RELATIONSHIPS BETWEEN MALARIA PREVALENCE AND MALARIA-RELATED MORBIDITY IN SCHOOL CHILDREN FROM TWO VILLAGES IN CENTRAL AFRICA

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Abstract. To investigate the relationship between parasite prevalence and malaria-related morbidity, we carried out a comparative study among cohorts of school children from two villages, Dienga, Gabon, and Pouma, Cameroon, both located in malaria-endemic areas. Seven to 17 year-old children attending primary schools were similarly followed-up at each site to evaluate the frequency of malaria attacks. Follow-up involved daily temperature recording (and blood smears in the case of fever) and preparation of blood smears every two weeks. In Pouma, 186 children were followed-up for six months. In Dienga, 228 children were followed-up for nine months. The mean prevalence rate of *Plasmodium falciparum* infections (as assessed by the blood smears) was twice as high in Pouma compared with Dienga (45.2% versus 26.8%; $P < 0.0001$), whereas the monthly malaria attack rate (as assessed by the daily surveillance) was twice as high in Dienga compared with Pouma (21.5% versus 41.4%; $P = 0.003$). The possible implication of several parameters that may differ between the two areas, such as the malaria transmission level, the economical and social status of the inhabitants, the characteristics of infecting parasite strains, and the genetic background of the population, is discussed.

Malaria is a leading cause of mortality and morbidity among young children in sub-Saharan Africa. Infants are reported to have only mild symptoms of the disease.[1] Death from malaria mainly occurs among young children,[2] and a marked decrease of severe malaria is observed after five years of age.[3] Similarly, malaria-related morbidity is reported to gradually decrease later in life,[4] probably as a consequence of the gradual development of protection related to immunologic mechanisms following repeated *Plasmodium falciparum* infections.[5] Few studies have investigated the relationship between parasite prevalence and malaria-related morbidity in children from malaria-endemic areas.[3,6] Moreover, different case definitions of malaria attacks have been used and it is difficult to compare the results between studies.[3,6,7] Therefore, we carried out a comparative study among well-defined cohorts of school children in two villages of Gabon and Cameroon, in whom parameters of malaria infection were recorded longitudinally to optimally assess the occurrence of malaria-related morbidity.

SUBJECTS AND METHODS

Study areas. Dienga, a village of 1,200 inhabitants, is located in southeastern Gabon, near the Congo border, approximately 100 km (3-hr drive) from Franceville. This equatorial African forested zone is a mesoendemic to hyperendemic region for *P. falciparum* malaria where parasite transmission is perennial with seasonal increases during the rainy season (from March to June and from September to December). *Anopheles gambiae s.l.* and *An. hancocki* are the mosquito species involved in *P. falciparum* transmission, with a mean 100 infective mosquito bites per human per year, as assessed by an entomologic survey in 1995–1996 (Elissa N, unpublished data). The village of Pouma is located in central Cameroon, 150 km west of Yaounde and 100 km east of Douala. This forested zone is a mesoendemic area for *P. falciparum* malaria. The equatorial climate allows pe-

rennial transmission of *P. falciparum* with seasonal increases during the rainy season from September to December and from April to June.

Field methods. In each area, all children of the primary schools of the two villages were followed-up longitudinally in a similar manner to evaluate the frequency of malaria attacks. In Pouma, children were followed-up from April to December 1995. In Dienga, children were followed up from February 1995 to March 1996. Each school day morning, the axillary temperature of each child was recorded at the school. Children who were absent from school were visited at home. On Saturdays and Sundays, a passive case detection was carried out at the field base of each village. During the summer school vacations (three months in Cameroon and four months in Gabon), the follow-up was interrupted, since many children were traveling outside the study area. Thus, the effective duration of follow-up was six months in Pouma and nine months in Dienga. In all cases of fever (axillary temperature $> 37.5°C$), a clinical examination was performed and a thick blood smear was made and examined for malaria parasites. In both areas, a *P. falciparum* malaria attack was defined using identical criteria: presence of fever and a *P. falciparum* parasitemia $> 5,000$ parasites/μl of blood and no apparent other cause of fever. In addition, regardless of the clinical status, thick blood smears were obtained every two weeks from each child (10 times in Cameroon and 16 times in Gabon) to assess the presence of asymptomatic malaria infection. Venous blood was collected during the follow-up for hematologic and anti-malarial antibody measurements. Urine was collected twice (once during the first month of the study, and once during the month following summer school vacation) to determine the presence of quinolines derivatives by the Saker-Solomons test.[8]

Antipyretics were given to all febrile children. In addition, children presenting with a malaria attack were given an antimalarial curative treatment. Children in Cameroon were treated with amodiaquine (25 mg/kg over a three-day peri-
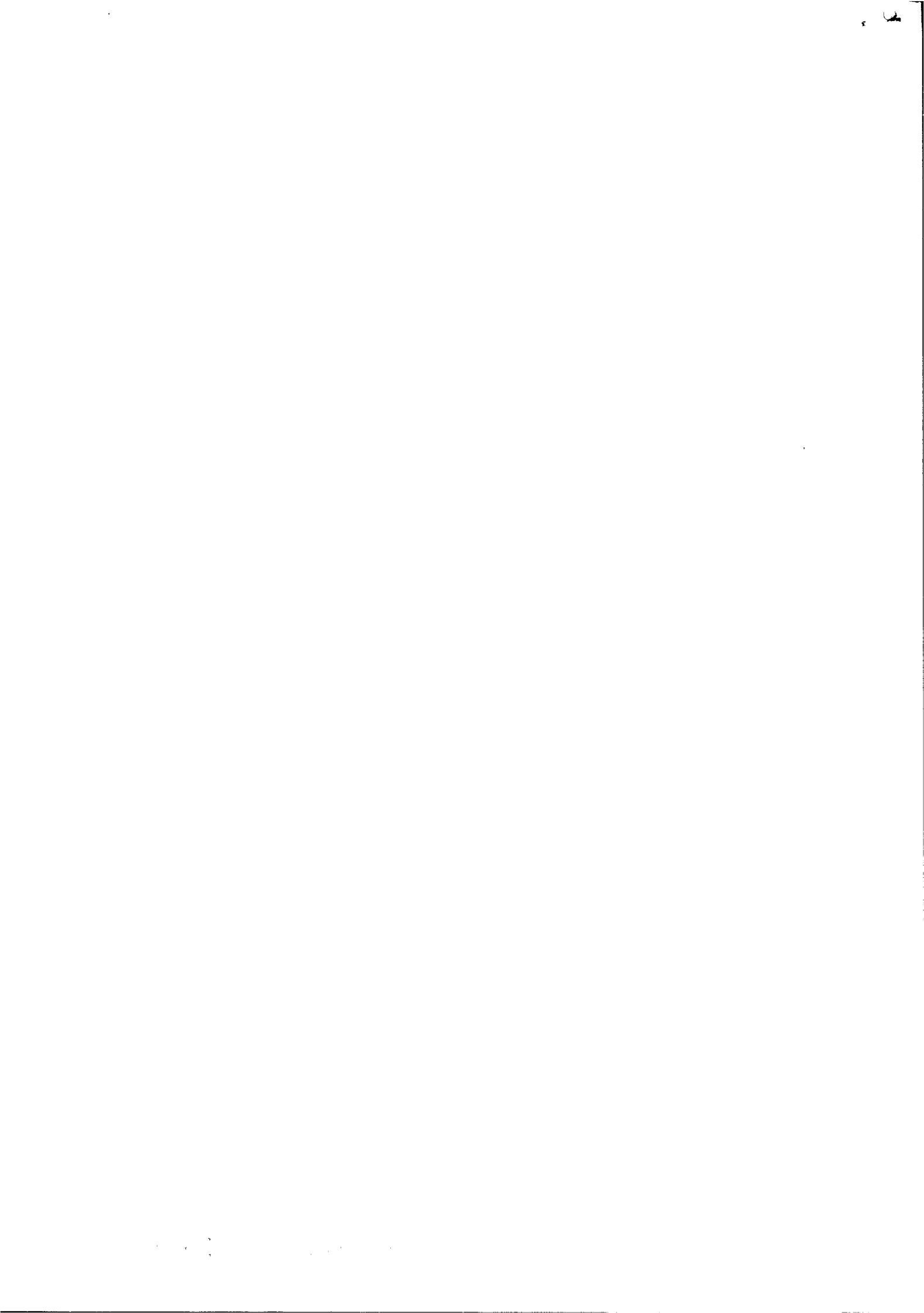
		Pouma	Dienga
Number		186	228
Sex ratio (M/F)		1.16	1.33
Mean ± SD age (years)		9.50 ± 2.65	10.84 ± 2.46
Ethnic group	Bassa	163 (87.6%)	0
	Nzebi	0	223 (97.8%)
	Other	23 (12.4%)	5 (2.2%)
Sickle cell trait carriers		30 (20.7%)	49 (23.1%)
Blood group	O	67 (46.2%)	124 (54.3%)
	A	42 (30.0%)	62 (27.2%)
	B	29 (20.0%)	40 (17.4%)
	AB	7 (4.8%)	2 (1.1%)

od). Children in Gabon were treated with quinine (8 mg base/kg given as Quinimax® tablets; Elf Sanofi Winthrop, Paris, France) and clindamycin (8 mg/kg) given twice a day for three days.[9] The study was approved at each site by the local ethic committee, the Ministry of Public Health, the Governor of the Province, and the local Prefet. Approval was also obtained from the director of the two schools, as well as from the parents of each child for the enrollment and the follow-up of the children.

Laboratory testing. All thick blood smears were stained with Giemsa and examined against 200 leukocytes if positive or against 400 leukocytes prior to being declared negative. Parasite densities were recorded as the number of parasites per microliter of blood, assuming an average leukocyte count of 8,000/μl; thus, the threshold of sensitivity was 20 parasites/μl. A routine quality control ensured the re-examination of a random sample of blood smears, without being aware of the previously recorded result.

Specific antibodies to *P. falciparum* were measured by an ELISA using a lysate from *in vitro*-cultured parasites (Palo Alto strain) as described.[10] Positive and negative control serum pools were included in each plate, and results were expressed in arbitrary units calculated from the formula 100 ×

Ln (absorbance of the test serum) − Ln (absorbance of the negative pool)/Ln (absorbance of the positive pool) − Ln (absorbance of the negative pool).

Data processing and analysis. Differences in proportions were analyzed using the chi-square test. Parasite densities and confidence intervals were calculated using geometric means and compared by the Student's *t*-test using Statview 4.5 (Abacus Concepts, Berkeley, CA). A stepwise logistic regression using the maximum likelihood ratio method (BMDP LR; University of California, Los Angeles, CA) was performed in the multivariate analysis to examine the association between blood smear results and demographic characteristics (ethnic group, living area). The stepwise procedure was used as the independent variables were initially biologically interrelated. The level of significance was a P value < 0.05 for the entire analysis.

RESULTS

In Pouma, 186 children (100 boys and 86 girls) were followed-up. They had a mean ± SD age of 9.5 ± 2.65 years (Table 1). A total of 20.7% had the sickle cell trait and 94.3% had plasma antibody to *P. falciparum* schizont extract. During the follow-up, thick blood smears were obtained 10 times every two weeks for the detection of asymptomatic infections. A total of 1,578 blood smears were collected, giving a mean of 8.5 blood smears per child. At least seven blood smears were obtained from 145 (78.0%) children. Overall, 717 (45.2%) blood smears were positive for *P. falciparum* (Table 2); however, the prevalence rate varied from a low of 28% in October–November to a high of 67% in May. All but six children (97%) had at least one positive blood smear, and at least half of the blood smears obtained were positive in 101 (55.5%) children. The frequency of asymptomatic infection was related to blood group (P = 0.02), being highest (47%) in children with group O and group A, intermediate (39%) in children with group B, and lowest (27%) in children with group AB.

Twenty children presented with at least one clinical ma-

TABLE 2

Malaria-related follow up data of both passive and active case detection in schoolchildren from Pouma (Cameroon) and Dienga (Gabon)

	Pouma	Dienga	P*
Number	186	228	ND
Follow-up duration (months)	6	9	ND
Prophylaxis (%)†			
April	19.3	21.3	0.7
September	9.6	5.6	0.2
Mean ± SD *Plasmodium falciparum* antibody titer (AU)	67.7 ± 24.5	73.7 ± 18.3	0.007
Cross-sectional surveys			
Number of surveys	10	16	ND
Mean parasite prevalence (%)	45.2	26.8	<0.0001
MGPD (95% CI) (/μl)‡	718 (649–795)	206 (179–237)	<0.0001
Active case detection			
Children with malaria attack	21	61	ND
Malaria attacks	24	85	ND
Monthly malaria attack rate (% per one thousand)	21.5	41.4	0.003

* ND = not done.
† Assessed by the Saker-Solomons test in urine.
‡ MGPD = mean geometric parasite density of positive slides; CI = confidence interval.

laria attack (four of them presented with two attacks), giving a monthly malaria attack rate of 21.5% per one thousand. Peripheral blood parasitemia always remained symptomless in all other children. Malaria attacks were observed in each month of the follow-up. The occurrence of a malaria attack was higher in young children ($P = 0.05$), in girls ($P = 0.02$), and in children whose father ($P = 0.007$) or mother ($P = 0.002$) were civil servants. After logistic regression, only age remained related independently to the occurrence of a malaria attack ($P = 0.04$).

In Dienga, 228 children (130 boys and 98 girls) were followed-up. They had a mean ± SD age of 10.8 ± 2.46 years (Table 1). The mean age of the children was one year older in Dienga than in Pouma ($P = 0.001$) although they were attending similar school classes. This is probably an indirect consequence of the higher overall rate of schooling in Gabon. Among the children in Dienga, 23.1% had the sickle cell trait and 98.7% had plasma antibody to *P. falciparum* schizont extract. During the follow-up, thick blood smears were obtained 16 times every two weeks for the detection of asymptomatic infections. A total of 3,014 blood smears were obtained, giving a mean of 12.8 blood smears per child. At least seven blood smears were performed in 208 (91.2%) of the children. Overall, 809 (26.8%) blood smears were positive for *P. falciparum* (Table 2); however, the prevalence rate varied from a low of 3.1% in May to a high of 43.0% in December. Similarly, the mean geometric parasite density of positive blood smears was much higher ($P < 0.0001$) in Pouma than in Dienga. All but 21 children (90.8%) had at least one positive blood smear, and at least half of the blood smears obtained were positive in 28 (12.3%) children. The frequency of asymptomatic infection was not related to sex, age, blood group, or hemoglobin phenotype.

Sixty-one children presented with at least one clinical malaria attack (among them 13, 2, 1, and 1 presented with 2, 3, 4, and 5 attacks, respectively). The monthly malaria attack rate was 41.4% per one thousand, almost twice the value observed in Pouma ($P = 0.003$). Malaria attacks were not clustered and were observed in each month of the follow-up. However, the monthly attack rate varied during the year from 50–65% per one thousand in February–March of both years (beginning of the rainy season) to 20% per one thousand in December, and ranged between 30%–40% per one thousand during the rest of the year. The occurrence of a malaria attack was related to age, being higher in young children, but the difference was not significant ($P = 0.07$). No other factor was related to the occurrence of a malaria attack.

DISCUSSION

We followed two cohorts of children from two villages in Cameroon and Gabon. Enrollment criteria for the follow-up procedures were similar, except that the follow-up lasted six months in Pouma (Cameroon) and nine months in Dienga (Gabon). The follow-up procedures were likely to identify a great proportion of malaria attacks occurring during the follow-up period. Indeed, children were seen every morning at school for a temperature recording and a thick blood smear was obtained from all those who were febrile. Since acute disease is a major cause of school absenteeism, absent chil-

dren were visited at home. Our intervention and even our mere presence precluded studies of mortality or severe morbidity, and may have resulted in underestimating the extent of malarial disease in the overall population. However, our objectives were to compare malaria clinical and parasitologic parameters in groups of children of same age subjected to similar follow-up but who were living in different areas.

The prevalence rate of *P. falciparum* infection, as well as the attack rate, varies with the area. However, the prevalence rate of *P. falciparum* infection was twice as high in Pouma than in Dienga, whereas the malaria attack rate was twice as high in Dienga. This is even more surprising since the children from Dienga, who were older than those from Pouma, would be expected to have a higher level of immunity. However, the mean age difference was only one year, and is probably insufficient to be responsible *per se* for a large difference in the protection level. In a malaria holoendemic village in Senegal, the monthly malaria attack rate was 52.6% in children of similar ages (7–14 years) during the maximum malaria transmission season, while the mean parasite prevalence was 85%.[7]

Our two study areas may differ by several parameters, including malaria transmission level, economic and social features of the inhabitants, virulence of the infecting parasite strains, and genetic background of the population. All of these factors may affect the occurrence of both malaria parasitemia and malaria clinical attack.[11] Entomologic surveys conducted in Dienga at the same time showed that each individual received a mean of 100 infective mosquito bites per year. We did not conduct such a study in Pouma. However, the levels of antibody to schizont extract were in the same range in Pouma and Dienga (the small difference in titers was likely related to the difference in age), suggesting that individuals had had similar previous exposures,[5] and that transmission is not different between the two areas.

In both areas, chemoprophylaxis does not appear to have resulted in any reduction in the parasite prevalence rate. In addition, prophylactic drug use, as assessed by the Saker-Solomons test, was similarly infrequent in Cameroon and Gabon (Table 2), and was further reduced a few months after initiation of the study, probably as a consequence of the presence of the team. Although bed nets are rarely used in the two villages, the impact of additional methods, such as burning coils or insecticide sprays, is difficult to assess.

Since the genetic diversity of *P. falciparum* varies according to geographic location,[12] *P. falciparum* parasites circulating in Dienga and Pouma may differ in several phenotypic characteristics, including both virulence[4,13] and antigenicity.[14] Accordingly, strains from Dienga should be more virulent and induce malaria attacks more often than those from Pouma. Indeed, *P. falciparum* genetic diversity (with regard to the merozoite surface protein-1 [MSP-1] and MSP-2 genes) was as high in Dienga as in Pouma, but several alleles were observed at one site but not at the other (Ntoumi F, unpublished data). Similarly, the strains may differ in the nature or the repertoire of the antigens expressed, and the immune response elicited in the population may differ in intensity and/or specificity. Alternatively, the genetic background of the human populations (belonging to different ethnic groups) may also differ, and has been shown to being responsible for variation in susceptibility to infection[15] or to

certain parasite strains.[16,17] This has been clearly shown for the sickle cell trait[15] and for defined HLA types,[18] but may also involve additional genetic characteristics of the host.[19]

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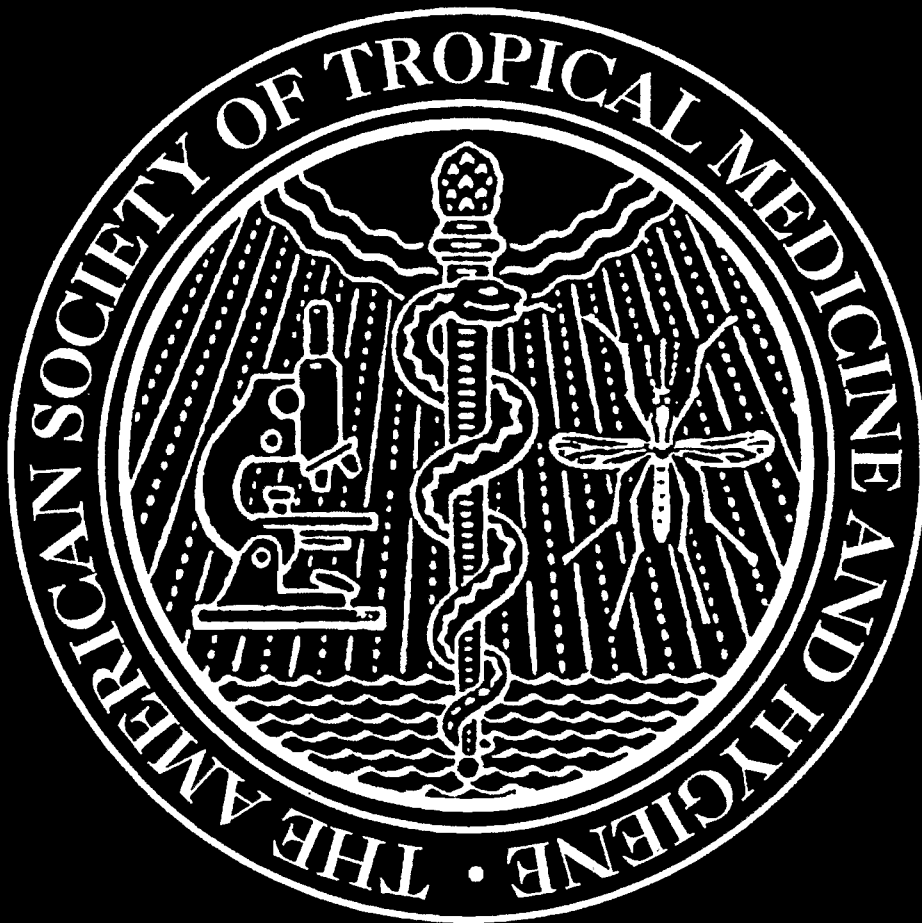
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