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High Malaria Transmission Intensity in a Village Close to Yaounde, the Capital City of Cameroon

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ABSTRACT A 2-yr longitudinal malaria study was undertaken in a suburb of Yaounde, the capital city of Cameroon, in the village of Simbock, ≈2 km from the city limits. This study allowed assessment of malaria transmission intensity and dynamics in this region before implementation of pyrethroid impregnated bed nets through the national vector control program. Anophelines were captured on human volunteers by pyrethrum spray collections and in resting sites outdoors. Malaria vectors were *Anopheles funestus* Giles, *Anopheles gambiae* s.s. Giles (M and S forms), *Anopheles moucheti* Evans, and *Anopheles nili* Theobald. *An. moucheti* was the most abundant mosquito captured during the study, accounting for >54% of total anophelines caught. The annual *Plasmodium falciparum* Welch entomological inoculation rates measured by enzyme-linked immunosorbent assay were 277 infected bites per human for the first year and 368 for the second year. *An. gambiae* s.s., *An. funestus*, *An. moucheti*, and *An. nili* were responsible for 23.8%, 26.8%, 39.2%, and 10.2% of malaria transmission, respectively. Malaria transmission is perennial throughout the year. All these vectors were highly anthropophagous because only two out of 566 mosquitoes blood-meal tested were not taken on humans.

KEY WORDS *Anopheles gambiae*, *Anopheles funestus*, *Anopheles moucheti*, *Anopheles nili*, malaria, anopheline

IN CAMEROON, AS in many other countries of the central African region, major vectors of malaria are *Anopheles gambiae* s. s. Giles and *Anopheles funestus* Giles present all over the country, *Anopheles nili* Theobald in borders of rivers and streams, *Anopheles moucheti* Evans in forest areas, and *Anopheles arabiensis* Patton in the northern part of the country (Mouchet and Gariou 1966, Carnevale et al. 1992, Njan Nloga et al. 1993). Some vectors considered as minor take a locally active part in malaria transmission. *Anopheles melas* Theobald is probably a good vector in the coastal region. *Anopheles paludis* Theobald, *Anopheles pharoensis* Theobald, and *Anopheles hancocki* Edwards have been found infected by *Plasmodium falciparum* Welch in Cameroon (Pajot and Segers 1964, Robert et al. 1992, Fontenille et al. 2000). *Anopheles wellcomei* Theobald and *Anopheles marshallii* Theobald (or/and *Anopheles hargreavesi* Evans) might occasionally transmit malaria to humans. Many studies conducted in the forest area of south Cameroon in the 1990s proved that malaria vectors relative abundance and transmission intensity differ greatly from one village to another (Fondjo et al. 1992, Manga et al. 1992, Le Goff et al. 1993, Meunier et al. 1999). Significant differences can also be found from year to year in the same locality, especially in rapidly changing environments as is the case in the suburbs of Yaounde, where deforestation is increasing and urbanisation is rising (Manga et al. 1995). Since the Cameroon government is engaging itself in the fight against malaria by implementing vector control programs under the Roll back malaria initiative, we have conducted a longitudinal study of malaria transmission in the village of Simbock, nearby Yaounde, the capital city of Cameroon.

Materials and Methods

Study Area. This study was carried out in a quarter called "block 6" of the village of Simbock (3° 50′ N, 11° 30′ E). About 300 inhabitants were living in this area situated in a rural forest region of Cameroon, only 2 km from Yaounde city. Most of the houses are built in the traditional style with mud walls and roofs of corrugated iron. As time goes by, modern urbanized dwellings come closer and closer to the village. The inhabitants stay inside their houses from ≈2100 hours, and rarely use mosquito netting. Most of the people living in Simbock work in Yaounde town. They go to

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work early in the morning every day, and are back in the evening. The animal life in the village consists of few dogs, occasional goats and pigs, and chickens. There are no bovids. The village is built on the slope of a hill between 700 and 750 m above sea level. The river Mefou, which is ≈100 m away, creates a permanent swamp. Ditches of sand are dug in the valley. The climate is equatorial. There are two rainy seasons, extending from March to June and from September to November. Average annual rainfall during the last 18 yr was 1,466 mm. During the study, rainfalls varied annually: 1,480 mm in 1998, 1,792 mm in 1999, and 1,554 mm in 2000. The average minimum and maximum monthly temperatures, recorded by the national meteorological service, were constantly high, ranging from 18–25°C in July to 20–29°C in March.

Mosquito Collections. Adult mosquitoes were captured monthly from November 1998 to September 1999 and every 2 mo from November 1999 to September 2000. The following four collection techniques were used: (1) adult volunteers captured mosquitoes landing on their legs from 1900 to 0600 hours at the same sites, in three different indoor locations in the village, for two consecutive nights every survey (two teams of volunteers collecting the mosquitoes during each night: one from 1900 to 0100 hours, the other from 0100 to 0600 hours). In total, 132 indoor human-nights were conducted during the study. The human biting rate was expressed as the number of mosquito bites per person per night during each survey sample. (2) From November 1999 to September 2000, collectors were also positioned outdoors at each of the three sites. All volunteers gave their consent for capturing mosquitoes and were given malaria prophylaxis. (3) Pyrethrum spray collections were made in the afternoon inside three to four bedrooms, which were different from the houses used for human bait collections. (4) Outside, resting mosquitoes were collected from a pit shelter, a deserted animal shelter, and in an empty 200-liter barrel.

Field Processing of Anophelines. Anophelines were identified using morphological characteristics according to the identification key of Gillies and De Meillon (1968), and Gillies and Coetzee (1987). Ovaries from a portion of female anophelines captured on human volunteers were dissected to determine parity (Detinova 1962). All the anophelines were stored individually in numbered tubes with desiccant for laboratory processing in Yaounde.

Laboratory Processing of Anophelines. Bloodmeal sources of a sample of females captured by pyrethrum spray were identified by an enzyme-linked immunosorbent assay (ELISA) (Beier et al. 1988). The technique identified human, bovine, ovine (sheep and goat), equine (horse and donkey), pig, or chicken host. The head and thorax of female anophelines were tested for the presence of circumsporozoite protein of *Plasmodium falciparum* welch, *Plasmodium malariae* Laveran, and *Plasmodium ovale* Stephens by ELISA, as described by Burkot et al. (1984) and modified by Wirtz et al. (1987). *Plasmodium vivax* Grassi & Feletti is not present in this region of Africa. The circumsporozoite protein rate and the 95% CI were calculated. The entomological inoculation rate was calculated by multiplying the human biting rate by the circumsporozoite protein rate for each sampling period. A previous work conducted in the same village had shown that CSP Elisa is a valuable method for measuring malaria transmission (Fontenille et al. 2001). Females belonging to the *An. gambiae* complex were identified to species using the polymerase chain reaction (PCR) technique described by Scott et al. (1993). A leg or a wing was placed directly into the reaction mixture containing the species-specific primers, dNTPs, buffer, and polymerase. The length of the amplified sequences was 315 nucleotides for *An. arabiensis*, 390 for *An. gambiae*, and 464 for *An. melas*. Specimens identified as *An. gambiae* were then tested for M and S molecular forms following the recently described diagnostic PCR-based assay (Favia et al. 2001).

Table 1. Number of anophelines captured from November 1998 to October 2000 in Simbock

Species	Feeding		Resting		Total
	Indoors	Outdoors	Bedrooms	Shelters	
An. coustani	1	0	0	1	2
An. funestus	611	92	595	8	1306
An. gambiae	330	58	65	1	454
An. hancocki	2	0	1	0	3
An. moucheti	2,204	440	284	7	2,935
An. nili	493	65	53	1	612
An. paludis	38	6	1	0	45
An. ziemanni	28	2	0	1	31
An. smithii	0	0	0	1	1
Total	3,707	663	999	20	5,389

Results

From November 1998 to September 2000, 5,389 *Anopheles* specimens were captured (Table 1). Of the *An. gambiae* complex females captured, 324 were identified by PCR, and all were *An. gambiae*, 90.5% being from the M molecular form, the remainder being from the S molecular form (Wondji et al. 2002).

The human indoor biting rate for each species varied temporally, depending on the seasons. *An. moucheti*, the main vector in the area, was present throughout the year with a biting rate almost always >10 bites/night (Fig. 1).

Night-biting cycles were similar for every species with an increase in the second part of the night (Fig. 2), and did not vary during the year (data not shown). In total, 80% of the bites of *An. gambiae*, 77% of *An. funestus*, 68% of *An. nili*, and 65% of *An. moucheti* occurred after midnight.

When capturing mosquitoes, both indoors and outdoors, during the same night, from March to September 2000, it was shown that 60.1% of *An. gambiae*, 61.5% of *An. funestus*, 51% of *An. moucheti*, and 56.1% of *An. nili* were captured indoors, suggesting these anopheline populations were mainly endophagic. There was no difference between species, with the exception of *An.*

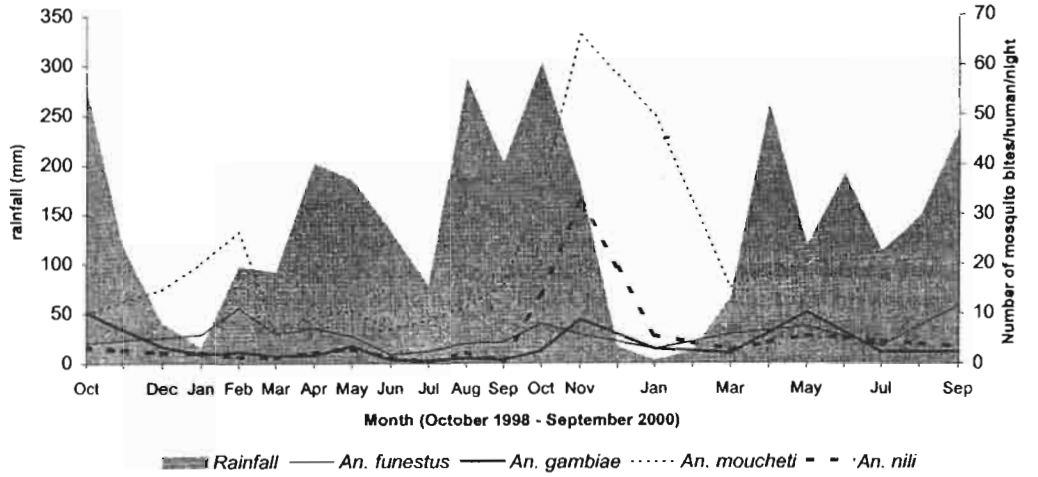

Fig. 1. Rainfall and indoor human biting rates for each vector species in Simbock, from October 1998 to September 2000.

funestus being significantly more endophagic than *An. moucheti* ($\chi^2 = 8.77$, df = 1, $P = 0.003$).

Blood meals from 39 resting *An. gambiae*, 299 *An. funestus*, 192 *An. moucheti*, and 36 *An. nili* were tested by ELISA. All fed on human. Two of them also fed on pig or bovine.

In total, 1,548 anophelines were classified into parous or nulliparous females. The parous rates were the following: *An. gambiae* 78.6% (70.9–85.1 CI), *An. funestus* 77.4% (72.9–81.5 CI), *An. moucheti* 76.4% (73.3–79.3 CI), and *An. nili* 74.2% (67.7–79.9 CI).

The circumsporozoite protein rate was calculated monthly for each species. Overall, 97.8% of identified *Plasmodium* were *P. falciparum* and 2.2% were *P. malariae*. No *P. ovale* infection was found. Among mosquitoes captured landing on human, 5.6% of *An. gambiae*, 4.1% of *An. funestus*, 1.5% of *An. moucheti*, and 1.6% of *An. nili* were tested positive for *P. falciparum*. All four species taken together, these differences were highly significant ($\chi^2 = 35.6$, df = 3, $P < 10^{-6}$), whereas infection rates were similar between *An. gambiae* and *An. funestus* ($P = 0.27$) and between *An. nili* and *An. moucheti* ($P = 0.84$). The CSP rates were not significantly different when calculated only on mosquitoes captured on human, or on resting mosquitoes (Table 2). No other mosquito species were found positive in Simbock.

The annual entomological inoculation rate was 277 (infected bites/humans/yr) from October 1998 to September 1999 and 368 from October 1999 to Sep-

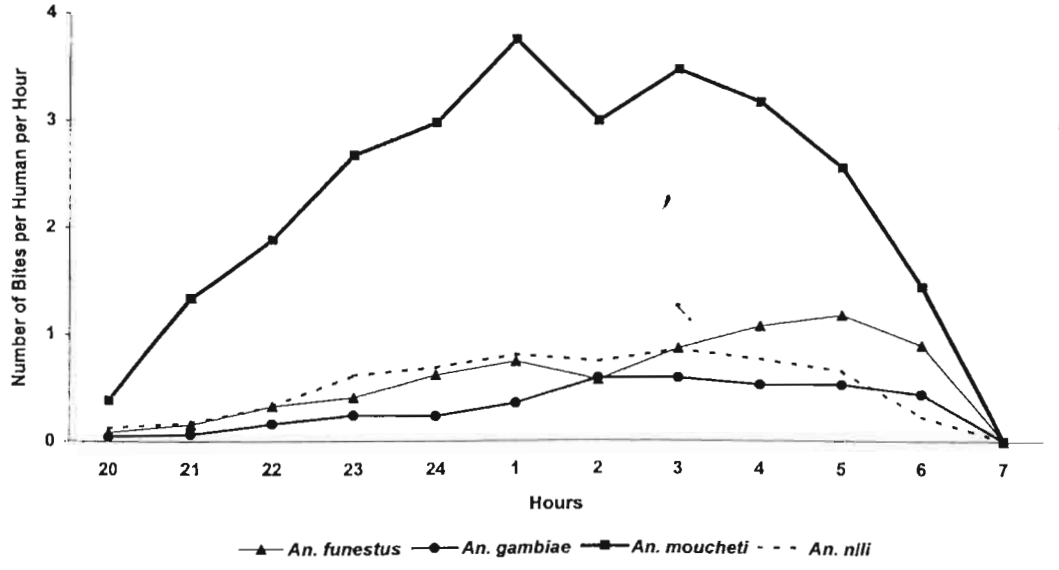

Fig. 2. Indoor night biting cycle (number of bites per human per hour) for each vector species in Simbock, calculated from the 2 yr of captures.

Table 2. Infection rate for *Plasmodium falciparum*, calculated by ELISA from the head-thoraces of mosquitoes captured resting in houses or after landing on human

Species	Resting mosquitoes			Landing mosquitoes		
	No. tested	Positive	CSP rate (95% CI)	No. tested	Positive	CSP rate (95% CI)
An. funestus	593	36	6.07% (4.3–8.3)	682	28	4.11% (2.7–5.9)
An. gambiae	63	2	3.17% (0.38–11.0)	356	20	5.62% (3.5–8.5)
An. moucheti	284	4	1.41% (0.38–3.57)	2425	36	1.48% (1.04–2.04)
An. nili	53	1	1.89% (0.05–10.1)	564	9	1.6% (0.73–3.01)

tember 2000 (Table 3). *An. gambiae, An. funestus, An. moucheti,* and *An. nili* were responsible for 23.8, 26.8, 39.2, and 10.2% of *P. falciparum* transmission, respectively. Transmission was continuous, and reached its peak in January 1999 and May 2000 when an average of 1.65 infected bites per human per night was observed indoors. Peaks of transmission of the four vector species did not occur at the same time as shown in Fig. 3.

Discussion

Rainfall almost every month during the 2 yr of our survey and the presence of different kinds of mosquito larval development sites (e.g., a permanent river, a marsh, borrow pits, rain pools) explain the sympatric presence of the four main African malaria vectors in Simbock. Density cycles of *An. gambiae, An. funestus, An. moucheti,* and *An. nili* depend partly on rainfall. For each species, the lower human indoor biting rates were observed at the end of the dry season, around March.

Table 3. Annual entomological inoculation rates (number of infected bites per human per year) per vector in Simbock

Species	First year	Second year	% (over 2 yr)
An. funestus	88.1	85	26.8%
An. gambiae	58.4	95.1	23.8%
An. moucheti	112.2	140.4	39.2%
An. nili	18.1	47.4	10.2%
Total	276.8	367.9	

In Simbock, as in most of central Africa, sporozoite rates were three times higher in *An. gambiae* and *An. funestus* than in *An. moucheti* and *An. nili* (Fontenille and Lochouarn 1999). However, the proportion of females fed on humans among mosquitoes resting inside as well as parity rates were similar between the different vector species, suggesting a higher vectorial competence for *An. gambiae* and *An. funestus.*

The total entomological inoculation rate was around 300 infected bites per human per year during both years surveyed. Even if CSP Elisa overestimates the true transmission level by a factor 1.12, as was shown recently (Fontenille et al. 2001), the level of the transmission remains very high for a village located in the suburbs of the capital city. *An. moucheti,* which is often considered a minor vector as compared with *An. gambiae* or *An. funestus,* was the main vector in Simbock. Previous studies conducted in Yaounde or in nearby closely suburbs reported annual entomological inoculation rate ranging from 3 to 33, generally only

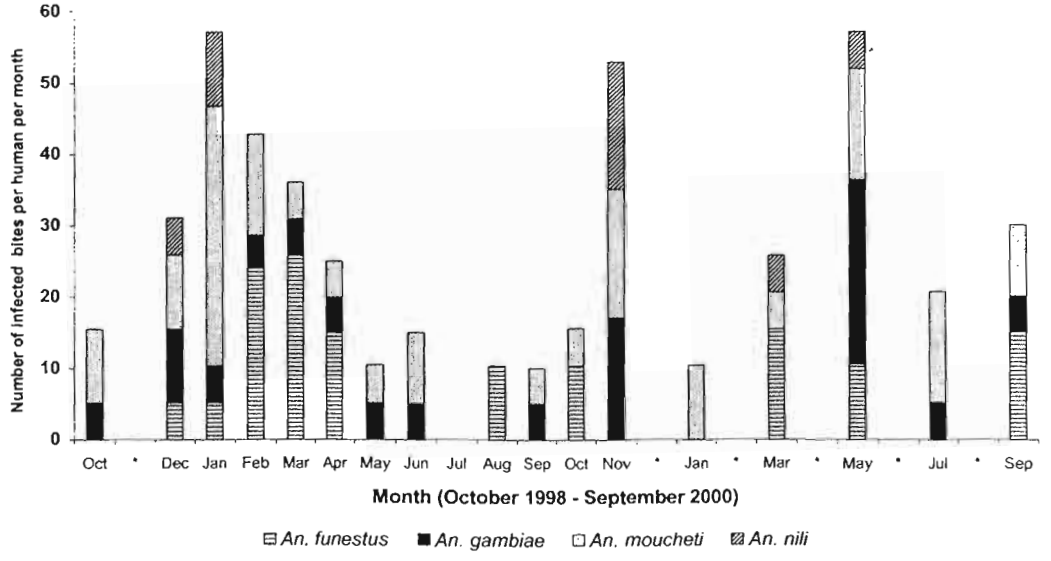

Fig. 3. Monthly entomological inoculation rate for each vector species in Simbock, from October 1998 to September 2000. (*No survey this month).

due to *An. gambiae*, the other being very rare vector species (Fondjo et al. 1992, Manga et al. 1992, Nimpaye et al. 2001).

Although transmission intensity is usually low in most African cities (Robert et al. 1986, Lindsay et al. 1990), such a high level of transmission in suburban areas has been observed in several countries. In 1991 and 1992 in the town of Bouake, Cote d'Ivoire, Dossou-Yovo et al. (1998) observed an annual entomological inoculation rate reaching 155 in some areas. In Kinshasa (DRC former Zaire), Coene (1993) observed an annual entomological inoculation rate of 30 within the city, whereas the entomological inoculation rate reached 455 in a suburban village located 15 km away from the city center. In Brazzaville (Congo), Trape and Zoulani (1987) showed a high heterogeneity in malaria transmission, with an annual entomological inoculation rate >100 infective bites per human per year in some areas.

In areas like Simbock, because plasmodia are transmitted at a high rate and continuously throughout the year by four different vector species, implementation of an efficient vector control strategy could not be easily attainable, despite the high anthropophily and endophily of the vectors. The village, located ≈2 km from classic urban areas, constitutes a "reservoir" of parasites and vectors for the more urbanized neighborhoods (Quakyi et al. 2000). However, the rapid extension of typical urban settings around Yaounde could, very probably, modify the malaria pattern in Simbock in the near future, decreasing the transmission and reducing density of species such as *An. moucheti* and *An. nili*.

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