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HIGH HUMAN MALARIAL INFECTIVITY TO LABORATORY-BRED *ANOPHELES GAMBIAE* IN A VILLAGE IN BURKINA FASO

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Abstract. The malarial infectivity of an African village population was tested by selecting a demographically representative sample of individuals for study, regardless of parasitemia or gametocytemia. The infectivity of this population people to laboratory-bred mosquitoes was investigated using membrane feeding techniques. Tests on 322 subjects (greater than four years of age) indicated that approximately 48.4% were capable of infecting mosquitoes. There were similar proportions of infectious individuals among gametocyte carriers (52.5%) and nongametocyte carriers (46.6%). All age groups appeared to contribute equally to this infective reservoir. Most of the infections resulted in low oocyst loads (1.8 oocysts) on the midgut of the positive mosquitoes and only a few mosquitoes per batch were infected (11.5%). A previous entomologic survey estimated 90 infected bites/person/year and a low parity index in *Anopheles gambiae* (< 60%) as well as in *An. funestus* (< 40%), the two main malaria vectors in this region. This low parity index could indicate a low life expectancy for infected mosquitoes and could therefore explain an inoculation rate lower than expected considering the high degree of infectivity of the human population studied.

An estimation of the infectiousness of *Plasmodium falciparum* gametocyte carriers to mosquitoes is of both theoretical and practical interest for understanding the epidemiology of malaria and its changes after application of control measures. There are many ways to estimate this infectiousness. A direct approach is to feed batches of mosquitoes on a demographically representative human population, regardless of parasitemia or gametocytemia, either by membrane feeding,[1,2] or by feeding laboratory-bred mosquitoes directly on the skin of individuals.[3] Another approach is to determine the mosquito infection probability.[4] A third is to measure the age-specific sporozoite rates in anopheline vectors.[5]

In this report, we have used the first approach, which has only been applied one other time in Africa, 30 years ago.[3] This allowed us to estimate the proportion of infectious individuals in an African village population near Bobo-Dioulasso in southwestern Burkina Faso.

MATERIALS AND METHODS

Study area

This study was conducted in Barre, a savanna village near Bobo-Dioulasso, Burkina Faso.

Malaria information

Anopheles gambiae s.l. is the most important malaria vector in this area, which consists almost entirely of *An. gambiae* s.s. with a few *An. arabiensis*.[6] *Anopheles funestus* serves as an important secondary vector species. Transmission is probably maintained at a low level during much of the year, with a recrudescence during the rainy season (June–October) and at the beginning of the dry season (November–December). The level of transmission is between 25 and 350 infected bites/person/year.[7] *Plasmodium falciparum* is the dominant parasite species, followed, to a lesser extent, by *P. malariae.*

Patients

The human subjects whose malarial infectivity to mosquitoes was tested consisted of 107 families (15% of the population) selected from those volunteering to participate after being informed of the nature of the study.

Since the size of the mosquito batch is an important factor in determining the accuracy of this type of survey, only batches with more than 14 surviving mosquitoes were dissected. In these conditions, the mean ± SD number of surviving mosquitoes per batch was 33 ± 8.

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Three hundred twenty two subjects (age range 5–67 years) were tested. No feedings were made on children less than five years of age. The age distribution of those studied was the same as the overall population of the village ($\chi^2 = 6.98$, degrees of freedom [df] = 4, $P > 0.05$). The human population sample was divided into three main groups: group I, 120 children (age range 5–14 years); group II, 113 adolescents and young adults (age range 15–29 years); and group III, 89 adults (> 29 years old).

Experimental infection

The infectivity of individuals to laboratory-bred mosquitoes was studied by membrane feeding. The laboratory-reared mosquitoes used were from a colony of *An. gambiae* s.s. established from wild-caught mosquitoes and maintained for several years.

Every morning, four individuals were brought to the laboratory, where a venal puncture was performed and two blood samples (2 × 7 ml) were drawn into either heparinized or dry tubes. The heparinized blood was immediately presented to approximately 200 *An. gambiae* that had been maintained without sucrose solution for 12 hr through a water-jacketed (37°C) glass feeder using a Parafilm® (American Can Co, Greenwich, CT) membrane. Thick and thin blood smears were immediately prepared, and after coagulation and centrifugation (4,000 × *g* for 10 min) of the blood, the serum was removed, aliquoted, and frozen at −20°C for other studies.

In the afternoon, unfed mosquitoes were removed and engorged females were kept in an insectary at 26–28°C and a relative humidity of 70–80% for nine days with a continuous supply of 10% sucrose solution and a rabbit blood meal on the fifth day. The midguts of surviving mosquitoes were then dissected on the ninth day and immediately examined without staining at 400× for oocysts.

To test the reliability of the examiners (two experienced technicians), examination of a positive batch control (mosquitoes fed on a blood sample from a high gametocyte carrier) was performed at the beginning of the study. Six negative batches (mosquitoes fed on calf blood samples) were examined as double-blind controls at a different time during the study.

A total of 336 mosquitoes survived from the control groups fed on calf blood. All were declared negative in the double-blind control activity. Therefore, the specificity and reliability of the examination for oocysts was excellent.

Since the percentage of infected mosquitoes theoretically depended on gametocyte density, we have estimated the infectivity of one gametocyte (PI). PI = % of infected mosquitoes/number of gametocyte/mm³.

With the proportion of each age group (A) in the human population (census 1985) and the percentage of infectious individuals (B), it was possible to evaluate the contribution of each age group to the human reservoir of infection by multiplying A by B.

Parasite counts

The thin and thick blood films were dried for at least 18 hr, stained with Giemsa, and examined at 500×. The density of asexual forms was estimated after reading 50 fields of the thin smear (800 red blood cells/field = 0.01 mm³). The density of the sexual forms was estimated after reading 75 fields of the thick blood film (35 white blood cells/field = 0.33 mm³). The detection thresholds were 100 infected red blood cells or three gametocytes/mm³, respectively.

Statistical analysis

The distribution of the quantitative variables (parasite, gametocyte, and oocyte densities) did not fit well with a normal distribution. The differences between means were tested with nonparametric tests (Kruskal-Wallis [means > 2] or Kolmogorov-Smirnov [means = 2]). To relate a dependent quantitative variable to an independent one, we used the Spearman test (rank correlation coefficient).[8]

RESULTS

Infectivity

The proportions of infectious individuals in each age group are shown in Figure 1. Of 322 individuals tested, 156 were found to be infective (48.4%). Approximately the same proportion was observed in the three age groups ($\chi^2 = 4.18$, df = 2, $P > 0.05$). Paradoxically, there were approximately the same proportion of infectious individuals among the gametocyte carriers

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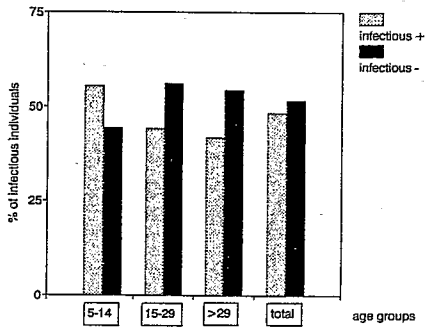

FIGURE 1. Distribution, according to age, of infectious or noninfectious individuals after experimental infection with laboratory-bred *Anopheles gambiae*. No significant difference was observed between the age groups among the infectious individuals ($\chi^2 = 4.18$, degrees of freedom [df] = 2, $P > 0.05$) or the noninfectious individuals ($\chi^2 = 4.03$, df = 2, $P > 0.05$).

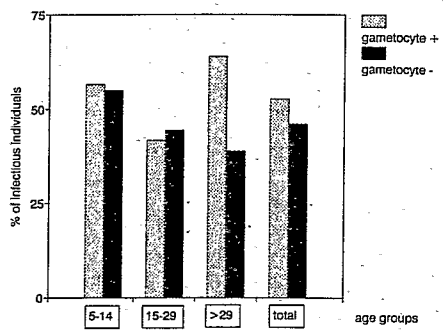

FIGURE 2. Distribution, according to age, of infectious gametocyte or nongametocyte carriers after experimental infection with laboratory-bred *Anopheles gambiae*. No significant difference was observed between age groups among the gametocyte carriers ($\chi^2 = 4.15$, degrees of freedom [df] = 2, $P > 0.05$) or the nongametocyte carriers ($\chi^2 = 2.84$, df = 2, $P > 0.05$).

(52.5%) as among what were apparently nongametocyte carriers (46.6%) (Figure 2).

There was a positive correlation between gametocyte density and the percentage of infected mosquitoes (Spearman's rank correlation coefficient $r_s = 0.11$, df = 320, $P < 0.05$). The mean infectiousness per gametocyte and per age group is shown in Table 1. There was no difference among the three age groups.

We have classified subjects as having a high infectiousness (PI + 2 SD), a low infectiousness (PI − 2 SD = 0), or a normal infectiousness 2 SD < PI < + 2 SD). There was no useful discriminating parasitologic variable for predicting into which group (high, low, or normal infectiousness) a case was most likely to be included. In particular, there was no relationship between the parasite density of asexual forms and the subject's infectiousness to mosquitoes.

Mean parasite densities

Sixty-three percent of the individuals were infected with asexual forms of *P. falciparum* and 17% with *P. malariae*. As expected, the prevalence decreased with age (77.5%, 61%, and 46% in 5–14-, 15–29-, and > 29-year age groups, respectively). The mean densities of asexual forms also decreased significantly with age. In addition, there was a negative correlation between *P. falciparum* densities and the age of the individuals ($r_s = -0.36$, df = 320, $P < 0.001$). A positive correlation was observed between parasite densities and gametocyte loads ($r_s = 0.26$, df = 320, $P < 0.001$).

The mean prevalence of the gametocyte carriers in this village was 52.5%. It did not decrease with age, despite a lower parasitemia in adults than in children (Table 2). The prevalence of gametocyte carriers was not significantly different in infectious or noninfectious subjects (Table 2). The mean gametocyte densities were slightly higher in infective (2.6 gametocytes/mm³) than in noninfective individuals (1.8 gametocytes/mm³), but the difference was not significant (Kolmogorov-Smirnov coefficient = 0.52, $P > 0.05$). There was a negative correlation between the *P. falciparum* gametocyte densities and the corresponding ages of individuals ($r_s = -0.15$, df = 320, $P < 0.01$). The mean ratio of female:male gametocytes was 4.8:1 (range 0–19:1).

Mean percentage of infected mosquitoes

The mean percentage of infected mosquitoes was 11.5% (Table 3). There was no significant difference among the three age groups (Kruskal-Wallis coefficient = 3.63, df = 2, $P > 0.05$), nor did the mean oocyst densities vary significantly with the age of the donor (Table 3). There were only 1.89 oocysts per infected mosquito (range 1–56). There was no correlation between the gametocyte densities and the corresponding oocyst loads in mosquitoes ($r_s = 0.04$, df = 320, $P > 0.05$).

Human reservoir infectious to mosquitoes

The different age groups contributed almost equally to the total number of infectious individuals in the population (Table 4). For each age group, this contribution represented approximately 13%. We could estimate that approximately 40 ± 8.4% of the population (greater than four years of age) was infectious to mosquitoes.

DISCUSSION

Almost half (48.4%) of the individuals in all age groups (5–70 years of age) in the savanna village studied were infectious to mosquitoes. Muirhead-Thomson found only 9.2% infectious individuals among a representative sample of the population (including children with an age range of 0-4 years) in a Liberian village.[3] Another study on malarial infectivity of human populations to mosquitoes was conducted in the Madang area of Papua New Guinea by Graves and others.[4] These investigators observed that only 4% of the individuals studied were infectious to mosquitoes.

This discrepancy might be explained by several factors. First, infections in mosquitoes fed through membranes usually equal or exceed infections in mosquitoes fed directly on the animal.[9] In the study of Muirhead-Thomson, mosquitoes were fed directly on the skin of individuals,[3] while in our study, they were fed under experimental conditions through a water-jack-

TABLE 1

Mean infectiousness of one gametocyte, according to subject age, after experimental infection on laboratory-bred Anopheles gambiae*

	Subject age (years)		
	5–14	15–29	>29
No. of infectious individuals	120	113	89
mPI	0.040	0.047	0.056
SD	0.049	0.068	0.082
Minimum PI	0	0	0
Maximum PI	0.264	0.333	0.411
PI + 2 SD	6	6	4
PI − 2 SD	13	18	2

* mPI = mean infectiousness of one gametocyte; PI = % of infected mosquitoes/gametocyte density. All the infectious nongametocyte carriers were considered as positive with at least two gametocytes/mm³ (one male + one female). PI + 2 SD = no. of individuals with a high (PI > mPI + 2 SD) infectiousness to mosquitoes; PI − 2 SD = no. of individuals with a low (PI = 0) infectiousness to mosquitoes. No significant difference was found among age groups (Kruskal-Wallis coefficient = 4, degrees of freedom = 2, $P > 0.05$).

TABLE 2

Prevalences (%) and densities of the asexual (infected individuals) or sexual (infectious individuals) forms of Plasmodium falciparum *in population of a savanna African village**

		Subject age (years)		
		5–14	15–29	>29
All individuals	Number	120	113	89
	Density	153	29	10
	Confidence interval	114–204	22–38	8–14
Infected individuals	Number	93	69	41
	Density	660	254	157
	Confidence interval	544–796	212–299	126–192
Infectious individuals	Number	67	50	39
	Density	3	2.4	2.2
	Range	0–108	0–141	0–42
Noninfectious individuals	Number	53	63	50
	Density	2.1	2	1.2
	Range	0–45	0–24	0–12

* Densities of asexual or sexual forms are expressed as logarithms. Confidence interval = mean (log) ± 2 SD.

TABLE 3

Mean prevalences and mean oocyst densities in infected mosquitoes, according to subject age groups, after experimental infection on laboratory-bred Anopheles gambiae

	Subject age (years)			
	5–14	15–29	>29	Total
No. of infectious individuals	67	50	39	156
Mean % of infected mosquitoes	11.4	13.4	9.9	11.5
Range	1.5–56.2	2.5–85.7	3–41.1	1.5–85.7
Mean oocyst density	1.76	1.70	2.11	1.89
Range	1–38	1–31	1–56	1–56

eted glass feeder using an artificial membrane. Second, the size of the mosquito batch is an important factor in determining the accuracy of this type of survey. We used larger batches of mosquitoes (mean number = 33) than Muirhead-Thomson (mean number = 19.4), and this larger number probably allowed us to detect a larger number of positive mosquitoes. In the study of Graves and others, the size of the mosquito batches was also lower (1,610 dissected mosquitoes in 201 batches = eight mosquitoes/batch).[4]

Recently, Lines and others[5] have estimated human malaria infectiousness in a Tanzanian village by measuring the age-specific sporozoite rates in anopheline vectors. They have estimated that 21% of the blood meals absorbed by mosquitoes in natural conditions were infectious. For comparison, the same model, applied to the data of Gillies and Wilkes[10] in the same village 25 years earlier, estimated that only approximately 8% of the mosquitoes were infected in each gonotropic cycle. The investigators concluded that during the 1960s, human infectiousness to mosquitoes was probably partially suppressed by the widespread use of chloroquine. In contrast, the recent development of drug resistance during the 1980s could explain the higher parasitemias and gametocytemias observed in local populations and, therefore, the increase of infectiousness to mosquitoes. Similarly, Ichimory and others showed that chloroquine might have a selective effect on the parasite population, enhancing the production and/or infectivity of gametocytes of *P. yoelii*.[11] However, in the Bobo-Dioulasso area, drug resistance, which is still rare in this region (Guigemde TR, unpublished data), could not be the likely explanation.

Muirhead-Thomson estimated that all age groups contributed about equally to the infective reservoir found in this study.[3] The adult group (more than 15 years of age) formed a substantial proportion (30%) of the total reservoir, despite having a lower gametocyte rate than in children. In our study, we observed the same tendencies but to a greater degree.

In Liberia, among mosquitoes feeding on infectious individuals, the percentage that became infected and the mean density of oocysts per infected mosquito were almost identical for all age groups (approximately 20% and two or three oocysts per midgut, respectively).[1] We observed a mean percentage of 11.5% infected mosquitoes per age group, which did not vary with age. Similarly, the mean oocyst density was approximately the same in the three age groups (1.8/midgut).

TABLE 4

*Infectiousness of different subject age groups to mosquitoes in a community exposed to stable malaria transmission in West Africa and contribution of each age group to the human reservoir of infection**

Age group (years)	Percentage of the human population represented by each age group (A)	Percentage of individuals infectious to mosquitoes (B)	Percentage of mosquitoes infected after feeding on infectious individuals	Contribution of each age group to human reservoir of infection (A × B)
0–4	16.6	ND	ND	ND
5–14	28.5 ± 0.2	55.8 ± 9.0	11.4	15.9 ± 2.7
15–29	25.6 ± 0.2	44.2 ± 9.3	13.4	11.3 ± 2.5
>29	29.3 ± 0.2	43.8 ± 10.5	9.9	12.8 ± 3.2
Total				Sum = 40 ± 8.4 (total human reservoir of infection)

* Values, where appropriate, are the mean ± SD. ND = not determined.

In our study, 52.5% of the population sample were gametocyte carriers, with a detection threshold of three gametocytes/mm³. The high gametocyte rate, associated with a high infectiousness, should be responsible for a high transmission level. An entomologic study, conducted five years prior to our study (unpublished data), allowed us to estimate the mean inoculation rate as 90 infected bites/person/year, which was a medium-level inoculation rate for this area.[7] This observation was surprising when compared with the high infectiousness of individuals. However, the proportion of gravid females was low compared with those in other villages in the surrounding savanna (mean parity index < 60% for *An. gambiae* and < 40% for *A. funestus*). From these proportions, the probability of survival through one day (p) could be estimated (p = the square root of the parity index), and from that, the mean expectation of life of local *Anopheles* could be derived (probability of survival after 12–15 days of the sporogonic cycle). Therefore, these low parity indices observed in this village could explain a low mean life expectancy for both mosquito species that are primarily responsible for transmission. Under natural conditions, it is probable that only a few *Anopheles* survived long enough to become infective to humans. This could explain a relatively low transmission level, despite the high level of infectiousness in the local population.

To define some discriminating factors that allowed the grouping of individuals with high and low infectiousness, we selected 16 subjects with a high infectiousness (mean PI + 2 SD) and 33 with a low infectiousness (mean PI − 2 SD). Unfortunately, no discriminating parasitologic variable was determined for predicting into which group (low or high infectiousness) a new case was most likely to be included.

There has been a strong indication in avian,[12] rodent,[13] and simian[1] models that the period of high asexual parasitemia is associated with reduced infectivity of the gametocytes to mosquitoes. In our study, there was no negative correlation between the densities of asexual forms and the infectiousness per gametocyte. In addition, the mean parasite loads had the same level in the high infective group as in the low infective group. Therefore, we could not verify the experimental results and we could not explain the low or high levels of infectiousness by the level of asexual parasite loads.

In experimental conditions, it has been demonstrated that high levels of antibodies to gamete-specific antigens[14, 15] and probably some cytokines[16] could reduce the infectivity of gametocytes to mosquitoes. It has been recently shown that specific transmission-blocking antibodies could be present in natural conditions, primarily in adults.[17] However, immune recognition of the gamete surface antigens is rare and generally weak.[18] Since the development of a transmission-blocking immunity takes many years to develop, we expected that only adults could present a reduced infectiousness to mosquitoes. Unfortunately, the proportions of low infectious individuals were the same in children (< 15 years old) or adults (> 15 years old). In these conditions, the possible role of a transmission-blocking immunity on the reduction of infectiousness might not be important, but supplementary studies are necessary to verify this hypothesis.

Some investigators have demonstrated that the suppressive effect of some inhibiting sera on infectiousness to mosquitoes was correlated with gamete antibody titers.[19–21] The level of gamete antibodies could be a potentially discriminating variable for predicting low or high infectiousness in local populations. The search for inhibitory or promoting factors in sera is being investigated. These factors may be able to explain the discrepancy between gametocyte density and infectiousness.

In conclusion, a high proportion of individuals infectious to mosquitoes has been observed in a savanna village in West Africa. This high level of infectiousness could be explained by several factors that are under investigation. Although progress has been made in laboratory studies, there remains a great need for information on basic aspects of natural transmission-blocking immunity with respect to the intensity of malaria transmission.

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MOLECULAR KARYOTYPE CHARACTERIZATION OF *LEISHMANIA PANAMENSIS, LEISHMANIA MEXICANA,* AND *LEISHMANIA MAJOR*–LIKE PARASITES: AGENTS OF CUTANEOUS LEISHMANIASIS IN ECUADOR

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Abstract. Molecular karyotypes of *Leishmania* isolates from patients with cutaneous leishmaniasis in Ecuador were analyzed by pulsed-field gel electrophoresis (PFGE) and Southern blot hybridization. The DNA karyotypes of *L. major*–like parasites were similar between two human isolates from a lowland coastal and a highland Andean region, but were apparently different from those of eleven World Health Organization reference strains including *L. major.* The smallest chromosome of 240 kilobases in *L. major*–like parasites was found to belong to the 715-class of small linear chromosomal DNAs, which have been shown to appear in some lines of *Leishmania.* Chromosome banding patterns of *L. mexicana* isolates exhibited a novel, ordered, chromosomal ladder, and were identical among four human isolates and one canine isolate from a restricted geographic region in the Andes. On the other hand, minor chromosome size polymorphisms were observed among three *L. panamensis* isolates from different endemic regions near the Pacific Coast. Chromosomal locations of dihydrofolate reductase-thymidylate synthetase and P-glycoprotein genes revealed further differences in chromosomal organizations among these *Leishmania* species in Ecuador. These results indicate that karyotype analysis by PFGE is useful for epidemiologic studies of leishmaniasis in Ecuador.

Leishmaniasis is widespread and is a considerable public health problem in Ecuador. We have carried out epidemiologic studies of leishmaniasis in Ecuador since 1982 and have found a large number of cutaneous cases and a small number of mucocutaneous ones.[1] In tropical and subtropical lowland areas near the Pacific Coast, we have isolated *Leishmania panamensis* from patients with cutaneous lesions.[2] Recently, we discovered a new endemic area of cutaneous leishmaniasis (Paute) in the Andes (Figure 1).[3] By zymodeme, schizodeme, and serodeme analyses, the causative species were determined to be as *L. mexicana* and an unusual parasite closely related to *L. major.*[3]

The advent of pulsed-field gel electrophoresis (PFGE) has enabled us to study chromosomal organizations of protozoa including *Leishmania.* This technique has distinguished karyotypes among *Leishmania* species, subspecies, and strains.[4–8] Despite this variability, the molecular karyotype analysis can be used for grouping closely related species and for identification of new isolates in epidemiologic studies.[9–11] To further characterize *Leishmania* isolates from Ecuador, we have extended our preliminary study[12] by analyzing their molecular karyotypes using pulsed-field gradient gel electrophoresis in combination with Southern blot hybridization.

MATERIALS AND METHODS

Parasites

Ten *Leishmania* isolates from Ecuador and eleven World Health Organization reference strains of the same genus were used in the present study (Table 1). The Ecuadorian isolates have been classified as *L. panamensis, L. mexicana,* and *L. major*–like parasites by isoenzyme electrophoresis, kinetoplast DNA fingerprints, and reactivity against monoclonal antibodies.[2, 3, 13] The geographic distribution of these isolates is shown in Figure 1.