endemic areas, low differences exist in the time to reappearance of malaria parasites after treatment.

SUBJECTS, MATERIALS, AND METHODS

Study area. The Niakhar study area is located in the Sahel, 120 km southeast of Dakar, Senegal, in the heart of the Senegalese ground-nut basin. This rural area of 29,000 inhabitants has, for several decades, been a regional observatory for population and health studies.[6,7] Rain is concentrated over a three-month period from July to the beginning of October. Annual rainfall averaged 444 mm from 1984 through 1996. During 1996, the study year, annual rainfall was 545 mm and the last rain occurred on October 16.

Entomologic surveys in this area showed that malaria transmission, almost exclusively by *Anopheles arabiensis*, was strictly seasonal and concentrated in September and October. A survey carried out in 1995, a year of heavy rainfall (614 mm), indicated an entomologic inoculation rate (EIR) of 12 and 9 infective bites per person per year, respectively, in Diohine village (the place of the present study) and in another representative village of the Niakhar area.[8] The parasite rate in children is usually less than 50% most of the year, but can reach 84% at the end of the rainy season, with rates of 82%, 15%, and 0.2% for *P. falciparum*, *P. malariae*, and *P. ovale*, respectively.[9] Malaria mortality is concentrated in children less than 10 years old, with approximately 80% of the malaria deaths among children 0–4 years of age. From 1988 to 1991, the average malaria mortality rate was 4.0 per thousand per year among children between 0 and 9 years of age. The emergence of chloroquine resistance in 1992 was associated with a dramatic increase in malaria mortality, which averaged 8.2 per thousand per year during the period 1992–1995.[7] The proportion of RII and RIII responses has increased from 10% in 1993 to 29% in 1996.[10,11]

Study subjects and protocol. The study was conducted from October 4, 1996 to November 29, 1996 among a cohort of 48 adults volunteers from Diohine village. Criteria for inclusion in the study were living continuously in the village during the whole surveillance period, being in good health, and not taking malaria chemoprophylaxis, and not having left the village until the end of November. The study protocol was carefully explained to the assembled village population and informed consent was individually obtained from

each volunteer. Approval for the study was obtained from the Ministry of Health of Senegal (Direction de la Pharmacie et des médicaments). After a medical examination and measurement of the axillary temperature, a thick blood film was made on Day 0 (October 4) and was immediately followed by a regimen of quinine (Quinimax®; Sanofi, Gentily, France; 25 mg/kg/day divided into three equal oral doses administered every 8 hr over a three-day period by a medical field worker). The study subjects were then monitored weekly for eight weeks for malaria parasitemia and axillary temperature. In addition, each subject was visited every day for clinical surveillance and thick blood films were made in case of fever or other symptoms. Thick blood films were stained with Giemsa. Those collected during fever episodes were immediately examined and treatment was given according to diagnosis to patients who developed fever or other symptoms. Weekly thick blood films were examined at the end of the study. Subjects who remained asymptomatic when parasites reappeared were not treated. A total of 200 microscopic oil-immersion fields was examined on each slide (approximately 0.5 μl of blood). The ratio of trophozoites to leukocytes was established by counting the trophozoites until 200 leukocytes were observed (ratio > 0.01) or from the total number of trophozoites observed on the 200 fields and an estimation of the average number of leukocytes per field (ratio ≤ 0.01). The gametocytes were recorded separately.

Malaria transmission was monitored weekly before and during the study period from September 20, 1996 to November 29, 1996. During this 11-week period, landing mosquitoes were collected one night each week from 7:00 PM to 7:00 AM by four groups of two collectors (two indoors and two outdoors). Each collector alternately worked and rested for 1 hr (4 person-nights per week). After collection, anopheline mosquitoes were separated from other Culicidae. Weekly EIRs were calculated from the observed values of the human-biting rate and a presumed value of 1% for the sporozoite index, an estimate derived from the whole entomologic data from the Diohine and Niakhar areas.[8]

Statistical analysis. The cumulative rate of reappearance of parasites in the peripheral blood rate as a function of time was graphically represented using the Kaplan-Meier method (seven-day interval). Differences in the reappearance rate according to age group and initial parasitemia were tested using a Cox model. Mantel and Cox tests (log rank test) were used to evaluate the relationship between age groups and parasitemia before treatment. The BMDP 7.0 (Statistical Software, Inc., Los Angeles, CA) and SPIDA (Statistical Computing Laboratory, Eastwood, Australia) software packages were used for analysis.

RESULTS

Malaria transmission. Among 44 person-nights of captures over an 11-week period, 224 anophelines were caught, including 216 *An. gambiae* s.l. (i.e., an average of 4.9 bites per person per night during the follow-up period). Vector density has decreased during follow-up. The daily EIR was estimated to be 0.095 from September 20 to October 11 (i.e., one infective bite per person every 10.5 days), 0.035 from October 12 to November 4 (i.e., one infective bite per person every 28.6 days), and 0.014 from November 5 to 29 (i.e., one infective bite per person every 71.4 days).

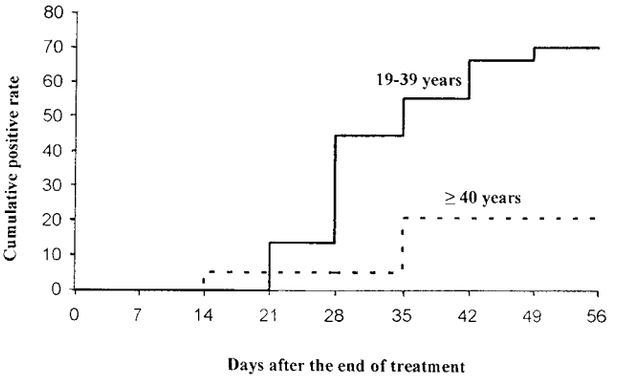

FIGURE 1. Cumulative positive rate of *Plasmodium falciparum* as a function of time according to age groups.

Parasitologic and clinical observations. A total of 48 adults (26 males and 22 females) 19 to 66 years of age (median age = 36 years) received a full course treatment, 47 of which were monitored during the whole eight-week follow-up period (one subject traveled after five weeks of monitoring). Among them, 31 (65%) had a positive thick blood smear at inclusion. *Plasmodium falciparum* was the only species and gametocytes were the only stage observed in eight cases. The trophozoite:leukocyte ratio was generally very low, less than 0.01 (18 cases) or between 0.01 and 0.2 (4 cases), but reached 2.1 in one case who developed a clinical attack a few hours after the first dose of treatment. Subjects with a *P. falciparum* infection before treatment were significantly younger (median age = 31 years) than those who were negative (median age = 40 years; $P = 0.03$ by Mann-Whitney test). All were negative on Day 7. The first reappearance of trophozoites in the blood was detected on Day 14 in a 45-year-old man who had a positive blood smear with a trophozoite:leukocyte ratio of 0.004 on Day 0. The proportion of individuals reinfected at Days 28, 35, and 56 was 25%, 38%, and 54%, respectively. Figure 1 shows that on Day 56, only 21% (4 of 19) of the subjects ≥40 years old were reinfected compared with 76% (22 of 29) of the subjects less than 40 years old ($P < 0.01$). The median time to reappearance of *P. falciparum* after the last day of treatment was 44 days for the whole cohort and 28 days for young adults (<40 years old). The time to reappearance of malaria parasites was significantly longer among subjects with a negative thick blood smear at the beginning of the study than in those who were positive ($P = 0.04$).

We used the stratified log rank test to test the effects of the variables age and parasitemia before treatment. When adjusted for age, the effect of parasitemia before treatment disappeared ($P = 0.21$). However, even after adjustment for parasitemia before treatment, adults ≥40 years old had a longer delay before reappearance of parasitemia than younger adults ($P = 0.002$). Differences in the reappearance rate according to age were tested using a Cox model. When controlled for the effect of prior parasitemia, the weekly risk of reappearance was 5.3 times (95% confidence interval = 1.8–15.4) higher in young adults than in those ≥40 years old ($P = 0.002$).

When parasites reappeared (first positive thick smear and/or subsequent thick smear taken less than eight days later), the trophozoite:leukocyte ratio was greater than 0.01 in 16 cases. It was between 0.2 and 1 in four cases and greater than 1 in two cases who both developed a clinical attack (ratios = 2.6 and 2.1 in two adults 58 and 19 years of age, respectively). These two patients received malaria treatment and were rapidly cured. Two other subjects who presented symptoms after parasite reappearance (headache and abdominal pain or vomiting 5 and 11 days after the first positive weekly thick smear, respectively) were not considered as malaria attacks since their trophozoite:leukocyte ratios were low (maximum = 0.05 and 0.09, respectively). One patient was given symptomatic treatment, and the other was given malaria treatment in addition to symptomatic treatment; in both cases, symptoms disappeared within one day. Six additional subjects had illnesses during follow-up (headache: 4 cases; abdominal pain and diarrhea: 1 case; high fever with a cough: 1 case), but thick smears were negative and illnesses occurred more than eight days before reappearance of parasites. Of the 23 reinfected subjects who did not received malaria treatment, six spontaneously cured their parasitemias and 17 still had malaria parasites at the end of follow-up (gametocytes only in six cases). The trophozoite: leukocyte ratio was less than 0.01 in two cases, between 0.01 and 0.2 in eight cases, and greater than 0.2 in one case.

DISCUSSION

The most interesting observation of this study was the high proportion of young adults who were rapidly reinfected. This occurred despite the high level of protective immunity they were presumed to have after dozens of years of exposure to moderate seasonal transmission since birth, and the relatively low levels of malaria transmission that occurred in Diohine during follow-up. In a human, there are usually 8–14 days between the inoculation of *P. falciparum* sporozoites by a vector and the time of the first detectable parasitemia. The median time to reappearance of *P. falciparum* in adults $<$40 years old was 28 days. This theoretically corresponded to 0.8 consecutive challenges by infected mosquito bites during the period starting at the end of treatment and ending 14 days before the first positive thick smear (i.e., 7–14 days before patency because of the weekly monitoring). Even if quinine was unlikely to kill or remove all pre-existing parasites in the liver (0.9 infected bites per person were theoretically received over the 10 days preceding the end of treatment), or radically cure all patent infections (a three-day regimen in symptomatic Senegalese children was nearly as effective as a seven-day regimen for curing parasitemia,[12] and in the present study, a positive thick blood film at Day 0 had no significant effect on the time to reappearance of malaria parasites when age was taken into account), our data strongly suggest that a low number of infected *Anopheles* bites were sufficient to cause a patent infection in most young adults.

Only 50% of non-immune volunteers bitten by one or two anophelines carrying sporozoites in their salivary glands developed a blood infection.[13] Thus, it is likely that most young adults in this study were no more protected against reinfection than naive individuals, despite the high level of premunition they developed, as shown by the low incidence of clinical attacks they had when *P. falciparum* reappeared (and the absence of malaria deaths after the age of 10 in the area). In contrast, adults from Dielmo village, an area of intense perennial transmission, resisted an average of 20 natural challenges before being reinfected.[5] The median time to reappearance of malaria parasites was 39, 48, and 74 days, respectively, in subjects 7–14, 15–39, and $\geq$40 years of age from this village where the EIR averages 200 infected bites per person per year. This suggests that for a wide range of transmission levels in endemic areas, relatively low differences occur in the time to reappearance of malaria parasites after treatment. It is a consistent view with the general framework of the relationships between transmission, infection, morbidity, and mortality, which has for some years emerged from research conducted in the Congo, Senegal, and east Africa.[14–18] As soon as malaria transmission reaches a few infective bites per person per year, a plateau is reached in the burden of malaria. Unfortunately, the Niakhar area shows high malaria mortality rates among children despite transmission levels much lower than in many other rural parts of tropical Africa.[7]

One of the purposes of this study was to establish the size of cohorts and duration of follow-up that would be required for testing the efficacy of malaria vaccines against pre-erythrocytic and asexual blood stages of *P. falciparum.*[19] The rapid reappearance of parasitemia among young adults both in Dielmo and in Niakhar suggests that small, low-cost trials could be very informative regarding the potential of new vaccine candidates over a wide range of transmission levels in tropical Africa.

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Authors' addresses: Cheikh-Sadibou Sokhna, Farba B. K. Faye, Hamady Dieng, and Jean-François Trape, Laboratoire de Paludologie, Institut de Recherche pour le Développement, BP 1386, Dakar, Senegal. André Spiegel, Unité d'Epidémiologie, Institut Pasteur, BP 220, Dakar, Senegal.

Reprint requests: Cheikh-Sadibou Sokhna, Laboratoire de Paludologie, IRD, BP 1386, Dakar, Senegal.

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