Annals of Tropical Medicine and Parasitology, Vol. 85, No. 4, 377–385 (1991)

Epidemiology of *Plasmodium falciparum* in a rice field and a savanna area in Burkina Faso: seasonal fluctuations of gametocytaemia and malarial infectivity

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Received 23 July 1990, Accepted 28 September 1990

For a better understanding of the epidemiology of *Plasmodium falciparum* in an African savanna area, the authors have: (a) defined the real gametocyte reservoir in the native population; (b) followed the fluctuations of gametocytaemia throughout the transmission period; and (c) measured the infectiousness of malarious individuals to mosquitoes.

The transversal surveys, in different villages of this endemic area, have shown that gametocyte carrier rates decreased with age and malaria experience; 10·9% of the whole population were potentially infectious to mosquitoes, and of these 73% were children and only 27% were adults. The longitudinal studies have shown that the *P. falciparum* gametocyte rate depends on the equilibrium between the gametocyte conversion rates and the density of the asexual forms. When there are large numbers of children who become carriers of the sexual stage of the parasite and at the same time a small number who lose their gametocyte infection, the gametocyte rate increases in the population; and vice versa. The circumstances under which gametocytes are produced are not well-known. Two factors seem to be important: the level of the parasite density and immune mechanisms.

The infectiousness of malarious individuals was estimated by the 'mosquito infection probability'. The percentage of mosquitoes infected after feeding on gametocyte carriers (which may partly reflect the infectiousness of a human population to mosquitoes) was multiplied by the percentage of gametocyte carriers in the population. This indicated that, in this endemic area, 4% of biting mosquitoes would become infected; but this theoretical mosquito infection probability is over-estimated. Not all the gametocyte carriers are infectious, and the proportion of mosquitoes experimentally infected on heavy gametocyte carriers does not reflect the true percentage of mosquitoes found positive in natural conditions. It is concluded that the best method for estimating the infectiousness of a human population is (a) to feed laboratory-reared *Anopheles* on a representative population sample (without regard to the presence of gametocytes), or (b) to feed these *Anopheles* only on the gametocyte carriers detected in the population (without regard to gametocyte density). The second method allows estimation of the proportion of gametocyte carriers infectious to mosquitoes in each age-group. By multiplying this proportion by the mean percentage of infected mosquitoes, a more realistic mosquito infection probability can be evaluated.

A comparative study of malaria transmission and its consequences for human individuals was carried out in a rice field and a typical savanna village in Burkina Faso. The parasitological data (Boudin *et al.*, in press) have shown that the rapid increase in the anopheline population, at

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the beginning of the rainy season in May, was immediately followed by a parallel increase in the malaria inoculation rate. This observation indicated that a potentially infectious human reservoir persisted throughout the long dry season, from November to April, in spite of low parasite densities in infected subjects.

For a better understanding of the epidemiology of *Plasmodium falciparum* in this area, investigations were made to (a) define the true gametocyte reservoir, (b) follow the fluctuations of gametocytaemia throughout a cycle of malaria transmission, and (c) measure the infectiousness to mosquitoes of malarious individuals.

The prevalence of gametocyte carriers is relatively easy to evaluate, by examining large numbers of blood smears from representative samples of the human population at different periods of the malaria transmission cycle. It is more difficult to estimate the infectiousness of malarious individuals. There are two methods: first, to feed laboratory reared *Anopheles* on a representative sample of the population, without regard to parasitological status (Muirhead-Thomson, 1957); and second, to define the 'mosquito infection probability' (Graves *et al.*, 1988).

As the first approach is very exacting, we preferred to use the second method. The mosquito infection probability is the proportion of mosquitoes, biting a human population on any one night, which can become infected. It can be estimated by multiplying the mean infectivity of gametocyte carriers (the mean percentage of infected mosquitoes obtained by feeding them on known gametocyte carriers) by the prevalence of gametocyte carriers in the human population (which theoretically reflects the proportion of infectious individuals).

In this paper the results obtained are compared to those of previous studies in other endemic areas.

MATERIALS AND METHODS

Study Area

The study was carried out in a West African savanna area, near Bobo-Dioulasso, Burkina Faso. This geographical region is characterized by a rainy season from May to October, with an average rainfall of 1000 mm year^{-1}. Eighteen transversal surveys were carried out between 1985 and 1987 in different villages in this area, during both the dry and rainy seasons. In parallel, two longitudinal studies were carried out in a rice field and in a typical savanna village.

Human Population

During the transversal surveys, nearly all the children of each village and some adults who were present at the time of the survey were examined. During the longitudinal studies, consecutive surveys were organized every two months; only the children of 31 volunteer families in the village were examined.

Survey Methods

Peripheral blood was taken from each individual and thick and thin smears were prepared. After staining with Giemsa, 50 fields of each thick film were examined to estimate gametocyte, densities. The detection threshold was five gametocytes mm^{-3}, and the density was calculated as follows: number of gametocytes seen/number of leucocytes seen $\times 7500$ (the mean concentration of leucocytes mm^{-3} in African populations).

The infectivity of individuals to mosquitoes was studied by membrane feeding. A 10 ml sample of blood was taken from some of the identified gametocyte carriers, heparinized, and immediately offered to *A. gambiae* through a Parafilm membrane in water-jacketed glass feeders. Engorged mosquitoes were sorted and kept in an insectary at ambient temperature for 12–14 days with a continuous supply of 10% sucrose solution. Their salivary glands were then dissected out and examined at a magnification of $\times 20$ for sporozoites.

The entomological methodology has been described elsewhere (Robert *et al.*, 1985).

RESULTS

Transversal Surveys

A total of 5582 individuals were examined. The proportion of gametocyte carriers was found to decrease with age (Table 1). Among the infected children there were some with more than 50 gametocytes mm^{-3}, whom we called 'heavy carriers'. No child under one year of age was a heavy carrier. The percentages of heavy carriers decreased also with experience of malaria; only three adults (0·8%) had more than 50 gametocytes mm^{-3}. One of these presented with an acute viral hepatitis, another had chronic lymphatic leukaemia, and the third was a pregnant woman. During the rainy season (May to October) 19·1% of the individuals examined were gametocyte carriers, and during the dry season (November to April) there were 15·6% ($\chi^2 = 12{\cdot}04$, $P < 0{\cdot}001$). There were 2·9% and 2·2% of heavy carriers during the rainy and dry season respectively; and there was no significant difference ($\chi^2 = 2{\cdot}43$, $P > 0{\cdot}05$). The *P. falciparum* gametocyte rates, after adjustment for age, were similar between villages (data not shown).

TABLE 1

Distribution of Plasmodium falciparum *gametocyte prevalences and densities, according to age, in individuals living in a savanna area of West Africa*

	Gametocyte carriers (>5 g mm^{-3})		*'Heavy carriers' (>50 g mm^{-3})*		
Age groups	*No. examined*	*Prevalence %*	*No. of positives*	*Prevalence %*	*Density* (g mm^{-3})*
<1 year	167	20·3	0	0	10·2
1–4 years	2216	20·7	101	4·5	21·9
5–9 years	1929	15·1	30	1·5	19·7
10–14 years	897	15	5	0·5	11·6
⩾15 years	373	5·6	3	0·8	5·8
Total	5582	17·1	139	2·5	19

* = Log average of the gametocyte densities in infected children.

The geometric mean of gametocyte densities in infected individuals varied from 5·8–21·9 gametocytes mm^{-3} according to age (Table 1).

The distribution of individuals in the different age groups was different from the demographic profile of the population. The theoretical number of gametocyte carriers in each age group could be estimated by multiplying the proportion of the population in that age group by the percentage of individuals of that age who showed gametocytes. For example, 4·01% of the population was under one year old, and 20·3% of these infants showed circulating gametocytes. This potentially-infectious group thus represented 0·8% of the population. The equivalent percentages were 2·9, 2·6, 1·7 and 2·9% in the 1–4, 5–9, 10–14 and 14+ year old groups respectively. Of the whole population in this area, 10·9% were potentially infectious to mosquitoes and so epidemiologically 'dangerous'; 73% of them were children and only 27% were adults.

Longitudinal Studies

Details of the entomological and parasitological data were given previously (Robert *et al.*, 1985, 1988; Robert, 1989; Boudin *et al.*, in press). For a better understanding of these data, this present work reports on the fluctuations of the entomological inoculation rate (Robert *et al.*, 1985) and the *P. falciparum* prevalences and densities (Boudin *et al.*, in press) in both the savanna village (Fig. 1). and the rice field (Fig. 2).

The gametocyte conversion rates (the percentages of individuals who either became gametocyte carriers or ceased to be gametocyte carriers) during the interval between two consecutive surveys has been calculated. The proportion of individuals who became positive in a second survey but were negative in the first survey is given by $a = n(-/+)/N-$, and the proportion who became negative in the second

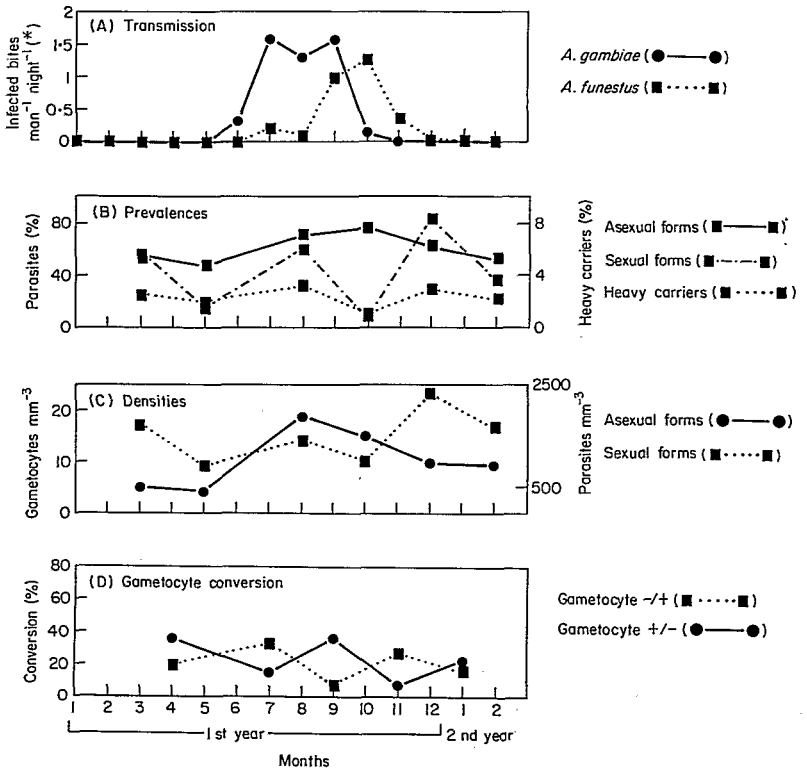

Fig. 1. The evolution, in an African savanna area, of: (A) the inoculation rate (Robert *et al.*, 1988); (B) the prevalences of the asexual and sexual forms of *Plasmodium falciparum* and of 'heavy' carriers (with more than 50 gametocytes mm^{-3}); (C) the parasite and gametocyte densities in infected children (log average); and (D) the gametocyte conversion rates (the proportions of children who become infected with gametocytes or who lose their gametocyte infection during the interval between two consecutive surveys).

survey but were positive in the first is given by $b=n(+/-)/N+$. The evolution of these proportions (a and b) are shown in Fig. 1 (savanna) and Fig. 2 (rice field).

Of the 240 children in the savanna village, 50% were examined at each of the six surveys. In the rice field 63% of the 286 children were examined.

In Savanna

At the beginning of the transmission period (May to August) the parasite prevalences and densities were rising, in parallel with the inoculation rate. The gametocyte prevalences and densities were also increasing; 33·3% of the children became gametocyte carriers, while only 14·7% lost their sexual infections.

From August to October parasite densities began to decrease, although transmission was still occurring. Both gametocyte prevalences and densities decreased; 36·3% of gametocyte carriers became negative, while only 8% of the negative children became positive, during the interval between the two surveys.

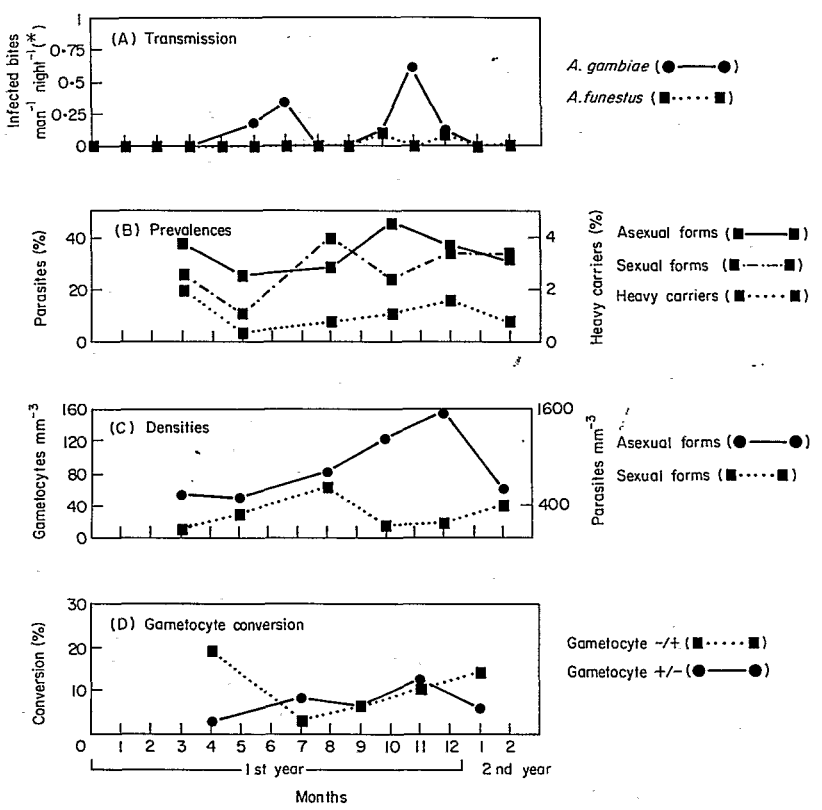

Fig. 2. The evolution, in an African rice field area, of: (A) the inoculation rate (Robert *et al.*, 1988); (B) the prevalences of the asexual and sexual forms of *Plasmodium falciparum* and of 'heavy' carriers (with more than 50 gametocytes mm^{-3}); (C) the parasite and gametocyte densities in infected children (log average); and (D) the gametocyte conversion rates (the proportions of children who become infected with gametocytes or who lose their gametocyte infection during the interval between two consecutive surveys).

From October to December the prevalences and densities of asexual forms decreased. However, the prevalences and densities of sexual forms were increasing; 27% of the negative children became positive, while only 7·2% of the gametocyte carriers became negative.

From December to May, a period without apparent transmission, the prevalences and densities of asexual forms were low. The gametocyte conversion rate (+/−) was about the same as the conversion rate (−/+), which might explain the stability of the gametocyte prevalences and densities.

In Rice Field

In this area the transmission is bimodal. The first period (April to July) coincides with the first flooding of the rice field at the end of the dry season. Overall parasite prevalences and densities increased slightly. Gametocyte prevalences and densities both increased; 8·3% of negative children became gametocyte carriers,

TABLE 2

Relationship between the infection of Anopheles gambiae, *the infectiousness of one gametocyte and the age of the* Plasmodium falciparum *gametocyte carriers*

Age	*1–4 years*	*5–9 years*	*10–14 years*	*⩾15 years*
No.	31	30	6	5
Mean of gametocyte densities	309±279	162±141	637±1238	983±1065
No. of mosquitoes	4709	2247	466	849
% Of infection*	40·7	37·9	27	22

Mean of gametocyte densities (gametocytes mm^{-3}).
*% Of mosquitoes infected with sporozoites.

while only 3·2% of gametocyte carriers became negative.

Between the two peaks of transmission the prevalences and densities of parasites continued to increase. The two gametocyte conversion rates in children were at the same level, but the gametocytaemia was not stable. The densities of the sexual forms decreased despite the increase in overall parasite loads.

During the second peak of transmission between October and December, the prevalences and densities of the asexual forms continued to increase. The gametocyte prevalences and densities were stable, possibly because of an equilibrium between the two conversion rates; 12·5% of the negative subjects became gametocyte carriers, and 10·4% of the gametocyte carriers became negative.

During the dry season (December to May) all the parasitological parameters decreased or remained stable. The gametocyte prevalences in particular were stable throughout this period, and the densities in infected children were high enough to infect the residual *Anopheles*.

Experimental Infection

In all, 72 experimental infections of *A. gambiae* were carried out in the laboratory, using the blood from 72 of 141 heavy gametocyte carriers. Four attempts failed, in spite of high gametocyte densities of 50, 190, 450 and 2225 gametocytes mm^{-3}. When fed on blood with levels of 0–50, 50–100, 100–200, 200–450 and 450+ gametocytes mm^{-3}, the percentages of mosquitoes which were infected were 7, 23, 36, 47 and 36% respectively (data not shown). The mean percentages of infected mosquitoes increased up to the level of 450 gametocytes mm^{-3}, and then decreased significantly (for more details, see Boudin *et al.*, 1989). The overall mean percentage of infected mosquitoes was 37·2%.

The mosquito infection probability (K) can be estimated by multiplying the mean proportion of gametocyte carriers by the mean percentage of infected mosquitoes. After experimental feeding on heavy carriers of gametocytes, 37·2% of mosquitoes became infected. The mean proportion of gametocyte carriers is 10·9%, which gives an estimate for K of 4%.

DISCUSSION

The transversal surveys, in this endemic area, have shown that gametocyte rates decrease with age and malaria experience. These results are not surprising, and confirm previous studies in different regions of the world (Bishop, 1955; Carter and Gwadz, 1980). No significant difference was found between the prevalences of gametocyte carriers in the 20 villages studied, after adjustments were made for age; and there is only a slight difference in gametocyte rates between the dry and the rainy season, which can be ignored for epidemiological purposes. These observations allowed an estimation, within good confidence limits, of the mean proportion of gametocyte carriers in the human population as 10·9%±0·5%. Comparison of our data with those observed during previous studies on other endemic areas is difficult, because the methods of estimating parasite numbers in blood smears are different. Nevertheless, Muirhead-Thomson (1957) in The Gambia and Molineaux and Gramiccia (1980) in Nigeria (with about the same levels of malaria transmission as in Burkina Faso) have observed similar infection levels in relation to age.

The longitudinal studies have shown that the *P. falciparum* gametocyte rate depends on the equilibrium between the gametocyte conversion rates and the density of the asexual forms. When a great number of children become infected by the sexual stages of the parasite and at the same time a small number lose their gametocyte infection, the gametocyte rate increases in the population, and vice versa.

At the beginning of the transmission period the parasite densities increase in both the savanna and rice field populations, who have probably lost part of their immunity during the long dry season; the gametocytaemia increases correspondingly. The densities of asexual forms are maximal during this period, and malaria attacks are frequent, especially in the savanna. The percentage of rings which develop into gametocytes is regulated by environmental conditions (Carter and Miller, 1979). High parasite densities are probably responsible for some disturbances which favour gametocytogenesis.

From the middle to the end of the transmission period, in savanna, parasite densities and prevalences both decreased while transmission was continuing. This phenomenon probably reflects the acquisition or the increase of an efficacious protective immunity in children (Boudin *et al.*, in press). This immunity is another factor unfavourable to parasite development, and an increased gametocytogenesis would represent an escape mechanism for the parasite (Brockelman, 1980). This may explain the increase of the 'heavy gametocyte' carriers and the gametocyte densities during this period. In the rice field, the interpretation of the data is more difficult.

During the dry season, generally, the gametocyte conversion rates (+/− or −/+) maintain the same levels. This could explain the stability in the numbers of sexual stages throughout this period. The gametocyte prevalences and densities remain high, and are probably sufficient to re-start transmission quickly, immediately after the appearance of the first mosquitoes at the beginning of the next rainy season.

The infectiousness to mosquitoes of the 72 gametocyte carriers studied appeared to decrease with age. The percentages of mosquitoes infected after feeding on adults or children 10–14 years old are lower than after feeding on younger children, in spite of high gametocyte densities. Certain individuals with high gametocyte densities did not infect mosquitoes. It is unlikely that this is due to the sporonticidal effects of antimalarial drugs, since Fansidar® and Primaquine® are not available to this population through local health services. The lower infectiousness to mosquitoes of adults and older children could be due to over-high gametocytaemias. In previous studies Rutledge *et al.* (1969) and Boudin *et al.* (1989) have recorded a gametocyte density threshold, at a density about which either individuals fail to infect mosquitoes or the sporozoite loads in infected mosquitoes are low, probably due to nutritional competition between developing oocysts.

Another inhibiting factor could be the appearance, in adolescents and adults, of a transmission-blocking immunity. Boudin *et al.* (1989) observed a specific gamete phagocytosis in the mosquito stomach following blood meals from some of the uninfectious carriers. No attempt has been made in this present study to examine the role of inhibiting antibodies in relation to the infectivity of gametocytes.

The mosquito infection probability (K) was estimated by multiplying the percentage of mosquitoes infected after feeding on gametocyte carriers (a reflection of the infectiousness of these carriers) by the percentage of gametocyte carriers in the population (see Graves *et al.*, 1988). It was found that 4% of the mosquitoes biting an individual in this endemic area are possibly infected. Muirhead-Thomson (1957) fed *A. gambiae* on randomly-selected members of a Liberian population, regardless of parasitaemia or gametocytaemia; 9·2% of these individuals were infectious to the mosquitoes, and 20·9% of the mosquitoes which engorged on

the infectious individuals developed an oocyst infection. In these conditions K could be evaluated as 1·9%. Graves *et al.* (1988), working in Papua New Guinea and using the same methodology, found 4% of infectious individuals and 37·9% of infected mosquitoes. This gives an estimate for K of 1·5%, but this figure was corrected to 1·3% by multiplying the mean gametocyte rate in the population by the percentage of mosquitoes infected after feeding on the gametocyte carriers.

The gametocyte rate observed during the present survey was higher than those found by Muirhead-Thomson (1957) in Liberia and by Graves *et al.* (1988) in Papua New Guinea. This gametocyte rate is probably realistic (see previous discussion), but it does not necessarily reflect the true proportion of individuals infective to mosquitoes. Graves *et al.* (1988) observed that 45·2% of their gametocyte carriers were not infectious to mosquitoes, while in the present study only four of 72 'heavy' gametocyte carriers were not infectious. The gametocyte density in the blood is a poor indicator of infectiousness to mosquitoes.

The percentage of infected mosquitoes observed in this survey also is higher than those observed by Muirhead-Thomson and Graves *et al.* This could be due either to the methodology of experimental infection or to an over-estimation of the sporozoite rate. The mosquitoes were engorged on 'heavy' carriers who were not representative of gametocyte carriers in the whole population.

An evaluation of the mosquito infection probability after biting is of both theoretical and practical interest for understanding the epidemiology of malaria. This evaluation depends on the choice of population samples for estimating the gametocyte rate in man and the sporozoite index in engorged mosquitoes. It appears that the most realistic and exacting method would be to feed groups of mosquitoes on a demographically representative sample of the human population, without regard to parasitological status. However, there is an easier approach. The percentage of infectious gametocyte carriers can be estimated by feeding laboratory *Anopheles* on a representative sample of gametocyte carriers. This percentage can then be multiplied by the proportion of gametocyte carriers in the population to produce an estimation of the proportion of the population infectious to mosquitoes. An analysis by age would allow evaluation of the responsibility of each age group for transmission of *P. falciparum*. Although some individuals, apparently without gametocytes, can infect mosquitoes, and are not taken into account in the estimation of the proportion of individuals infectious to mosquitoes, nevertheless this approach is sufficient for epidemiological studies.

ACKNOWLEDGEMENTS. This work was supported by a grant from the World Health Organization (Tropical Disease Research). We gratefully acknowledge the entomological team of the Muraz Center in Bobo-Dioulasso for their technical help, and we are indebted to Dr. F. Santoro for his critical reading of the manuscript.

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