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Plasmodium falciparum clinical malaria: lessons from longitudinal studies in Senegal

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Abstract. Development of new antimalaria strategies and particularly vaccines, needs an in-depth understanding of the relationships between transmission, infection, immunity, morbidity and mortality. The intensive and longitudinal collection of entomological, parasitological and clinical data from the Senegalese populations of Dielmo (250-300 inhabitants), exposed to a perennial and intense transmission (about 200 infective bites/person/year) and of Ndiop (300-350 inhabitants) exposed to a seasonal transmission (about 20 infective bites/person/year), allows to respond to many questions about this subject. The acquisition of an antimalaria immunity as one gets older appears to reduce parasite density, complexity of infection, risk of new patent infection after a suppressive treatment but does not reduce the prevalence (as assessed by PCR) of infection which is commonly chronic and asymptomatic. The existence of a pyrogenic threshold effect of parasitaemia allows the individual diagnosis of malaria attacks. *P. falciparum* genotyping suggests that successive malaria attacks are due to distinct recently inoculated parasite populations that multiply initially without restriction, a dominant population is generally responsible of the clinical manifestations and all new populations do not trigger systematically attacks. The initial intensity of clinical manifestations does not differ perceptibly among children and adults, is not related to the duration of the attacks, does not allow the distinction between several types of attacks, is not predictive of their severity, and the clearance of parasites and manifestations is longer among youngest persons. The risk of malaria attacks is lower as one gets older and among carriers of AS haemoglobin, is higher when transmission increases and during pregnancy up to three months after delivery, and vary between children. The risk of malaria attack per infective bite is negatively related to the intensity of transmission. Because of their high sensitivity in malaria case detection, this type of small community-based studies are powerful and useful for the identification of protective immunological mechanisms as well as for testing rapidly and cheaply the clinical efficacy of any intervention such as antimalarial vaccines and drug therapy or prophylaxis. As a lot of vaccine candidates and drug combinations will be screened or tested in the perspective of the 'Roll-Back Malaria' programme, more attention must be given to longitudinal studies of this type.

Key words: *Plasmodium falciparum*, morbidity, epidemiology, transmission, immunity.

For as long as the fight against malaria was mainly based on vector control and chemoprophylaxis, illness and death due to malaria were largely ignored as epidemiological research topics. Now that reduction of morbidity and mortality are the main goals and are attempted through various means, a fuller understanding of their determinants is needed. With this aim in view, the Institut Pasteur and IRD ex-ORSTOM are carrying out a longitudinal study of the relationships between malaria transmission, infection and morbidity in Dielmo and Ndiop, two villages of Senegal which differ in their endemicity. This leads to a precise assessment of the malaria burden in these areas and of the potential effect of various interventions. Here, we present the current main lessons from these longitudinal studies in Senegal.

Study of the relationships between malaria transmission, infection and morbidity

The populations of Dielmo and Ndiop have been enrolled in a longitudinal study of malaria, respectively since 1990 and 1993 (Trape *et al.*, 1994; Rogier and Trape, 1995). Similar entomological surveys and identical strict clinical surveillance programs are carried out, including a daily home visit to each person and the presence, night and day, of a medical team in the villages. Any pathological episodes are diagnosed and treated. The perception of the symptoms, the medical vocabulary, the knowledge, the attitudes and the practices related to feverish diseases were almost the same in the two populations (Coulibaly, 1994). Malaria transmission intensity differs considerably in these two villages that are only 5 kilometres apart (Konate *et al.*, 1994; Fontenille *et al.*, 1997a, 1997b). In Dielmo, transmission is in-

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tense, about 200 infective bites per person per year, and perennial (with seasonal fluctuations) due to the presence of a stream which serves as a permanent breeding site. In Ndiop transmission is about eight to ten times lower and only occurs during the four months of the rainy season.

Measuring malaria morbidity

Measuring the incidence of malaria clinical attacks presupposes a definition of the cases. Because of the high prevalence of asymptomatic malaria infections and the non-specificity of signs and symptoms of the disease, the diagnosis of clinical malaria presents difficult methodological problems in highly endemic areas. In the past, these difficulties were a major obstacle in the evaluation of malaria control strategies. Investigating longitudinally the intrinsic nature of the relationship between parasitaemia and fever at the individual level and the variations in tolerance of parasitaemia among individuals in the population of Dielmo, evidence for an age-dependent threshold effect of the parasitaemia was obtained (Rogier *et al.*, 1996b). The level of this threshold varied from 2.45 trophozoites per leukocyte, maximum at one year of age, to 0.5 trophozoites per leukocyte, minimum after 60 years of age. When the parasite density of a person crossed the threshold level corresponding to his or her age, the individual's risk of fever was multiplied by 44 (95% confidence interval = 13.6-114.8). The occasional observation of very high parasitaemia in asymptomatic individuals has often been an argument against using parasite density for the individual diagnosis of malaria attacks. In fact the observation of these asymptomatic carriers in whom parasitaemia is above the threshold is very rare (0.3%), and parasitaemia peaks are usually accompanied by fever or symptoms linked to fever during the preceding or following hours. As a result of this threshold effect, the parasite density measurement makes it possible to distinguish malaria attacks from other causes of fever at the individual level. This diagnostic criterion for malaria attacks is usable for both identification of the mechanisms of acquired antimalaria immunity (Aribot *et al.*, 1996) in highly endemic areas, antimalarial drug trials (Rogier *et al.*, 1996a) and clinical studies.

In Ndiop, malaria attacks are also associated with a sharp increase in parasite density but the level of parasitaemia triggering fever is lower and its variations according to age are less pronounced.

Clinical presentation and severity of attacks

From 689 *Plasmodium falciparum* malaria attacks observed and treated during a three year period among 226 inhabitants (78% of cases <6 yrs old) of Dielmo, neither convulsion nor life-threatening anaemia were recorded and only one case was severe and died despite receiving correctly administered quinine less than 12 hrs after the beginning of symptoms. This illustrates both the efficiency of the rapid treatment of sick persons to avoid or limit complications of malaria and the fact that the risk of development of severe forms cannot be totally prevented and/or that certain individuals may be predisposed to this type of outcome.

The symptom frequencies observed during these 689 malaria attacks were tested against age, gender and parasite density and a study of distinguishable clinical presentations was carried out by multi-correspondence analysis (Rogier *et al.*, 1999). There was little difference between the severity of symptoms during the initial course of attacks in young children and adults, and this severity was not correlated with the duration of the pathological episode. It was not possible to distinguish objectively different malaria attack types according to the severity of clinical manifestations. In contrast, the duration of fever, symptoms, and parasite clearance were significantly longer among the youngest children than among the oldest children and adults. These findings suggest that of the two components of protective immunity – anti-parasite and anti-toxic immunity – only the first would play a major role as age increases. They suggest also that the initial clinical presentation of malaria attacks is not predictive of the level of protective immunity. These clinical findings are important both for the adaptation of the malaria case management at the population level or in the peripheral health centres and for the case definitions used in malaria epidemiology.

The level of transmission as a risk factor for malaria morbidity

The malaria morbidity in Dielmo and in Ndiop, under different transmission level conditions, has been compared (Trape and Rogier, 1996). As shown in Figure 1, the patterns of age-dependent variations in mild malaria attack incidence differ markedly in the two populations. The proportion of attacks arising during adulthood was respectively 23% and 41% in Dielmo and in Ndiop. According to these observations, at the age of 60 the inhabitants experience an

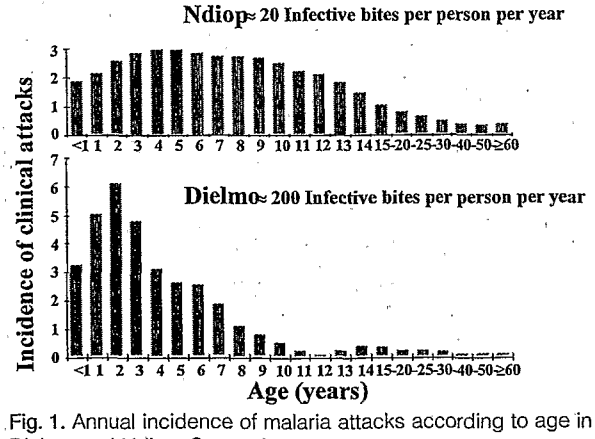

Fig. 1. Annual incidence of malaria attacks according to age in Dielmo and Ndiop, Senegal.

average of 43 and 62 malaria attacks in Dielmo and in Ndiop, respectively, whereas they are respectively exposed to about 200 and 20 infective bites/person/year.

The comparison of these populations indicates that the burden of malaria morbidity is similar in populations that vary by as much as a factor of 10 in exposition to transmission. It is notable that the observations made among older children and adults, generally not considered at risk and then too often discarded from the trials and malaria studies, are decisive for a correct assessment of the burden of malaria morbidity in the whole community.

In most of African tropical areas, the level of malaria transmission varies according to the seasons and the rainfall. Figure 2 compares the fluctuations

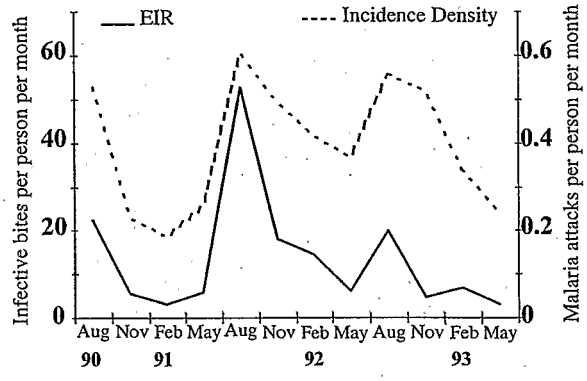

Fig. 2. Entomological Inoculation Rate and Incidence Density of malaria attacks. Children ≤6 yrs, Dielmo, Senegal.

of the entomological inoculation rate and the incidence density of malaria attacks in children in Dielmo. These fluctuations are closely related, and a ten-fold decrease or increase in malaria transmission is associated, in the following weeks, with a two-fold decrease or increase in malaria morbidity. The yearly decrease of malaria transmission in Dielmo, at the end of the rainy season, can be compared to the implementation of a bednet programme, as a ten-fold reduction of transmission is close to the maximum reduction in malaria transmission that has been achieved by impregnated bednets in tropical Africa. Moreover, the corresponding two-fold variation in incidence of malaria morbidity is similar to the variation in parasite incidence or in incidence of malaria attacks that have been observed in the short term trials.

In terms of transmission-morbidity relationship prospects, the concordance between these natural observations and the experimental results of malaria transmission reduction is striking and suggests that, in tropical Africa, a long-term reduction of the burden of the malaria morbidity could only be achieved by a considerable reduction of transmission. This reduction should be much higher than what can presently be obtained on a large scale, or be maintained for more than a few years.

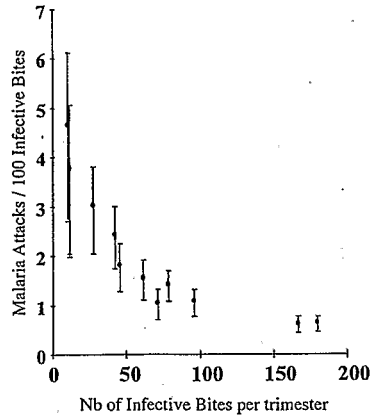

Fig. 3. Risk of malaria attacks per 100 infective bites (confi dence interval 95%) according to the intensity of transmission Children <10 yrs, Dielmo, Senegal.

In addition, there are pieces of evidence that th susceptibility to malaria infection – e.g. the risk o parasitaemia associated with the exposure to an in fective bite – is negatively related to the intensity o transmission (Molineaux and Gramiccia, 1980). Fig ure 3 shows the relationship between the risk o clinical malaria attacks per 100 infective bites an the intensity of transmission. This relationshi seems to follow an inverse exponential function wit a maximum risk when the intensity of transmissio is low and a minimum risk reached with higher lev els of transmission. The same pattern of relationshi is observed whatever the age groups, from childre aged less than 1 year up to adults whose risk is low er. The adjustment of this clinical resistance to th level of transmission seems to be rapid (some day to some weeks). These observations suggest that on or several mechanisms control parasitaemia and oc currence of clinical attacks in a density-dependen manner. These mechanisms may be related to rapidly acquired and short lived density-dependen immune response (against pre-erythrocytic stage? or/and to parasite competition (including an hypo thetical active inhibition of parasite populations b others or a competition between parasites for recep tors, mediators or nutrients).

Incidence, risk and predictive factors

The observations made during short periods in Diel mo suggest that the acquisition of clinical protectio in areas where malaria is highly endemic involves progressive and homogeneous decrease of the prob ability of having a malaria attack (attacks occur les frequently as age increases, in all children), rathe than the acquisition of complete protection by an in creasing number of children (Rogier and Trape 1993). However longitudinal data collected for sev eral years from Dielmo and Ndiop show importan differences between children in acquiring anti-para site and anti-disease immunity. Some children ha only one malaria clinical episode before the age o

two years while others experienced 20 times more malaria attacks during the same period. These differences were undetectable through studies lasting only a few months, as most epidemiological studies of malaria, and were not explainable by the phenotype of haemoglobin, the G6PD activity level, the HLA groups (Dieye *et al.*, 1996, 1997), the use of bednets or the location in the village (manuscript in preparation).

The evidence of the clinical protective effect of cytophilic antibodies (mainly IgG3) against *P. falciparum* (Aribot *et al.*, 1996; Sarthou *et al.*, 1997) and their predictive value for the incidence of severe malaria (J.F. Trape and C. Roussilhon observations) offer the opportunity to test this kind of biological marker in selecting persons who are particularly at risk and in targeting malaria control interventions.

It is well known that women are more susceptible to malaria during pregnancy. Analysing 44 histories of women from Dielmo who were monitored daily during the 33 month-period which covered twelve months before conception, the whole duration of pregnancy, and twelve months after delivery, the incidence rate of *P. falciparum* malaria attacks was, on average, 5.5 (CI95%: 2.6-11.7) times higher during the second and third trimester of pregnancy than during the year preceding conception. This increase in risk of clinical malaria was greater among primigravidae than multigravidae but was detectable up to the fifth pregnancy (Diagne *et al.*, 1997). The possible persistence of this phenomenon during *postpartum* was also investigated. High incidence of malaria attacks persisted three months after delivery. During this period, the incidence rate of malaria attacks was, on average, 7.1 (3.3-15.4) times higher than observed during the year which preceded pregnancy and during the 4th-to-12th month after delivery (Diagne *et al.*, manuscript in preparation). Parasite prevalence and mean asymptomatic parasitaemia were also increased during pregnancy and early *postpartum* compared to other periods. These findings indicate that women are highly susceptible to malaria both during pregnancy and early *postpartum*, and support the hypothesis that pregnancy-associated immuno-suppression, but not only parasite sequestration in the placenta, is the leading mechanism involved in maternal malaria. They suggest also that antimalarial chemoprophylaxis would be useful for the women up to the third month after delivery.

Although it is claimed that health policy must be evidence-based, little is known about the epidemiology and the clinical presentation of mild malaria attacks in the populations, by far the most frequent presentation of clinical malaria. These observations from Dielmo and Ndiop illustrate the power and the usefulness of intensive and longitudinal collection of entomological, parasitological and clinical data from small populations exposed to different levels of transmission for an in-depth understanding of the relationships between transmission, infection and morbidity but also of parasite genetics (Ntoumi *et al.*, 1995; Bottius *et al.*, 1996; Contamin *et al.*, 1996; Daubersie *et al.*, 1996; Ntoumi *et al.*, 1997; Zwetyenga *et al.*, 1998), parasite biology (Pradines *et al.*, 1998, 1999), and the mechanisms of anti-malaria immunity (Behr *et al.*, 1992; Scherf *et al.*, 1993; Thomas *et al.*, 1994; Toure-Balde *et al.*, 1996), as requirements for the development of new anti-malaria strategies. Because of its high sensitivity in malaria case detection (Trape and Rogier, 1995), this type of small community-based studies is also powerful and useful for testing rapidly and cheaply the clinical efficacy of any intervention such as antimalarial vaccines and drug therapy or prophylaxis. As a lot of vaccine candidates and drugs combinations will be screened or tested in the perspective of the 'Roll-Back Malaria' programme, more attention must be given to longitudinal studies of this type.

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