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 mosquitos 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 0.76% with P. malariae; the corresponding proportions for A. funestus were 6.45% and 0.47%. 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 immunoassay (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 parasitic females (parity rate, PR) was calculated for each survey (S). The percentage of infected glands (parasitological sporozoite rate, s) was also determined. The human biting rate (m) was estimated by determining the mean number of bites per person per night, and reflects the dangerous needle-bearing habits of the vectors. 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 (b/h).

**Enzyme-linked immunosorbent assay**

Mosquitoes from the second lot were tested with an two-site ELISA, using the monoclonal antibodies (MAb) 3SP2 and 6B10-1P2 against P. falciparum and P. malariae sporozoites, respectively. Details of the ELISA have been described previously (17). They are presented here only in outline. Head-thoraxes and abdomens were separately ground in two-site ELISA, using the monoclonal antibodies (MAb) for 1.5 hours at room temperature. After rinsing the incubation, the supernatant was tested on microtiter plates by ELISA. 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 peroxidase-anti-peroxidase complex was added and the mixture incubated for 30 minutes. After the plates had been washed, the substrate (o-phenylenediamine in a mixture of citrate buffer (pH 5) and hydrogen peroxide) was added and the plates 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 $\lambda = 492$ nm.

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

**Estimation of the sporozoite load**

In each microtiter plate sensitized with 3SP2 MAb, serial dilutions of a P. falciparum sporozoite suspension (0.2–100 x 10^6) were added to eight wells and tested. On each plate, a standard curve of absorbance was obtained. By comparing the absorbance of the mosquitoes 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 (×100) to detect and quantify malaria parasites. Fifty fields (about 2000 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 plasmodial 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 mosquitoes in the study area, the following were the potential malaria vectors: A. gambiae, A. funestus, and A. nili. A total of 3078 mosquitoes 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 500 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 (IR) against P. falciparum and anti-P. malariae MAb are shown in Table 1. Altogether 4.8% of dissected A. gambiae were infected with Plasmodium spp. and 5.9% were positive in the ELISA (5.82% with P. falciparum and 0.16% with P. malariae). In addition, 4.22% of A. funestus were infected with Plasmodium spp. and 6.86% were positive in the ELISA (6.45% with P. falciparum and 0.41% with P. malariae). A total of 1.15% of A. nili had sporozoites in their salivary glands and 13.5% were positive in the ELISA (10.8% with P. falciparum and 2.7% with P. malariae). There was a significant difference between the sporozoite rates estimated using the two techniques ($P < 0.05$). When compared by survey, the percentage distributions of the parasitological and immunological sporozoite rates were similar.

During the first part of the transmission period (June–September), almost all the infected A. gambiae were detected by either dissection (43/45) or ELISA (34/35 mosquitoes infected with P. falciparum and one with P. malariae). Only a few A. funestus were positive by either dissection (12/33) or ELISA (6/31 mosquitoes infected with P. falciparum and none with P. malariae). The situation with A. nili was intermediate (1/2 infected mosquitoes, by dissection, and 1/4 infected with P. falciparum and 1 with P. malariae, by ELISA).

During the second half of the transmission period (October–December) only two infected A. gambiae out of 45 were diagnosed by dissection, while ELISA only detected one out of 35 infected with P. falciparum and none with P. malariae. In addition, the percentage distributions of the parasitological and immunological sporozoite rates were similar.
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. nillii mosquitoes were also occasionally infected: 3/4 mosquitoes infected with P. falciparum were detected by ELISA and 1/2, by dissection.

For A. gambiae, it 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 night. For A. nillii it was 0.72 infectious bites per person per night. 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, Fr, and I) 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 x 15r) 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 (IFP) fluctuated from 35.4% in the dry season to 82.5% in the rainy season (Fig. 2). The annual averages were 63%, 59.5%, and 63.5% for 0–4, 5–9, and 10–14-year-olds, 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. falciparum 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 unsuccessful 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. falciparum vector in this area.

The results of the ELISA test showed that A. gambiae, A. funestus and A. nillii were vector 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 rains in May the densities of A. gambiae increased, probably because larval habitats are greater in number, 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 P. falciparum sporogonic cycle and occasionally that 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.
June–August, the densities of *P. falciparum* increased in parallel to the *A. gambiae* inoculation rates. From August to December they decreased, more rapidly 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 in *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 during the long period from December to May when transmission was very low.

*P. malariae* densities increased, whereas 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 both species. *P. malariae* seems to be effective against *P. falciparum* induced by infection with *P. malariae*. Thus, parasitological indices of *P. malariae* may 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 high (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, 10); the antigenic stimulus is too episodic to produce specific immunoprotection and to limit the parasite loads. These observations also explain the chronicity of *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 mosquitoes rarely occur;—
- gametocyte densities are generally low in local populations; and—
- although they are sensitive to infection, *A. gambei*, *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.

**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, en ville, et 3 fois par mois, en village. Les espèces de moustiques anthropophiles et hémato- phages capturés sur hommes ont été identifiées et les *Anopheles* ont été divisés en 2 lots. L’un des lots a été disséqué et l’autre testé en *ELISA* contre des antigènes monoclonaux spécifiques de l’antigène membranaire CSP des *Plasmodium falciparum* et *P. malariae*. Cela a permis de déterminer l’indice sporozoitaire global et spécifique d’espace. 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. gambei*, *A. funestus* et *A. nili* ont été les 3 vecteurs leucux du paludisme, mais *A. nili* est un vecteur secondaire. La parasite est transmise de juin à décembre. *A. gambei* est le vecteur principal pendant la première moitié de l’année (juin–octobre), puis le paludisme est pris par *A. funestus*. A la saison de transmission, 4,48% des *A. gambei* et 4,02% des *A. funestus* ont des sporozoïtes dans leurs glandes salivaires. Les indices sporozoïtiques immunologiques sont supérieurs aux indices parasitologiques. Parmi les *Anopheles* positifs en *ELISA*, 5,62% sont parasites 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 infectés subissent chaque année environ 206 piqûres d’*Anopheles* infectés par *P. falciparum* et seulement 52 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 1500 parasites/1 (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/1, 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 prémunition 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 immunoprotéction antigénique 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 2 espèces.

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**References**