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 gametocytsemia. The infectivity of this population 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 gametocytemic carriers (22.5%) and non-gametocytemic 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 gametocytsemia, 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. 6 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*. 7 *Anopheles funestus* serves as an important secondary vector species. Transmission is theoretically dependent on the size of the sexual forms was estimated after reading 75 fields of the thick smear (53 white blood cells/field = 0.33 mm²). The detection thresholds were 100 infected red blood cells or three gametocytes/mm², 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 non-parametric tests (Kruskal-Wallis or Kolmogorov-Smirnov (means = 2)). To relate a dependent quantitative variable to an independent one, we used the Spearman test (rank correlation coefficient).

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 infectious (48.4%). Approximately the same proportion was observed in the three age groups (x² = 4.18, df 2, P > 0.05). Parasitologically, there were approximately the same proportion of infectious individuals among the gametocyte carriers.

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 (x² = 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 x 7 ml) were collected and stored in heparinized or dry tubes. The heparinized blood was immediately prepared for membrane feeding, while the blood samples were immediately prepared for membrane feeding and the blood samples were immediately prepared for 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 x 7 ml) were collected and stored in heparinized or dry tubes. The heparinized blood was immediately prepared for membrane feeding, while the blood samples were immediately prepared for 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 x 7 ml) were collected and stored in heparinized or dry tubes. The heparinized blood was immediately prepared for membrane feeding, while the blood samples were immediately prepared for membrane feeding.

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(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 in infectiousness per gametocyte and per age group.

The mean percentage of infected mosquitoes was slightly greater in infectious or noninfectious subjects (Table 2). The prevalence of gametocytes carriers was not significantly different in infectious or noninfectious subjects (Table 2). The mean gametocyte densities were slightly higher in infective (2.6 gametocytes/mm$^3$) than in noninfective individuals (1.8 gametocytes/mm$^3$), but the difference was not significant (Kolmogorov-Smirnov coefficient $= 0.22, P > 0.05$).

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 male/female gametocytes was 4.8:1 (range 0-19:1). 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 compared with the younger age groups.

No significant difference was observed between the age groups among the gametocyte carriers ($x^2 = 4.18, df = 320, P > 0.05$). Among the individuals in the population, the time of developmental maturation in mosquitoes: $t = -8.4%$ of the population (greater than four years of age) was infectious to mosquitoes. The mean percentage of infected mosquitoes was 7.8% among what were apparently nongametocyte carriers ($x^2 = 320, P > 0.05$).

**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.

**Malarial Infectivity to $An. gambiae$**

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.

**TABLE 1**

<table>
<thead>
<tr>
<th>Subject age (years)</th>
<th>No. of infectious individuals</th>
<th>mPI</th>
<th>SD</th>
<th>Minimum mPI</th>
<th>Maximum mPI</th>
<th>PI ± 2 SD</th>
<th>PI ≤ 2 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-14</td>
<td>120</td>
<td>0.040</td>
<td>0.047</td>
<td>0.045</td>
<td>0.056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29</td>
<td>113</td>
<td>0.049</td>
<td>0.068</td>
<td>0.056</td>
<td>0.082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* mPI = mean infectiousness of one gametocyte. PI = % of infected mosquitoes/gametocyte density. All the infectious gametocyte carriers were considered as positive with at least one gametocyte present. mPI = mean; PI = sum of individuals with a positive mPI. No significant differences were found among age groups (Kruskall-Wallis coefficient = 4, degrees of freedom = 3, $P > 0.05$).

**TABLE 2**

<table>
<thead>
<tr>
<th>Subject age (years)</th>
<th>All individuals</th>
<th>Infected individuals</th>
<th>Infectious individuals</th>
<th>Noninfectious individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Density</td>
<td>Confidence interval</td>
<td>Number</td>
</tr>
<tr>
<td>5-14</td>
<td>120</td>
<td>153</td>
<td>148-204</td>
<td>93</td>
</tr>
<tr>
<td>15-29</td>
<td>113</td>
<td>29</td>
<td>22-38</td>
<td>600</td>
</tr>
<tr>
<td>≥30</td>
<td>89</td>
<td>10</td>
<td></td>
<td>544-796</td>
</tr>
</tbody>
</table>

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

**FIGURE 1.** Distribution, according to age, of infectious or noninfectious individuals after experimental infection with laboratory-bred $An. gambiae$. The mean densities of the three age groups is shown in Table 1. There was no difference among the three age groups.

**FIGURE 2.** Distribution, according to age, of infectious gametocyte or nongametocyte carriers after experimental infection with laboratory-bred $An. gambiae$. There was a negative correlation between the age groups among the infectious individuals ($r = 4.18, df = 2, P > 0.05$) and the noninfectious individuals ($r = 4.03, df = 2, P > 0.05$).

**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 ≥30 years, 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 ($r = -0.36, df = 320, P < 0.001$). A positive correlation was observed between parasite densities and gametocyte loads ($r = 0.26, df = 320, P < 0.001$). There was a 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 ($r = 0.04, df = 320, P > 0.05$).

**Human reservoir 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. Another study on malarial infectivity of human populations to mosquitoes was conducted in the Madang area of Papua New Guinea by Graves and others. 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. In the study of Muirhead-Thomson, mosquitoes were fed directly on the skin of individuals, while in our study, they were fed under experimental conditions through a water-jack.
Mean prevalences and mean oocyst densities in infected mosquitoes, according to subject age groups, after experimental infection on laboratory-bred Anopheles gambiae

<table>
<thead>
<tr>
<th>Subject age (years)</th>
<th>No. of infectious individuals</th>
<th>Mean % of infected mosquitoes</th>
<th>Mean oocyst density</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-12</td>
<td>57</td>
<td>15.4</td>
<td>1.70</td>
<td>1-38</td>
</tr>
<tr>
<td>13-29</td>
<td>44</td>
<td>20.2</td>
<td>2.11</td>
<td>1-31</td>
</tr>
<tr>
<td>30-49</td>
<td>62</td>
<td>12.3</td>
<td>3.11</td>
<td>1-56</td>
</tr>
<tr>
<td>&gt;50</td>
<td>116</td>
<td>11.5</td>
<td>1.89</td>
<td>1-56</td>
</tr>
<tr>
<td>Total</td>
<td>268</td>
<td>15.6</td>
<td>2.11</td>
<td>1-56</td>
</tr>
</tbody>
</table>

In our study, 52.5% of the population sample were gametocyte carriers, with a detection rate of 15%. The high gametocyte rate, associated with a high infectiousness, should be responsible for high transmission levels. 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. 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 parasite index < 60% for An. gambiae and < 40% for A. funestus). From these proportion data, the probability of survival of a new transmission (after 12-15 days of the sporogonic cycle). Therefore, these low parasite 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 level of 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, rodent, and simian 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 sexual parasite loads.

In experimental conditions, it has been demonstrated that high levels of antibodies to gamete-specific antigens and probably some cytokines 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. However, immune recognition of the gamete surface antigens is rare and generally weak. 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 possibility 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. 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 the basic aspects of natural transmission-blocking immunity with respect to the intensity of malaria transmission.

Acknowledgments: We thank the entomologic team of the Centre Muras in Bodo-Doulasso for technical assistance.
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. In tropical and sub-tropical lowland areas near the Pacific Coast, we have isolated Leishmania panamensis from patients with cutaneous lesions. Recently, we discovered a new endemic area of cutaneous leishmaniasis (Puerto) in the Andes (Figure 1). By means of pulsed-field gel electrophoresis and biochemical analyses, the causative species were determined to be L. mexicana and an unusual parasite closely related to L. major.

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. In this study, the molecular karyotype analysis can be used for grouping closely related species and for identification of new isolates in epidemiologic studies. To further characterize Leishmania isolates from Ecuador, we have extended our preliminary study 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, kinesin DNA fingerprints, and reactivity against monoclonal antibodies. The geographic distribution of these isolates is shown in Figure 1.

REFERENCES


