

Malaria Transmission and Vector Biology on Sainte Marie Island, Madagascar

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ABSTRACT A 17-mo longitudinal malaria survey (November 1988–March 1990) was carried out on Sainte Marie Island, an area on the east coast of Madagascar which is frequently visited by tourists. During 706 man-nights of capture, 46,401 mosquitoes belonging to 32 species were caught. Sporozoite rates were determined by ELISA and incriminated *Anopheles gambiae* Giles s.s., *An. funestus* Giles, and *An. mascarensis* De Meillon as vectors of malaria. *An. gambiae*, the main vector, was highly anthropophilic but largely exophilic. Transmission by this species occurred mainly from November to April; the overall circumsporozoite antigen positivity rate was 1.7%, with a maximum of 3.2% in March–April. The nightly peak of transmission occurred between midnight and 0400 hours. The annual inoculation rate was calculated to be 100 infective bites per human, of which 92 were of *Plasmodium falciparum*. *An. funestus* played a minor role in transmission. *An. mascarensis*, an anopheline endemic to Madagascar, was incriminated for the first time, as a malaria vector. The sporozoite rate in this species varied from 0.4 to 0.9% as shown by both ELISA and salivary gland dissections. Parasite indices in humans up to 20 yr of age fluctuated during the year from 64 to 80%. Bed nets are recommended for malaria protection for the local population and tourists.

KEY WORDS Insecta, *Anopheles* spp., malaria transmission, Madagascar

MALARIA TRANSMISSION was well studied in Madagascar until the 1960s. Long-term studies have not been carried out since 1962, after which effective vector control programs were conducted (Chauvet et al. 1964). The known vectors on Madagascar are *Anopheles gambiae* Giles s.l. and *An. funestus* Giles (Grjebine 1966). Depending on the region, malaria transmission ranges from seasonally discontinuous with one or less infective bites per year to perennial with >100 infective bites per year (Fontenille 1991).

Following a malaria outbreak, which occurred on the High Plateau in the tropical altitude region from 1985 to 1989, investigations were initiated to evaluate concurrently the malaria transmission rates near Antananarivo, the capital city on the highland (Fontenille et al. 1990), and on Sainte Marie Island off the wet tropical eastern coast. This article describes the results of a 17-mo entomological and parasitological survey on Sainte Marie Island from November 1988 to March 1990. Malaria transmission has been considered to be perennial in this region, with *An. gambiae* s.l. as the only vector (G. Chauvet, unpublished data; Grjebine 1966). A short-term

parasitological survey was conducted on the island in August 1988 (Lepeers et al. 1989).

During the past 5 yr, tourism has increased on Sainte Marie Island, and accurate data on malaria ecology will be necessary before control measures can be initiated. The specific objectives of the present study were to describe the biology of the vectors, to determine the prevalence of the *Plasmodium* species, and to estimate the sporozoite, inoculation, and infection risk rates.

Materials and Methods

Study Area. Sainte Marie is an island 7 km off the east coast of Madagascar, 50 km long and 7 km wide, which is inhabited by about 16,000 humans and 600 bovines. The climate is similar to that of the east coast of Madagascar, with 3,500 mm of rainfall per year and no dry season. The mean minimum and maximum temperatures, respectively, are >20 and 24°C during the cool season and >24 and 28°C during the warm season from October to May.

The only two villages of the island with a health center and a secondary school were selected for the study: Ambodifotatra, the subprefecture (17° 00' S, 49° 88' E) where the hospital is located, and Lonkinty, 17 km to the north. Most dwellings were constructed of wood with roofs of palm thatch or corrugated iron. Occasional entomological investigations also were carried out at

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Table 1. Total number of mosquitoes biting man on 706 human-nights from November 1988 to March 1990 on Sainte Marie Island, Madagascar

Species	Indoors	Outdoors	Total	Bites/human/night
No. human nights	353	353	706	—
<i>An. coustani</i>	1,407	3,712	5,119	7.2
<i>An. squamosus-cydippis</i>	108	548	656	0.9
<i>An. gambiae</i> s.l.	5,754	5,878	11,632	16.5
<i>An. mascarensis</i>	4,783	7,979	12,762	18.1
<i>An. funestus</i>	230	99	329	0.5
<i>An. flavicosta</i>	270	964	1,234	1.8
<i>An. brunnipes</i>	34	205	239	0.3
<i>An. pharoensis</i>	0	1	1	—
<i>An. maculipalpis</i>	0	2	2	—
<i>An. grassei</i>	0	3	3	—
Other Culicidae	4,526	9,898	14,424	20.4
Total	17,112	29,289	46,401	65.7

two hamlets, Saint Joseph and Marômandia, near the villages. Annual DDT insecticide spraying for malaria control was stopped in 1959. Although occasional, localized spraying occurred after 1960, these villages had not been sprayed with DDT for 3 yr before the study took place.

Mosquito Collection. Entomological surveys were conducted for 4–8 d/mo from November 1988 to March 1990. Mosquitoes were collected from 1800 to 0600 hours host-seeking indoors and outdoors at human bait (four groups of two baits each: two groups staying indoors and two staying outdoors at each of two selected houses). Collectors were replaced at midnight. Collections in each hour were counted separately. An average of 11 dwellings each month was sampled in early morning by hand catch. Two collections also were made in July and November 1989 by pyrethrum spraying in Saint Joseph, Marômandia, and an area of Ambodifotatra. Outdoor-resting mosquitoes were collected in crevices, a pit shelter, and under the thatched roof of a cow shed using mouth aspirators. Each mosquito was identified microscopically the morning after collection.

Mosquito Processing. Species of the *An. gambiae* complex were identified by examining polytene chromosomes of the ovarian nurse cells of 65 specimens caught in December 1988 and in January, February, and June 1989 (Coluzzi et al. 1979), and by polymerase chain reaction (PCR) by F. H. Collins (CDC, Atlanta), using abdomens preserved dry (Paskewitz & Collins 1990). Origin of the blood meals of 93 mosquitoes caught resting indoors was determined by ELISA, using the method described by Beier et al. (1988). The parity rate was estimated each month by ovarian dissection of random samples of biting mosquitoes (Detinova 1962). Heads and thoraces of all specimens of *An. gambiae* s.l. and *An. funestus* were preserved in registered vials with desiccant. The infected mosquitoes were identified by an ELISA using monoclonal antibodies against the circumsporozoite protein (CSP) of the four human *Plasmodium* species, as described by

Burkot et al. (1984) and modified by Wirtz et al. (1987). Specimens of *Anopheles mascarensis* De Meillon caught from September 1989 to March 1990 were tested, either by ELISA or by salivary gland dissection followed by ELISA, when sporozoites were observed microscopically. Specimens caught before September 1989 were not processed because this species was reputed to be a nonvector. The circumsporozoite rate (CS positivity index by ELISA = CSpi), inoculation rate ($h = ma * CSpi$, where ma is the number of bites per human per night), and monthly inoculation risk were calculated following Krafsur & Armstrong (1978). The hourly inoculation rate also was calculated as the number of bites per human per hour multiplied by the sporozoite rate observed during that hour.

Parasitology. Malaria infection was determined in November 1988 and in March and June 1989 of 867, 624, and 502 individuals, respectively (ranging from newborns to 20 yr of age), from Ambodifotatra and Lonkinty who were being medically examined at the schools. Thick films stained with Giemsa were examined until 1,000 leukocytes had been seen.

Results

Mosquito Bionomics. In 202 houses searched, 140 *An. gambiae* s.l. and 62 *An. funestus* were captured resting indoors. In 706 human-nights over 17 mo, 46,401 mosquitoes belonging to 32 species were caught at human bait (Table 1). The most abundant anopheline species were *An. mascarensis* (18.1 females per bait-night), *An. gambiae* s.l. (16.5 per bait-night), and *An. coustani* Laveran (7.3 per bait-night). Only 329 *An. funestus* were caught at human bait.

In total, 65 specimens of the *An. gambiae* complex, caught during the cool and the warm seasons, were unequivocally identified by examining the polytene chromosome from ovarian nurse cells; all were shown to be *An. gambiae* s.s. These identifications were confirmed on three additional specimens tested by PCR. Of 65 *An.*

Table 2. Monthly human-biting and *Plasmodium* CSP rates and inoculation risk for *An. gambiae*, *An. funestus*, and *An. mascarensis*

Date (No. human-nights)	<i>An. gambiae</i>				<i>An. funestus</i>				<i>An. mascarensis</i>				Monthly risk (g + η) ^d
	ma ^a	n ^b	CSPR	(95% CI) ^c	ma ^a	n ^b	CSPR	(95% CI) ^c	ma ^a	n ^b	CSPR	(95% CI) ^c	
Nov.-Dec. 1988 (96)	26.6	1,904	2.3	(1.6-3.0)	1.23	78	2.6	(0.4-8.4)	7.5	0	—	—	0.999
Jan.-Feb. 1989 (82)	47.5	2,274	0.9	(0.5-1.3)	0.67	64	0	(0-5.6)	14.5	0	—	—	0.999
March-April 1989 (96)	8.2	787	3.2	(2.0-4.4)	0.35	37	0	(0-9.5)	16.6	0	—	—	0.999
May-June 1989 (96)	3.0	289	1.7	(0.6-3.5)	0.49	54	0	(0-6.6)	35.9	0	—	—	0.78
July-Aug. 1989 (112)	1.3	146	2.1	(0.5-5.1)	0.34	62	0	(0-5.8)	19.9	0	—	—	0.49
Sept.-Oct. 1989 (96)	2.7	272	0.4	(0.1-1.9)	0.18	21	0	(0-16.1)	11.5	503	0.4	(0.1-1.1)	0.25
Nov. 1990 (48)	28.9	1,443	1.6	(0.9-2.2)	0.17	9	0	(0-33.6)	6.4	248	0.8	(0.2-2.8)	0.999
Jan. 1990 (48)	29.5	1,424	1.5	(0.9-2.2)	0.17	9	0	(0-33.6)	33.3	545	0.9	(0.3-2.1)	0.999
March 1990 (32)	28.4	914	2.3	(1.3-3.3)	0.13	4	0	(0-60.2)	17.9	568	0.9	(0.3-2.0)	0.999

^a Number of bites per human per night.

^b Number of mosquitoes tested for sporozoites.

^c Mean circumsporozoite positivity rate (95% confidence interval).

^d Infection risk from *An. gambiae* and *An. funestus* calculated by month by the method of Krafsur & Armstrong (1978).

gambiae identified chromosomally, 62 yielded preparations that could be examined for chromosomal polymorphisms. The sample was found polymorphic only for the paracentric inversion 2La, including 20 standard homokaryotypes +/+, 31 heterokaryotypes +/a, and 11 inverted homokaryotypes a/a. These karyotype frequencies are consistent with the Hardy-Weinberg equilibrium ($\chi^2 = 0.029$, $df = 1$, $P > 0.8$).

The highest number of *An. gambiae* bites per human per night was observed in both villages in January (the warm season); the lowest number of bites was observed in August in Ambodifotatra and in September in Lonkinty. Specimens of *An. funestus* were rare throughout the year. *An. mascarensis* was most abundant at the beginning of the cool season from May to July (Table 2). The night-biting peak of both *An. gambiae* and *An. funestus* occurred between 0100 and 0400 hours, and *An. mascarensis* was collected most frequently between 2200 and 0200 hours.

Feeding and Resting Behavior. Endophagy of *An. funestus* was high; 70% of specimens were taken on human bait indoors. Corresponding proportions were 49.5% for *An. gambiae* and 37.5% for *An. mascarensis*. The proportion of indoor feeding by *An. gambiae* caught at human bait varied during the night from 54.6% before 2300 hours to 47.9% after 2300 hours ($\chi^2 = 36.21$, $df = 1$, $P < 0.001$). Of 78 *An. gambiae* and 15 *An. funestus* that were blood fed and collected resting indoors, all were found to have fed on humans. Although the study was not designed to measure the degree of endophily precisely, indoor-resting *An. gambiae* and *An. funestus* were sampled during each month in selected houses. From 161 bedrooms inspected, a mean of 0.18 *An. gambiae* per room (indoor resting density: IDR) were hand caught during the cool season (73 rooms from April to September) and 0.76 during the warm season (88 rooms). The corresponding estimates for *An. funestus* were 0.37 and 0.30. More efficient pyrethrum spray catches

in 41 bedrooms yielded 60 *An. gambiae* and 9 *An. funestus*. No mosquitoes were found in holes in walls or under the roof of a cow shed, and only two *An. gambiae* were caught in a pit shelter in January 1989.

Age Structure and Malaria Parasite Infection. The parity rate of females collected at human bait was estimated to be 82.2% for 2,642 *An. gambiae* and 86.1% for 137 *An. funestus* dissected from November 1988 to January 1990. The parity rates of *An. gambiae* varied among months from 73.8% in May-June 1989 ($n = 271$ females dissected) to 91.5% in July-August 1989 ($n = 141$). In November 1989, the parity rate among 99 *An. mascarensis* was 67%.

The presence of CSP was determined by ELISA for 9,453 *An. gambiae* s.l. and for 338 *An. funestus*. *An. mascarensis* specimens were tested only in September 1989 and from November 1989 to March 1990; 1,864 females were tested by ELISA, and 237 of these also were examined by salivary gland dissection (Table 2). One hundred and sixty-five *An. gambiae* were positive by ELISA; 92.1% of the positive mosquitoes were infected with *P. falciparum*, 7.3% with *P. vivax*, 1.8% with *P. malariae*, and 1.8% with *P. ovale*. Four had multiple infections. The identification of four of the circumsporozoite-positive *An. gambiae* was confirmed by the cytogenetic method or by PCR. The two positive *An. funestus* were infected with *P. falciparum*. Two of the 237 dissected *An. mascarensis* showed sporozoites in their salivary glands. After observation, the salivary glands were removed, preserved dry, and later tested by ELISA. Fourteen *An. mascarensis* specimens were CSP positive for *P. falciparum* (2 positive by dissection and 12 that were not dissected).

The CSP index for *An. gambiae* ranged from 0.4% in September-October (95% CI, 0.1-1.9) to 3.2% (2.0-4.4) in March-April 1989. Infected *An. funestus* were observed only in November 1988. The CSP index for *An. mascarensis* ranged

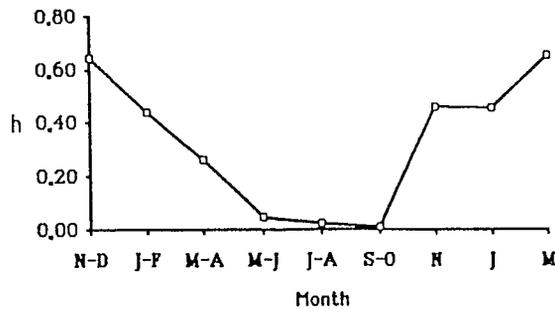


Fig. 1. Inoculation rate per night for *An. gambiae* from November 1988 to March 1990.

from 0.4% (0.1–1.1) in September to 0.9% in January (0.3–2.1) and March (0.3–2.0). Sporozoite inoculation rates were calculated for *An. gambiae* and *An. funestus* and ranged from 0.65 infected bites per human per night in November–December 1988 to 0.01 in September–October 1989 (Fig. 1). The mean number of infected bites per night averaged 0.29 for the 17-mo survey in Ambodifotatra and 0.28 in Lonkinty. The monthly infection risk from *An. gambiae* and *An. funestus* exceeded 0.99 during the warm season and ranged from 0.25 to 0.77 during the cooler months. The monthly *An. mascarensis* inoculation rate was 1.3 in September and 9.3 in January 1990, and the monthly infection risk was 0.72 and 0.99, respectively.

From the hourly human-biting rate and the hourly sporozoite rate, the *An. gambiae* inoculation rate was calculated for each hour of the night (Fig. 2). No infected bites were recorded before 2000 hours, and the peak of transmission occurred between 0200 and 0300 hours because of increases in both the sporozoite and the human-biting rates.

Table 3 shows parasite indices for all *Plasmodium* species and for *P. falciparum* by age group and by month. Parasite indices fluctuated during the year, with a peak (80.5% of 502 subjects) in June at the end of the warm season and a low value (63.8% of 873 subjects) in November at the end of the cool season ($\chi^2 = 49.9$, $df = 2$, $P < 0.001$). Gametocytic indices did not follow similar seasonal variations. Highest parasite indices were observed in the 10–15-yr age group and in the 5–9-yr age group for gametocytic indices. *P. falciparum* represented 82% of all the *Plasmodium* observed, whereas *P. vivax*, *P. malariae*, and *P. ovale* represented 2, 15, and 1%, respectively. Double infections were observed in 18.8% of positive blood slides for *P. falciparum* + *P. vivax*, 0.8% for *P. falciparum* + *P. ovale*, and 18.1% for *P. falciparum* + *P. malariae*. Except in June, parasite indices were significantly higher in Lonkinty than in Ambodifotatra.

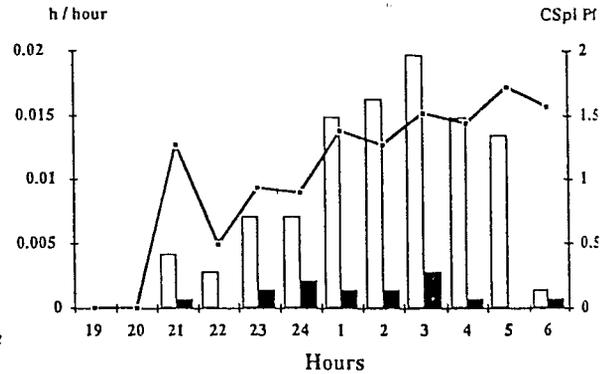


Fig. 2. Circumsporozoite positivity index for *P. falciparum* (CSpi Pf) (■), mean inoculation rate per hour by *An. gambiae* for *P. falciparum* (□), and for other *Plasmodium* (*P. vivax*, *P. malariae*, *P. ovale*) (■).

Discussion

Malaria transmission and the mosquito fauna of Sainte Marie had not been well studied before this survey. *An. funestus* was recorded for the first time. Of the 46,401 mosquitoes caught on human bait, *An. mascarensis* was the most abundant species with peak abundance at the beginning of the cool season, whereas *An. gambiae* had peak abundance during the rainy season. Among the species of the *An. gambiae* complex, only *An. gambiae* s.s. was collected, agreeing with the previous identification of this species on Sainte Marie Island by Chauvet (1969). The chromosomal constitution of the *An. gambiae* population was identical to that of populations from forest or humid savannah areas of eastern continental Africa. We did not find *Anopheles arabiensis* Patton, which has been recorded on the east coast of Madagascar opposite Sainte Marie (O. Ralisoa Randrianasolo & M. Coluzzi, personal communication). In Madagascar, *Anopheles merus* Doeritz has been reported only on the west coast (Chauvet 1969).

Only 140 *An. gambiae* specimens were collected resting indoors, whereas 5,754 were collected seeking hosts indoors at human bait. We felt that inefficient sampling methods were not responsible because in other areas of Madagascar, >100 anophelines per room were caught using the same methods (at Ankazobe) (D.F., unpublished data). *An. funestus* was more endophilic; 62 females were captured resting indoors but 230 were captured seeking hosts indoors on human bait. According to Molineaux et al. (1979), one can compare the degree of exophily of *An. gambiae* and *An. funestus* using two estimates of abundance: (1) the mean human-biting rate indoors (ma_i) of 16.3 and 0.65 females per bait per night, respectively; and (2) the indoor resting density (IRD, estimated by hand catch in the same villages on the mornings after

Table 3. Parasite and gametocyte prevalence by age and by month in Sainte Marie (Ambodifotatra and Lonkinty)

Date	Parameter ^a	Age, yr				All
		0-4	5-9	10-15	16-20	
Nov. 1988	n	175	269	293	136	873
	PI	48.6	66.2	74.4	55.9	63.8
	PIf	48.0	64.3	72.0	55.9	62.3
	GI	5.1	19.3	17.4	4.4	13.5
March 1989	n	122	212	167	123	624
	PI	64.7	82.1	82.0	64.2	75.2
	PIf	63.9	80.2	79.0	58.5	72.4
	GI	17.2	19.8	13.2	5.7	14.7
June 1989	n	7	232	236	27	502
	PI	28.6	78.0	85.2	74.0	80.5
	PIf	28.6	73.7	82.2	33.3	74.9
	GI	14.3	17.7	8.9	3.7	12.7

^an, number of children examined; PI, percent positive for all *Plasmodium*; PIf, percent positive for *P. falciparum*; GI, percent positive for gametocytes.

the night-biting collections) of 0.49 and 0.33 females per room, respectively.

Assuming that the above estimates of m_a and IRD are unbiased, or are biased to the same degree for *An. gambiae* and *An. funestus*, we calculate an IRD/ m_a ratio of 0.030 for *An. gambiae* and 0.53 for *An. funestus*. Assuming also that the *An. funestus* endophilic rate is 100%, the *An. gambiae* endophilic rate is $\approx 5.7\%$. This marked difference between these species is consistent with earlier observations by G. Chauvet (unpublished data).

The outdoor daytime resting places of these species are not known, although they are probably peridomestic. No mosquitoes were collected in a cattle shed that was built, and only two *An. gambiae* were collected in pit shelters.

The duration of the *An. gambiae* and *An. funestus* gonotrophic cycles is not known in this area. Grjebine (1966) estimated that, in Madagascar as a whole, the cycle lasted 3 d (including the pregravid meal) in nulliparous females of *An. gambiae* s.l. and 2 d in parous females. Using parity rates observed in the present study, the probability of daily survival (p) was estimated to be 0.91 for *An. gambiae* and 0.93 for *An. funestus* for the entire survey and 0.82 for *An. mascarensis* in November 1989. Hence, the expectation of life infective for *P. falciparum* at 24°C (sporogonic cycle duration of 11 d) was ≈ 3.5 d for *An. gambiae*, 6 d for *An. funestus*, and 0.6 d for *An. mascarensis* in November. From the high human blood index (a) of indoor-resting anophelines ($\approx 100\%$) and the long expectation of life, the index of stability (Macdonald 1956) was high (10.2 for *An. gambiae*). The number of infected bites per human per year by *An. gambiae* was calculated to be 100, of which 92 were with *P. falciparum*, with a seasonal peak during the warmer months.

For the first time, *An. mascarensis*, a mosquito endemic to Madagascar, was incriminated as a

malaria vector. Previous authors dissected salivary glands from 5,932 females from the eastern and western regions of Madagascar (but not from Sainte Marie) and never observed sporozoites (Grjebine 1966). For this reason, specimens of *An. mascarensis* were not processed by ELISA at the beginning of the survey. However, sporozoites were found in *An. mascarensis* in September 1989. From then until March 1990, the sporozoite indices were always $< 1\%$. However, because the human-biting rate was 33 per night in January 1990, the monthly inoculation rate was 9 for this month, nearly as high as for *An. gambiae*. The relative contribution to transmission by *An. mascarensis* may be particularly important during the cool months, when this species is much more abundant than *An. gambiae*.

The parasite indices in the human population varied concurrently with the inoculation rate. This rate is lowest from June to October, and the parasite index over all age groups was found to be lowest in November. Similar situations have been observed in other areas in western and eastern Africa (Fontaine et al. 1978, Gazin et al. 1988). The high malaria transmission level, mainly of *P. falciparum*, may have a serious effect on tourist development currently under way in Sainte Marie. Insecticide spraying of village houses is likely to be ineffective because of the exophilic resting behavior exhibited by this population of *An. gambiae*, even if some females sit on the house walls one moment before or after biting, and because of the difficulties of sustaining such a campaign in this country. With > 100 infective bites per year, the island's adult population is generally immune. The risk of developing patent infection exists mainly in young children and nonimmune tourists. Because villagers sleep indoors, impregnated mosquito nets could be used effectively to limit contact with vectors (Rozendaal 1989). In view of the marked late-night peak of transmission between midnight

and 0400 hours, if villagers slept under undamaged bed nets after 2200 hours, this may reduce their risk of an infective bite by 93%. The widespread use of impregnated bed nets in the villages may reduce the human-biting and sporozoite rates in the vector population. However, the efficacy of such a program, as measured by the decrease of prevalence of infection and incidence of clinical disease, must be evaluated in this area of high and continuous transmission. This control program also would reduce the risk of malaria transmission to tourists. Additional protection for this nonimmune group could be secured by individual protective measures (repellents and chemoprophylaxis) and by providing impregnated bed nets in hotels.

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