Malaria Vectors in the Municipality of Serra do Navio, State of Amapá, Amazon Region, Brazil

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We conducted a survey to determine the vectors of malaria in six localities of Serra do Navio municipality, State of Amapá, from 1990 to 1991. Malaria infection rates of 29.3%, 6.2% and 20.4% were detected by human blood smears in Colônia Água Branca, Porto Terezinha and Arrependido, respectively. There was no malaria infection detected in Serra do Navio. Fifteen species were identified among 3,053 anopheline mosquitoes collected by human bait and 64.4% were identified as Anopheles albitarsis *s.l., 16.7%* An. braziliensis, *9.5%* An. nuneztovari *and 5.8%* An. triannulatus. An. darlingi, *the main vector of malaria in the Amazon region of Brazil, was scare. Using enzyme-linked immunosorbent assay (ELISA), a total positive rate of 0.8% (23/2876) was found for six species: fifteen* An. albitarsis *s.l., four* An. nuneztovari, *and one of each:* An. braziliensis, An. triannulatus, An. oswaldoi *and* An. rangeli. *Nine of 23 positive mosquitoes were infected with* Plasmodium malariae, *eight with* P. vivax *VK210, three with* P. vivax *VK247 and three with* P. falciparum. *Since* An. albitarsis *s.l. was collected feeding on humans, was present in the highest density and was positive by ELISA for malaria sporozoites, it probably plays an important role in malaria transmission in this area.*

Key words: mosquito - *Anopheles* - malaria vector - *Plasmodium* - Brazil

Since the 80's, several studies on malaria transmission have been carried out in different parts of the Brazilian Amazon and, based on dissections, enzyme-linked immunosorbent assays (ELISA) and anthropophilic behavior, *Anopheles darlingi* Root, 1926 was found to be the main vector. Other species such as *An. albitarsis s.l.* Lynch-Arribalzaga, 1878, *An. deaneorum* Rosa-Freitas, 1989, *An. oswaldoi* (Peryassú, 1922), *An. mediopunctatus s.l.* Theobald, 1903 (now a complex of three species – Sallum et al. 1999) and *An. triannulatus* (Neiva and Pinto, 1922) are considered to be potentially regional vectors (Arruda et al. 1986, Tadei et al. 1988, Deane 1988, Lourenço-de-Oliveira et al. 1989, Klein et al. 1991, Branquinho et al. 1996) and, more recently, *An. marajoara* Galvão and Damasceno, 1942 has been determined to be a new emerging malaria vector near Macapá, State of Amapá (Segura 1998). Despite the high prevalence and wide distribution of human malaria in Brazil, there are areas or states such as Amapá where little information about abundance and identification of anopheline mosquitoes, or their vector status is available.

The present study was conducted to identify anopheline mosquitoes and to determine their role as potential vectors in each of six localities in the Serra do Navio (SNV) municipality, in Amapá.

MATERIALS AND METHODS

Study site - The study area is the municipality of SNV (0°57'00"S, 52°00'49"W; elevation 380 m) located 146 km north of Macapá, the capital of Amapá. The climate is that of a tropical rainforest with a rainy season in which 85% of the precipitation generally occurs, and a dry season. From December to March it rains several times every day with high intensity but short duration; from April to June the rain is continuous and heavy; and from July to November (the dry season) the rain is less frequent and of lower intensity. The mean rainfall for 1990 was 293.6 mm (January-June) and 129.1 mm (July-December).

SNV was created in 1947, when Icomi (Indústria e Comércio SA) received a license to explore mineral resources in this area for a period of 50 years (Gusmão 1991). From 1954 to 1960, the company constructed an industrial park and a residential village to accommodate a population of about 2,000-2,500 inhabitants. The administrative, industrial and residential areas (SNV treated area) were completely isolated from one another. Facilities included a water purification plant, a sewage treatment plant, rain water drainage, a rubbish incinerator and a hospital. A disease control program was initiated, aimed mainly at malaria and leishmaniasis, that consisted of water purification, houses with window and door screening, biannual residual insecticide (DDT) spraying, domestic animal control and administration of chloroquinised salt to every inhabitant. The three localities (CAB: Colônia Água Branca, PT: Porto Terezinha, and ARR: Arrependido) are small villages which have a standard of living in marked contrast with that of the population of SNV (the treated area). The houses in these villages are made of wood with roofs of zinc panels, strong plastic or dry palm leaves, and there has been no vector control to reduce parasite transmission.

Mosquito collection - Anopheline mosquito collections were carried out four times, twice in each year, for about 20 days during each of the wet and dry seasons. Different collection sites were chosen within each locality. These included SNV (the treated area), SNV the forest, and SNV Carpintaria, as well as CAB, PT and ARR. Carpintaria was separated from SNV because, although people from SNV work there during the day, no one stays there at the time of peak biting activity of anophelines. The forested area was part of SNV, located between SNV and CAB, PT and ARR. It was not treated. Each sampling site was visited for at least three days during each trip, by a protozoologist and three entomological technicians. Human bait catches were made peridomestically (indoors and outdoors) in the residential areas and light traps (Shannon and CDC) were used in the forest. Collection periods were 18-22 h and in addition, at least one 12 h collection was conducted in each locality. Mosquitoes were collected by pairs of collectors and a rotating catch timetable was used to prevent a bias due to personal differences in attraction and skill. The mosquitoes were separated at the time of collection into glass tubes. The identification of adult anophelines was carried out using taxonomic keys (Forattini 1962, Faran & Linthicum 1981).

Detection of sporozoites - All anophelines from light traps and human bait captures were tested for the *Plasmodium* circumsporozoite protein (CSP)

by ELISA (Wirtz et al. 1987). Specimens were identified, placed in capped vials and kept in plastic bags containing silica gel. In the laboratory at Evandro Chagas Institute, Belém, specimens were stored at -20°C until tested. The head and thorax of each specimen was triturated prior to use in the test. Positive controls for each *Plasmodium* species were placed in two wells of each test plate. Negative controls (uninfected mosquito homogenates) were placed in four wells and blocking buffer in four wells. The same batches of capture monoclonals, peroxidase-labelled mono-clonals and positive controls were used in all tests. Positivity was determined by both visual reading and at 405-414 nm using an ELISA reader 30 min after the substrate had been added. The cut-off was selected as twice the mean of negative controls (Wirtz et al. 1987).

RESULTS

Collection parameters - Ninety-five mosquito collections were made during four trips (two each in the dry and rainy seasons). The usual capture time was 4 h a day (from 18 to 22 h) based on the peak activity of the species in all localities as determined by the 12 h collections. The range of temperature during collections was 23°C to 31°C for both wet and dry seasons and the relative humidity ranged from 78 to 100% in the wet season and from 62 to 100% in the dry season with means of 96% and 80.3%, respectively. Collections were carried out during all four phases of the moon, on days with or without rain and wind. The number of bites/man/hour for each area during dry and rainy seasons is shown in Table I, with the highest index obtained in the dry season.

Distribution of mosquito species - Fifteen anopheline species were identified among 3,053 mosquitoes collected in the four localities. The most frequent species and their distribution by locality and season are shown in Table II. The majority (96.4%) of mosquitoes caught consisted of four species, *An. albitarsis s.l.* (64.4%), *An. braziliensis* (Chagas, 1907) (16.7%), *An. nuneztovari* Galbadon, 1940 (9.5%) and *An. triannulatus* (5.8%). The remaining 11 species [*An. oswaldoi, An. darlingi, An. peryassui* Dyar and Knab, 1908, *An. minor* Costa Lima, 1929 , *An. neivai* Howard, Dyar and Knab, 1903, *An. intermedius* (Chagas, 1908), *An. mediopunctatus s.l., An. rangeli* Galbadon, Cova-Garcia & Lopes, 1940, *An. evansae* (Berthes, 1926), *An. argyritarsis* Robineau-Desvoidy, 1827 and *Chagasia bonneae* Root, 1927] were uncommon (3.6%). Most mosquitoes (77.2%) were collected during the dry season.

Sporozoite rate by salivary gland dissection - We carried out 88 dissections of mosquitoes col-

TABLE I

Number of bites/man/hour for all areas in which anophelines were collected, during the dry and rainy seasons
(February and August 1990 and April and September 1991)

Study area	Dry season	Rainy season
Serra do Navio (SNV)-treated area	0	0
Serra do Navio forest	1.1	1.4
Serra do Navio Carpintaria[c]	6.5[a]	4.4
Colonia Água Branca	2.4	2.5
Porto Terezinha	4.7	1.1
Arrependido	14.6[b]	6.6[b]

a: P>0.05*,* Chi-square test; *b:* P< 0.05, Chi-square test; *c:* locality 3 km from SNV (the treated area), at the foot of the hill

TABLE II

Number of each *Anopheles* species collected during 1990-1991 in study areas Serra do Navio (SNV), Colonia
Água Branca (CAB), Porto Terezinha (PT) and Arrependido (ARR), by season

Anopheles species	SNV[a] W/D	CAB W/D	PT W/D	ARR W/D	Total W/D	Grand Total
An. albitarsis s.l.	91/91 (43.7)	1/14 (7.0)	71/90 (48.6)	200/1407 (76.8)	363/1602	1965 (64.4)
An. braziliensis	30/27 (13.7)	1/0 (0.5)	33/37 (21.1)	35/348 (18.3)	99/412	511 (16.7)
An. nuneztovari	8/41 (11.8)	61/70 (65.1)	14/31 (13.5)	6/50 (2.7)	89/201	290 (9.5)
An. triannulatus	88/16 (25.0)	7/20 (12.5)	21/16 (11.1)	7/2 (0.5)	123/54	177 (5.8)
An. oswaldoi	4/10 (3.4)	5/17 (10.2)	2/1 (0.9)	6/0 (0.3)	17/28	45 (1.5)
An. darlingi	0/6 (1.4)	0/4 (1.8)	1/1 (0.6)	0/4 (0.2)	1/15	16 (0.5)
An. peryassui	0/1 (0.2)	0/3 (1.4)	1/1 (0.6)	0/4 (0.2)	1/9	10 (0.3)
An. minor	0/0	0/1 (0.5)	0/8 (2.4)	0/1 (<0.1)	0/10	10 (0.3)
Other species[b]	0/3 (0.7)	0/2 (1.0)	0/5 (1.5)	4/15 (0.9)	4/25	29 (0.9)
Total	221/195	75/140	142/189	259/1832	697/2356	3053

a: consists of mosquitoes collected in the forest and Carpintaria (none was collected in the treated area); *b:* other *Anopheles* mosquitoes collected in low numbers included: *An. intermedius, An. mediopunctatus, An. rangeli, An. evansae, An. neivai, An. argyritarsis* and *Chagasia bonneae;* W: wet season; D: dry season; ( ): percentages determined in relation to the total number of mosquitoes collected by locality.

lected from human bait, of four species: 35 *An. albitarsis s.l.,* 20 *An. braziliensis,* 15 *An. nuneztovari,* 10 *An. triannulatus* and 8 *An. oswaldoi,* of which 46 (52.2%) mosquitoes were parous. Of those, three had sporozoites in the salivary glands (one each of *An. albitarsis s.l., An. braziliensis* and *An. nuneztovari*) and four had oocysts on the stomach wall (two were the same specimens that had sporozoites – *An. braziliensis* and *An. nuneztovari* – and two *An. oswaldoi*). The overall infection rate for the salivary glands was 3.4% and for stomachs 4.5%.

Infection with malaria parasites - The positive rate of the total number of mosquitoes tested by ELISA performed with CSP antigen for *P. falciparum, P. vivax* VK210, *P. vivax* VK247 and *P. malariae*, was 0.8 (23/2876) belonging to six mosquito species: 15 *An. albitarsis s.l.*, four *An. nuneztovari* and one of each: *An. braziliensis, An. triannulatus, An. oswaldoi* and *An. rangeli*. Nine were positive for *P. malariae* (five *An. albitarsis s.l.,* and one of each *An. braziliensis, An. nuneztovari, An. triannulatus* and *An. rangeli*), eight for *P. vivax* VK210 (seven *An. albitarsis s.l.*

and one *An. nuneztovari*), three for *P. falciparum* (two *An. albitarsis s.l.* and one *An. oswaldoi*) and three for *P. vivax* VK 247 (one *An. albitarsis s.l.* and two *An. nuneztovari*) as shown in Table III. Abdomens of all positive specimens were also retested by ELISA and only the *An. triannulatus* was negative for this second test.

No mosquitoes were collected in the residential and administrative part of the treated area (SNV).

P. malariae, found in some mosquitoes collected in the SNV-forest, could be *P. brasilianum* Gonder and Berenberg-Gossler, 1908 which is a monkey parasite (Deane 1992) that are indistinguishable by morphological techniques from *P. malariae* (Lal et al. 1988) and had already been detected in monkeys captured in these localities (Deane 1969, Deane & Ferreira Neto 1969, Deane et al. 1971).

DISCUSSION

Migratory movement has played an important role in the spread of malaria in Brazil (Marques 1986), since the exodus in Brazil has been from town to rural areas and within the rural areas in the Amazon region. This was initially a result of a Federal government program for populating and developing the Amazon region based on a belief that in the Amazon region everyone was entitled to land in order to facilitate economic development. This resulted in a major migration of people from all parts of Brazil to become farmers, goldminers or hydroelectric workers (Alecrim 1992). Deforestation and burning were carried out to provide space for settlements, resulting in drastic changes in the ecology of this region (Marques 1987). Those changes and the growth of settlements without provision for infrastructure, are thought to have contributed to the spread of malaria throughout the Amazon region by the formation of new breeding sites for *Anopheles* mosquitoes (Marques 1986).

In the 60's, companies such as Icomi established projects which created new residential localities and new jobs and this subsequently increased migration. Even companies that had made extensive preparations faced problems of morbidity and mortality from tropical diseases, mainly malaria and leishmaniasis. This was the stimulus for Icomi to establish a program for the control of malaria, the most important disease in the area (Gusmão 1991).

The prevalence of malaria infections in the localities (CAB, PT and ARR) during the period of this study (1990-1991) was 29.3%, 6.2% and 20.4%, respectively, and there were no malaria infections in SNV-treated area (Póvoa 1993). Data

TABLE III

Positive rate by enzyme-linked immunosorbent assay of *Anopheles* species collected in the localities [Serra do Navio[a] (SNV), Colonia Água Branca (CAB), Porto Terezinha (PT) and Arrependido (ARR)]

Anopheles species[b]	PF				PV1				PV2				PM				TIM/TTM
	SNV	CAB	PT	ARR	SNV	CAB	PT	ARR	SNV	CAB	PT	ARR	SNV	CAB	PT	ARR	(%)
An.albitarsis s.l	-	-	-	2	-	-	-	7[c]	-	-	-	1	3	-	-	2	15/1833 (0.82)
An. brazileinsis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1[c]	1/480 (0.20)
An. nuneztovari	-	-	-	-	-	1	-	-	-	2[c]	-	-	-	1	-	-	4/280 (1.40)
An. triannulatus	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1/177 (0.56)
An. oswaldoi	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1/45 (2.22)
Other species[d]	-	-	-	-	-	-	-	-	-	-	-	-	1[e]	-	-	-	1/28 (3.57)
Total	-	-	-	3	-	1	-	7	-	2	-	1	5	1	-	3	23/2876[f] (0.80)

a: consists of mosquitoes collected in the forest and in Carpintaria; *b: An. darlingi, An. peryassui* and *An. minor* were all negative; *c:* one of each of these mosquitoes was also positive by dissection (sporozoite); *d:* other *Anopheles* mosquitoes collected in low number included: *An. intermedius, An. mediopunctatus, An. rangeli, An. evansae, An. argyritarsis* and *Chagasia bonneae; e: An. rangeli; f:* total tested including negative specimens; PF: *Plasmodium falciparum;* PV1: *P. vivax* VK210; PV2: *P. vivax* VK247; PM: *P. malariae;* TIM: total of infected mosquitoes; TTM: total of tested mosquitoes; -: negative; ( ): percentages determined in relation to infected mosquito by the total number of collected mosquito.

from 1961 (prior to the control measures) show malaria infection in SNV (47.1%), then a sharp decrease in the rate to 4.3% immediately after the establishment of the daily intake of chloroquinised salt in 1963 as part of the malaria control program established by Icomi until 1993. Therefore, in the treated area at SNV, it was not surprising to find no evidence of malaria infection.

Unpublished records of the *Anopheles* species distribution in the SNV area obtained by Icomi malaria control program, show that in the 60's *An. darlingi* was found close to the SNV residential areas. After the implementation of their control program, this species disappeared from SNV as confirmed by Deane and Ferreira Neto (1969), and has only been occasionally reported from SNV Carpintaria (the present study).

Our findings support the Icomi data since we collected *An. darlingi* (n=16) from all localities except SNV (the treated area). In the forest connecting SNV residential and administrative areas to the other three localities, the *Anopheles* species collected during our study are those considered predominantly zoophilic (e.g. *An. triannulatus, An. rangeli* and *An. evansae*) (Consoli & Oliveira 1994).

Our results demonstrate that the number of bites/man/hour is significantly higher at SNV-Carpintaria during the dry season, possibly supporting the observation that most malaria transmission in the Amazon Basin occurs at this time (Deane 1988). However, in all other localities, no seasonal differentiation was observed. The locality ARR had significantly higher (Chi-square test, $P < 0.05$) bites/man/hour in both seasons compared with all other localities except Carpintaria and also a high malaria infection rate (20.4%). Because 14/23 of the mosquitoes positive by ELISA were collected at ARR, and only 4/23 at CAB (in spite of a malaria infection rate of 29.3%), we suggest that the highest risk for malaria transmission was for the inhabitants of ARR.

Reports on malaria vectors in Brazil are few. Based on studies carried out in the 40's and 50's, it was established that along the coast, malaria was transmitted with a low level of endemicity by the brackish-water breeder *An. aquasalis* and in all other areas where malaria transmission occurred, *An. darlingi* was the primary vector. However, species of the subgenus *Kerteszia* were also important on the transmission of malaria in the southern part of Brazil. Other species such as *An. albitarsis* and *An. braziliensis* were sometimes of secondary importance as malaria vectors (Deane et al. 1946, 1948). In our study we determined species carrying human malaria sporozoites detectable by ELISA, were the same as those reported in past

studies in Brazil (Arruda et al. 1986, Tadei et al. 1988, Lourenço-de-Oliveira et al. 1989, Branquinho et al. 1996), plus *An. braziliensis* and *An. rangeli*. The latter was positive only fo*r P. malariae/brasilianum*, but we do not believe that this specie take role on the transmission of both, human and simian malaria in the Brazilian Amazon.

Because *An. albitarsis s.l.* was caught with human bait, presented the highest density over localities, was positive by ELISA for the four tested species of *Plasmodium* and positive by salivary glands dissection for malaria sporozoites, it probably plays an important role in the epidemiology of malaria in these localities. It is also worth noting the number of *An. nuneztovari* caught in CAB, which, as in Venezuela (Gabaldón 1981, Rubio-Palis & Curtis 1992) and in Suriname (Rozendal 1990), probably plays a role in local malaria transmission.

All *Anopheles* mosquitoes positive for *P. malariae* were collected in the forest area, where on several occasions during the catches we saw small monkeys. Since *P. brasilianum* (monkey malaria parasite) is genetically and morphologically indistinguishable from *P. malariae* (Lal et al. 1988), probably a sylvatic cycle of this parasite occurs in this area. Our records did not show infection in man caused by *P. malariae* and the SNV hospital and Fundação Nacional de Saúde records show a very low prevalence of this parasite, yet a high positivity in mosquitoes was detected for *P. malariae* in SNV. The maintenance of this *Plasmodium* could therefore be through the monkeys.

Marques (1986, 1987) showed that the spread of malaria in Brazil started in the 60's with the building of new roads followed in the 70's with the establishment of hydroelectric projects and in 80's with the emergence of the gold prospecting sites. He classified transmission areas of malaria in Brazil as "stable malaria" (old transmission areas) and "frontier malaria" (recent transmission areas). The Meeting of World Health Organization (WHO) held in Amsterdam, in 1992, resulted in a global malaria control strategy which suggested that each country, based on its specific characteristics of malaria transmission, had to identify local problems and priorities in order to apply appropriate interventions (WHO 1992). Based on the WHO suggestions and considering the classification made by Marques, our localities, CAB, PT and ARR have the characteristics of areas of "stable malaria".

The control measures used at SNV aimed at decreasing the human/vector contact were very effective (i.e., we collected very few mosquitoes and they were primarily zoophilic, and no malaria cases were detected by blood smears). In contrast,

after the desativation of the control system at SNV, human malaria cases were registered and we collected infected mosquitoes within the residences. Similar institutional programs are likely to dramatically reduce malaria transmission in the Amazon region of Brazil.

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