Plasmodium falciparum and P. malariae epidemiology in a West African village

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Transmission of Plasmodium falciparum and P. malariae was studied in a village in Burkina Faso. Consecutive captures of mosquitoes were organized twice a month over a year and the species of the mosquitoes identified. Also, the prevalences and densities of Plasmodium spp. were determined every 2 months in a sample of children who lived in the village. Anopheles gambiae, A. funestus, and A. nili were the local vectors, but only the first two played a predominant role in both P. falciparum and P. malariae transmission. The parasitological sporozoite index (SI) was 4.48% for A. gambiae and 4.22% for A. funestus. The immunological SIs were higher: 5.82% of A. gambiae were infected with P. falciparum and only 0.16% with P. malariae, the corresponding proportions for A. funestus were 6.45% and 0.41%.

Transmission of Plasmodium spp. by A. gambiae was important during the rainy season (July–October) and by A. funestus at the beginning of the dry season (September–November). Each child in the study village could receive about 396 P. falciparum-infected bites per year but only 22 of P. malariae. The P. falciparum parasite indices were maximum during the middle of the rainy season (August), while those for P. malariae reached a peak during the dry season (February).

Introduction

After Plasmodium falciparum, P. malariae is the most important cause of human malaria in West Africa (6). The geographical distribution of both species is widespread, but P. malariae is localized in foci (7). The periods during which these two species are transmitted are different (12). P. malariae is responsible for some malaria morbidity and chronic infections in endemic areas, and can sometimes induce a nephrotic syndrome (9). In some foci it can therefore be important to follow P. malariae transmission in order to select specific control measures against this parasite species.

By microscopy, it is virtually impossible to differentiate P. malariae sporozoites and P. falciparum in the salivary glands of infected mosquitoes. Recently, however, a two-site enzyme-linked immunosorbent assay (ELISA) that uses a monoclonal antibody against a surface P. malariae sporozoite antigen has been employed to detect this parasite in infected mosquitoes (4). Specific vectors for P. malariae have already been characterized in Kenya (1), but there has been no investigation of the transmission of this species in West Africa.

Using the above-mentioned ELISA with monoclonal antibodies against either P. falciparum or P. malariae, as well as entomological and parasitological surveys, we carried out a longitudinal investigation of malaria transmission in a savanna area of Burkina Faso.

Materials and methods

Characteristics of the study area

The study was conducted in Karangasso, Burkina Faso, near the city of Bobo-Dioulasso. Karangasso is a typical savanna village with a semipermanent river but is sufficiently distant from Bobo-Dioulasso to preclude significant use of chloroquine by the local population. The area is characterized by a hot and rainy season from June to October (average rainfall, 1000 mm per year; mean temperature, >25 °C); a cold and dry season from November to February (minimum temperature, 15 °C); and a hot and dry season from March to May.

Entomological investigations

Consecutive surveys were carried out from January 1985 to February 1986. Eight indoor human night catches were performed in the village twice a month. The species of mosquitoes caught were identified and each batch of Anopheles gambiae, A. funestus, or A. nili, the main malaria vectors in the area (13, 14)
during the rainy season, was randomly divided into two lots: one for microscopy of salivary glands and ovaries and the other for immunological testing.

The proportion of parous females (parity rate, PR) was calculated for each survey (5). The percentage of infected glands (parasitological sporozoite rate, s) was also determined. The human biting rate (ma) was estimated by determining the mean number of bites per person per night, and reflects the dangerous anopheine density for humans. The inoculation rate (h), which reflects the intensity of transmission was calculated from the relationship

\[ h = ma \times s \]

and is expressed in infected bites per person per night (11).

**Enzyme-linked immunosorbent assay**

Mosquitos from the second lot were tested with a two-site ELISA, using the monoclonal antibodies (MAb) 3SP2 and 6B10-1F2 against *P. falciparum* and *P. malariae* sporozoites, respectively. Details of the ELISA have been described previously (17, 18), and are presented here only in outline. Head–thoraxes and abdomens were separately ground in Nonidet P-40 with an anti-enzyme solution. After incubation, the supernatant was tested on microtitration plates sensitized with each of the two MAb.

The plates were incubated for 2 hours at room temperature and then washed three times. Biotinylated MAb solution was then added and incubated for 1.5 hours at room temperature. After rinsing the plates three times, 50 µl of a pre-formed streptavidin–biotin–peroxidase complex was added and the mixture incubated for 30 minutes. After the plates had been washed, the substrate (o-phenylene-diamine in a mixture of citrate buffer (pH 5) and hydrogen peroxide) was added and the plates were incubated for a further 30 minutes. The reaction was stopped by the addition of a solution of 2 mol/l sulfuric acid, and the absorbance of the resulting solution was measured photometrically at λ = 492 nm.

The prevalences of the infected mosquitos in the parasitological and immunological tests were compared.

**Estimation of the sporozoite load**

In each microtitration plate sensitized with 3SP2 MAb, serial dilutions of a *P. falciparum* sporozoite suspension (0.2–100 × 10^3) were added to eight wells and tested. Each plate, a standard curve of absorbance, according to the sporozoite concentrations, was obtained. By comparing the absorbance of the mosquitos tested with that of the standard curve, we estimated the sporozoite load of each infected mosquito.

**Parasitological investigations**

Parasitological surveys were performed every 2 months on the same population sample of 31 voluntary families. No particular control measure was taken other than treating the malaria patients. Only children aged 0–14 years were examined. Samples of peripheral blood were taken from each child and thin and thick smears prepared. After being treated with Giemsa stain, the thick and thin films were examined microscopically using an oil immersion objective (×50) to detect and quantify malaria parasites. Fifty fields (about 1500 white blood cells and about 40 000 red blood cells) of thick and thin films were each examined. The detection thresholds were 5 parasites per µl and 100 parasitized red blood cells per µl in the thick and thin films, respectively.

The prevalences of the different plasmoidal species and the logarithmic means of parasite densities in infected children were estimated for the blood smears at each survey.

**Results**

**Entomology**

Of the ten species of mosquitos in the study area, the following were the potential malaria vectors: *A. gambiae*, *A. funestus*, and *A. nili*. A total of 3078 mosquitos were captured between January 1985 and February 1986.

*A. gambiae* and *A. funestus* were captured all year round, with important seasonal variations, while *A. nili* was caught only from June to October. The density of *A. gambiae* fluctuated from 0.2 to 38.1 bites per person per night, with a peak in July–August, the middle of the rainy season. The density of *A. funestus* varied from 0.3 to 25.2 bites per person per night, with a maximum in September–October, at the end of the rainy season, while that of *A. nili* fluctuated from 0 to 4.7 bites per person per night (Fig. 1a). Based on these biting rates, an individual living in this endemic area could receive about 3200 *A. gambiae* bites per annum, 2600 *A. funestus* and only 560 *A. nili*.

The parity rates, estimated when the mosquito densities were sufficiently high, fluctuated from 43% to 84% for *A. gambiae*, with a maximum in June and July; those for *A. funestus* varied from 58% to 90%, with a peak in November; and those of *A. nili* were similar to those for *A. funestus* (Fig. 1b).

The parasitological sporozoite rates of *Anopheles* spp. and the corresponding immunological sporozoite rates (ISR) with anti-*P. falciparum* and anti-*P. malariae* MAb are shown in Table 1. Altogether 4.48% of dissected *A. gambiae* were infected with *Plasmodium* spp. and 5.98% were
Fig. 1. Evolution of a) anopheline densities (bites per person per night); b) parity rates (%); and c) entomological inoculation rates (infected bites per person per night), for Anopheles gambiae, A. funestus, and A. nili, in an African savanna area.

Table 1: Distribution of infected mosquitoes: Anopheles gambiae (A.g), A. funestus (A.f), or A. nili (A.n), detected by either dissection of salivary glands or enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies against Plasmodium falciparum or P. malariae circumsporozoite protein

<table>
<thead>
<tr>
<th>Dates (month/year)</th>
<th>Dissection</th>
<th>ELISA (P. falciparum)</th>
<th>ELISA (P. malariae)</th>
</tr>
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<tr>
<td>1/1985</td>
<td>0(2)*</td>
<td>1(7)</td>
<td>0(0)</td>
</tr>
<tr>
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<td>0(10)</td>
<td>0(8)</td>
<td>0(0)</td>
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<tr>
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<td>0(0)</td>
</tr>
<tr>
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<td>0(10)</td>
<td>0(0)</td>
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</tr>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
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<td>0(3)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Total</td>
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<td>33(781)</td>
<td>2(174)</td>
</tr>
<tr>
<td>% of mosquitoes infected</td>
<td>4.48</td>
<td>4.22</td>
<td>1.15</td>
</tr>
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</table>

* Figures in parentheses are the number of mosquitoes tested.
contrast, 20 out of 33 *A. funestus* were diagnosed by dissection, and ELISA detected 25/31 infected with *P. falciparum* and two with *P. malariae*. *A. nili* mosquitos were also occasionally infected: 3/4 mosquitos infected with *P. falciparum* were detected by ELISA and 1/2, by dissection.

For *A. gambiae*, *h* became positive in June, reached a maximum in July–September (about 1.5 infected bites per person per day) and then decreased (Fig. 1c). The annual average value was 0.39 infectious bites per person per night. In contrast, for *A. funestus* the rate became detectable in July and reached its maximum (about 1.3 infectious bites per person per night) in October. An individual living in the village could therefore have received an average of 0.31 infectious bites per night. For *A. nili* it was difficult to analyse the fluctuations in *h* because of the small number of mosquitos in the sample. The annual mean value of the rate in the study village was 0.72 infectious bites per person per night. The evolution of the three entomological indices (*ma*, PR, and *h*) showed that *A. funestus* progressively replaced *A. gambiae* during the second part of the transmission period.

Theoretically, each individual living in the village could have received 263 infected bites per year (daily *h* × 365). The immunological inoculation rate (*h* = *ma* × ISR) was calculated from the immunological sporozoite rate. We estimate that during the year each child received about 396 bites infected with *P. falciparum* and 22 bites infected with *P. malariae*.

Finally, the sporozoite loads estimated by ELISA were greater in *A. gambiae* than in *A. funestus* (Table 2).

### Parasitology

The prevalence of *P. falciparum* (IPF) fluctuated from 35.4% in the dry season to 82.5% in the rainy season (Table 3). The annual means were 63%, 59.5%, and 65.3% for 0–4-, 5–9-, and 10–14-year-olds, respectively.

Parasite densities varied from 280 to 2980 parasites per µl, with the annual means being 1675, 854, and 596 per µl for 0–4-, 5–9-, and 10–14-year-olds, respectively. Parasitological loads reached their maximum in August–October (Fig. 2a), during the rainy season. Subsequently, they decreased more rapidly in groups II and III than in group I, despite a high level of transmission (2.5 infected bites per person per night in September and 1.4 in October).

The prevalences of gametocytes fluctuated from 9.2% to 39.7% (Table 3), with the annual means being 27.1%, 23.7% and 30.3% among 0–4-, 5–9-, and 10–14-year-olds, respectively. Gametocyte densities (in logarithmic units) varied from 7 to 32 per µl according to both the age and the season. Those aged 0–4 years had higher levels of gametocytes (21.6 per µl) than the older age groups (14.6 and 11.6 gametocytes per µl for 5–9- and 10–14-year-olds, respectively).

The prevalence of *P. malariae* fluctuated from 3.5% to 25% and the annual means were 14.2%, 14%, and 10.8% in groups I, II, and III, respectively (Table 3).

![Fig. 2. Evolution of a) *Plasmodium falciparum* densities among three age groups of children and b) *P. falciparum* and *P. malariae* densities among children (all age groups) during a malaria transmission cycle in an African savanna area.](image-url)
Parasite densities varied from 260 to 1400 parasites per μl in infected children, and the annual means were 642, 376, and 316 parasites per μl, in groups I, II, and III, respectively. Parasitological loads reached their maximum in May and February, during the dry season, and were lowest when transmission was at a maximum (Fig. 2b). However, the relatively small number of infected children was insufficient to compare the parasitological densities between age groups or surveys.

The prevalence of *P. malariae* gametocytes was low (<3%) all year round. A total of 97% of *P. malariae* carriers were also infected with *P. falciparum*, both during the rainy and dry seasons.

**Discussion**

Under experimental conditions, the *P. malariae* sporogonic cycle in mosquitoes lasts 14–18 days at 28 °C, while that of *P. falciparum* lasts only 9–10 days (7). The anopheline vector of *P. malariae* must therefore have a greater survival rate than that of *P. falciparum*. Attempts to produce *P. malariae* sporozoites by experimental infection of locally bred *A. gambiae* were successful in Bobo-Dioulasso (2), which indicates that under optimal conditions this mosquito is a possible *P. malariae* vector. Theoretically the survival rate of *A. funestus* is greater than that of *A. gambiae* (3). Therefore, *A. funestus* also could play a major role as a *P. malariae* vector in the wild.

The results of the ELISA test showed that *A. gambiae, A. funestus* and *A. nili* were local vectors, but at different periods of the transmission cycle. These findings are in accord with those reported by Beier et al. in Kenya (1). The succession of the species at different periods of the malaria transmission cycle can be explained by the ecological differences between species and the longevity of the vectors.

With the first rainfalls in May the densities of *A. gambiae* increased, probably because larval habitats are generally small ponds, newly flooded, without floating vegetation (13). From June to July, the parity indices were regularly over 80%; their longevity was therefore enough to permit complete development of the *P. falciparum* sporogonic cycle and occasionally that also of *P. malariae*.

At the beginning of the dry season (October–December) conditions were favourable for the *A. funestus* population. Mosquito densities reached their maximum level and the parity rates generally exceeded 80%. Their longevity was therefore sufficient to permit complete development of *P. falciparum* and probably also of *P. malariae* sporogonic cycles. In contrast, the *A. gambiae* parity indices decreased regularly to a level which, theoretically at least, did not permit development of the *P. malariae* sporogonic cycle.

In the study village no *A. arabiensis* was caught, while *A. gambiae* s.s. represented more than 97% of the collected *Anopheles* spp. Cytogenetic investigations showed that the *A. gambiae* comprised two distinct populations: the Mopti form (about 30%) and the savanna form (about 70%) (15). No investigation was made of the seasonal fluctuations of these forms, and only the malaria susceptibility of the Mopti form was studied (2). It was not possible to interpret the variations in the transmission of *P.
falciparum and P. malariae according to the mosquito cytotype.

At the beginning of the transmission period in June–August, the densities of P. falciparum increased in parallel to the A. gambiae inoculation rates. From August to December they decreased, more quickly in age groups II and III than in age group I, despite the importance of this transmission route. This phenomenon probably reflects the acquisition of an efficacious premunition after the peak P. falciparum density. In contrast, the rapid increase in the density of P. falciparum immediately after the beginning of the transmission period (June) could reflect the partial loss of immunoprotection in children during the long period from December to May when transmission was very low.

P. malariae densities increased, while those of P. falciparum decreased during the second half of the transmission period. The immunoprotection induced by infection with P. falciparum does not seem to be effective against P. malariae. The opposing fluctuations in P. malariae and P. falciparum densities have been described previously [10, 12], and could arise because of competition between both species.

P. falciparum parasite loads were low compared with the high intensity of transmission. Premunition appears to stabilize the development of P. falciparum. The prevalences and densities of P. malariae are consistent with the intensity of the specific transmission. Also, P. malariae densities in infected children seem to be relatively constant, even during the period of apparent non-transmission (January–May). The chronicity of this blood infection could be explained by an inefficacy or a delay in the appearance of specific immunoprotection. In several areas of the Sahel, the inoculation rates are very low (<30 infected bites per person per year) and are concentrated over two or three months of the year. Under such conditions, the parasite indices are high, even during the non-transmission period, and premunition is weak and delayed [8, 16]; the antigenic stimulus is too episodic to produce specific immunoprotection and to limit the parasite loads. These observations may also apply to P. malariae infection and could explain its chronicity.

In conclusion it can be stated that P. malariae transmission in the West African savanna study area is low compared with that of P. falciparum. This probably arises for the following reasons:

- the optimal conditions for a complete sporogonic cycle in mosquitos rarely occur;
- gametocyte densities are generally low in local populations; and
- although they are sensitive to infection, A. gambiae, A. funestus, and A. nili are probably not optimal vectors under natural conditions.

P. malariae infection exhibits three conflicting phenomena that cannot be readily accounted for:

- the opposing fluctuations in P. malariae and P. falciparum densities during the period of malaria transmission;
- the positive association with P. falciparum in children; and
- the chronicity of the infection.

In order to better understand the specific epidemiology of P. malariae and its consequences in local populations, similar studies should be carried out in different and typical epidemiological strata in Africa. The mosquito ELISA test with the anti-P. malariae monoclonal antibody opens new perspectives in this respect.

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Résumé

L’endémie à Plasmodium falciparum et P. malariae dans un village d’Afrique de l’Ouest

Une étude sur la dynamique de transmission de Plasmodium falciparum et P. malariae a été effectuée dans un village de la savane ouest-africaine au Burkina Faso. Des captures de moustiques ont été organisées 2 fois par mois, la nuit, dans le village. Les espèces de moustiques anthropophiles et hématophages capturés sur hommes ont été identifiées et les Anopheles ont été divisés en 2 lots. L’un des lots a été désossé et l’autre testé en ELISA contre des anticorps monoclonaux spécifiques de l’antigène membranaire CSP des sporozoïtes de P. falciparum et P. malariae. Ceci a permis de déterminer l’indice sporozoïtaire global et spécifique d’espèce. Une étude parasitologique longitudinale, avec enquêtes bimestrielles, sur un échantillon d’enfants de 0 à 14 ans, a été parallèlement conduite dans ce village.

A. gambiae, A. funestus et A. nili sont les 3 vecteurs locaux du paludisme, mais A. nili est un vecteur secondaire. La parasitose est transmise de juin à décembre. A. gambiae est le vecteur principal
pendant la première moitié de cette période (juin–octobre), puis le relais est pris par *A. funestus*. À la dissection, 4,48% des *A. gambiae* et 4,22% des *A. funestus* ont des sporozoïtes dans leurs glandes salivaires. Les indices sporozoïtaires immunologiques sont supérieurs aux indices parasitologiques. Parmi les *A. gambiae* positifs en ELISA, 5,82% sont parasités par *P. falciparum* et seulement 0,16% par *P. malariae*. Ces pourcentages sont respectivement de 6,45% et 0,41% chez *A. funestus*. Les enfants subissent chaque année environ 396 piqûres d’*Anopheles* infectés par *P. falciparum* et seulement 22 infectées par *P. malariae*.

Les prévalences de *P. falciparum* oscillent entre 35 et 82% chez les enfants, avec un maximum au milieu de la saison des pluies (août). Les densités parasitaires décroissent avec l’âge et vont de 400 à 1900 parasites/μl (moyenne logarithmique chez les sujets infectés). Les prévalences de *P. malariae* se situent entre 3,5 et 25% avec un maximum en février (saison sèche). Les densités parasitaires, comprises entre 200 et 1400 parasites/μl, sont presque aussi élevées que celles de *P. falciparum*. Ces résultats parasitologiques semblent en contradiction avec le faible taux de transmission de *P. malariae*. *P. malariae* pourrait induire une prévention moins efficace que celle de *P. falciparum*, ce qui expliquerait la chronicité de l’infection. Le faible taux d’inoculation, limité à une partie de l’année, pourrait être insuffisant pour relancer et maintenir, à chaque cycle de transmission, une immunoprotection spécifique solide. Les densités parasitaires de *P. malariae* chutent quand celles de *P. falciparum* augmentent et vice versa. Il pourrait exister un phénomène de compétition entre les 2 espèces.

References