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*Plasmodium falciparum* transmission intensity and infection rates in children in Gabon

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**Abstract** Several factors can determine the outcome of a malarial infection. Studies on susceptibility or resistance to malarial infection can be confounded by differences in transmission. In the present study, the relationship between vector abundance and *Plasmodium falciparum* infection rate of Gabonese children was studied. Indoor human bait catches were conducted in the houses of two groups of children, those who had been found earlier to be either frequently (> 3 infections per year) or rarely (< 0.5 infections per year) infected with *P. falciparum*. The human biting rate was 12 and 31 bites per person per night during the dry and the rainy season, with 3% and 16% *Anopheles*, respectively. *Anopheles gambiae* and *A. moucheti* were found to be the only vectors involved in the transmission of malaria in this area. No significant difference in the abundance and the rate of *P. falciparum* infection of the *Anopheles* mosquitoes was found among children rarely or frequently infected. Differences in transmission cannot account for differences in infection rates in our study group. Hereditary and immunological factors seem to be the primary determinants for the outcome of malarial infection.

**Introduction**

In areas of high malaria transmission, children below 6 years of age are highly susceptible to severe malarial disease leading to hyperparasitaemia, severe anaemia, hypoglycaemia or cerebral malaria. Even though virtually every child in these areas is infected several times during infancy and experiences malarial attacks, only a few children develop severe malaria. Both parasite virulence factors and host hereditary factors could account for this difference in susceptibility (Greenwood et al. 1991).

In a study in Gabon, 200 children with either severe or mild malaria were recruited and followed up actively to determine differences in infection characteristics. These studies showed, firstly, a high variation of infection rates, from over 4 per year in some children to virtually zero reinfection over 3 years of follow-up (Lell et al. 1999). Secondly, it was found that the infection rates depend on immunological and hereditary factors, such as interferon-γ and a nitric oxide synthase 2 promoter polymorphism (Kun et al. 1998a; Luty et al. 1999). Furthermore, children who had had a severe attack also had significantly more reinfections (Lell et al. 1999). Differences in transmission rates, however, could confound these findings, since it is possible that children with frequent malarial infections have a higher probability of developing severe malaria because of greater exposure to virulent parasites and not because of an inherent susceptibility of the host. We therefore undertook a study to compare malaria transmission rates and characteristics in households of children with high versus low infection rates.

**Materials and methods**

The study took place in Lambaréné, a town in the centre of Gabon near the equator. The different districts of the town lie along the Ogooué river and are surrounded by plantations, degraded forest and rain forest. The climate is tropical with 1,500 mm rainfall per year. The area is hyperendemic for *Plasmodium falciparum* malaria, with transmission year-round. The epidemiological and entomological characteristics of the study area have been described in detail elsewhere (Wildling et al. 1995; Sylla et al. 2000).

The study was approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital. All parents or guardians of the children gave informed consent.
The study population consisted of two groups of 100 children each, who were enrolled in 1995 and 1996 with initial severe or mild attacks of malaria. These children were subsequently followed up every 2 weeks (Kun et al. 1998b; Lell et al. 1999). At the time the entomological study took place, children had been followed up for a mean of 2.2 years. Eight children who had been frequently infected (incidence rate > 3 infections/year) and eight children rarely infected (incidence rate < 0.5 infections/year) were compared. These malarial infections refer to *P. falciparum* parasitaemia with signs or symptoms of malaria, including rectal temperatures above 38°C (Lell et al. 1999). The mean ages were 5.4 years and 7.5 years in the two groups, respectively. In these children’s houses mosquitoes were caught indoors during the dry season from July to September 1998 and during the rainy season from January to February 1999. The houses were randomly distributed throughout Lambaréné and were either made of wood (six houses per group) or out of brick (two houses per group). Bed-nets were either not used or ineffective because of damages. All mosquitoes landing on a catcher’s exposed leg were caught with an aspirator before biting. Human biting rate was calculated on the basis of bites per house per night, and all mosquitoes caught were considered to have bitten if not trapped. Catching was performed by a single catcher per house from 8 p.m. to 5 a.m. with a break of 10 min every hour, three times a week for a total of 15 nights. The catchers had experience from a former entomological survey in the area (Sylla et al., 2000). Frequent control visits were done by one of us (E.H.K.S.) and a helper to ensure the accuracy of the catching. Mosquitoes were sorted and the genus identified based on taxonomic criteria. The *Anopheles* species were identified with the key of Gillies and Coetzee (1987) and Gillies and De Meillon (1968). The ovaries of the unfed females were examined as described by Detinova and only the parous were analysed further (Detinova 1962).

Infected mosquitoes were identified by PCR amplification of sporozoites from mosquitoes. DNA was extracted only from the female parous *Anopheles* mosquitoes by the method of Collins et al. (1987). Briefly, head and thorax were ground with a plastic pestle in 0.08 M Tris-Cl, 0.16 M sucrose, 0.06 M EDTA and 0.05% SDS. Proteins were precipitated by adding 8 M potassium acetate to the mosquito homogenate, which was then incubated on ice and centrifuged at high speed. The DNA in the supernatant was purified by two steps washing in 95% and 70% ethanol and finally re-suspended in 50 μl sterile water. The PCR was performed by a modified method of Cheng et al. (1997) in 50 mM Tris-HCl, 3 mM MgCl₂, 200 nM of each nucleotide, and 50 ng primers (Interactiva, Ulm, Germany): 5' ACA TTA TCA TAA TGA (C/T)CC AGA ACT 3' forward; 5' GTT TCC AAT ATT TCT TTT TCT ATC 3' reverse.

Amplification of DNA was performed in capillary tubes using a Rapid Cycler (Idaho Technology) for 35 cycles. A denaturation step at 92°C for 0 s was followed by annealing at 55°C for 0 s and an extension step at 72°C for 15 s. Since times specify settings on the machine, the denaturation and annealing steps took place only during ramp time.

For statistical analysis the paired Wilcoxon test was employed and a *P*-value lower than 0.05 was considered significant.

### Results

The mean incidence rate of *P. falciparum* malaria was 0.17 and 4.25 infections per child per year for children rarely or frequently infected, respectively. Fifteen night-indoor catches in these 16 children’s houses were conducted in both seasons and a total of 2,831 mosquitoes were caught during the dry season and 7,412 mosquitoes during the rainy season, averaging 12 and 31 mosquitoes per house per night (human biting rate). *Anopheles* was the genus caught least often and represented only 3% of all mosquitoes during the dry season and 16% of all mosquitoes caught in the rainy season. On average, 0.3 and 4.9 *Anopheles* were caught per house per night during the dry and the rainy season.

The distribution of the human biting rate of the different mosquito species caught in the houses of rarely or frequently infected children (Table 1) shows no significant difference in the number of *Anopheles* sp. during the dry and the rainy seasons. There were significantly more mosquitoes caught from the children rarely infected in the dry season, but no difference was found in the rainy season.

*A. gambiae* was the only malaria vector found in the dry season and *A. moucheti* was the most frequent vector in the rainy season (Table 2). There were significantly more *A. gambiae* caught in the houses of children frequently infected in the rainy season, but the total number of *Anopheles* mosquitoes was not significantly different between the two groups of children.

A total of 900 (71%) *Anopheles* were parous and of these, 28 were found to be infected (2% of total *Anopheles*), 0.5 infected *Anopheles* per person per night. Seventeen infected *Anopheles* were caught in houses of frequently infected children and 11 in houses of rarely infected children. Table 3 shows the infection rate of the *Anopheles* with *P. falciparum*. There was no significant difference between the infection rate of *Anopheles* caught in the houses of the two groups of children during any season or for any species.

Segregation into groups of children originally admitted into the study with mild or severe malaria showed no difference in mosquito abundance, human biting rate or infection rate. Other factors associated with low re-infection rates in this study group, the NOS2* Lambaréné*