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Malaria vectors in two indigenous reserves of the Brazilian Amazon

ABSTRACT

OBJECTIVE: To describe the composition, ecological and behavioral characteristics and infectivity of *Anopheles* species in indigenous reserves of the Amazon region.

METHODS: The study was performed in villages of the Nhamundá-Mapuera and Cuminapanema indigenous reserves, in the state of Pará, Northern Brazil, in 2002. A total of three two-week collections were conducted in each reserve, with the capture of adult and immature forms. Adult *Anopheles* specimens were captured using a Castro sucking tube with human landing trap in indoor and outdoor environments, from 6.00 pm to 9.00 pm and from 6.00 pm to 6.00 am, and subsequently assessed to verify parity and infectivity by plasmodiums using dissection and ELISA. Water collections near the villages were surveyed using 500 ml ladles, with 20 ladlefuls for each 10 m, covering the maximum extent of 200 m of perimeter around the breeding spot.

RESULTS: Adding up the collections from both reserves, a total of 8,668 females were captured. *Anopheles darlingi* was the most frequent species, with higher frequency around the homes. In the Mapuera reserve, blood feeding activity was concentrated between 8.00 pm and 12.00 am, while, in Cuminapanema, it remained high until 12.00 am, decreasing after this time and increasing again early in the morning. Of all the 6,350 *An. darlingi* females analyzed, 18 were infected with *Plasmodium vivax* VK247, VK210, *P. falciparum* and *P. malariae*. In addition, other 1,450 females of other species were analyzed, but none was found infected. *An. nuneztovari* and *Chagasia bonnae* were the most frequent species in the breeding spots of the Mapuera and Cuminapanema villages, respectively. Immature *An. darlingi* forms were not located in Mapuera and were captured in only one of the collections of the Cuminapanema reserve.

CONCLUSIONS: *An. darlingi* populations in the two reserves showed exophilic behavior and intense nocturnal activity. The occurrence of immature forms was little frequent and larval density was low. Vector behavioral characteristics were not favorable for the usual vector control activities.

DESCRIPTORS: *Anopheles*. Insect Vectors. Amazonian Ecosystem. Malaria, prevention & control. Indigenous Population. Epidemiologic Surveillance. *An. darlingi*.

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INTRODUCTION

The legal Brazilian Amazon includes all the states of Northern Brazil, and the states of Mato-Grosso and Maranhão, covering an area of 5,217,423 km², of which about 80% is occupied by the Amazon Rain Forest. Malaria is one of the main health problems in this population, where 99% of Brazilian cases are notified. Of all the 457,570 malaria cases reported in 2007, 79.6% had

Plasmodium vivax as their etiological agent; 19.3%, *P. falciparum*; 0.1%, *P. malariae*; and 1.0% were mixed infections.

In addition to the cities' urban areas, the Amazon is inhabited by riverside populations and indigenous peoples with distinct levels of contact with those who are not indigenous. Malaria is an important and serious disease among indigenous peoples, representing the main cause of hospitalization and death in some ethnic groups.[12,19] Severe cases occur mostly in villages located further away from urban areas, where contact with non-indigenous populations is recent or reduced and transmission is stable. In these populations, exposure to the disease is lower and there is loss of immunological memory throughout time.[13]

Limitation of access to Indian reserves has hindered research aimed at shedding light on malaria transmission dynamics and its vectors. Many reserves are located inside the forest and far from cities, several days away by river; some are only accessible by air, with improvised runways. The scarce entomological information available usually results from few collections or was obtained from villages located near urban centers, where environmental changes are greater and indigenous populations have more contact with the non-native population.[10,18] Thus, vector control activities follow patterns that are little effective, as they do not consider local environmental patterns.

Knowledge about fauna composition and vector behavior in indigenous areas is essential for the adoption of alternative vector control strategies that are culturally and environmentally correct, aiming at preventing transmission. In addition to de *Anopheles darlingi,* other species were found naturally infected in the Amazon and incriminated as primary or secondary vectors, in particular situations.[7,16,21] The behavior of the vector species influences the local epidemiological pattern, apart from the fact that the same *Anopheles darlingi* population may show behavioral variations[2,24] in response to external changes, thus increasing the complexity of malaria transmission dynamics.

In August 2001, the *Plano de Controle da Malária* (Malaria Control Plan) began in the Nhamundá-Mapuera and Cuminapanema Indian reserves, in the state of Pará, in areas of migratory movements between Brazil and Suriname. In the following year, this Plan was incorporated into the Protocol of the *Cooperação Brasil/Suriname para Ações de Malária em Áreas de Fronteira* (Brazil/Suriname Cooperation for Malaria Actions along the Border). The goals of the Protocol were malaria reduction and control and knowledge about the *Anopheles* fauna in the area.

In 2000, the Annual Parasite Index (API) in the Mapuera reserve reached 500 cases per 1,000 inhabitants, decreasing to 18 and 37 in the two following years, while the Brazil/Suriname Cooperation Protocol was in effect. The Wai-Wai people, inhabiting the Mapuera village, participate in indigenous meetings and perform celebrations, welcoming ethnic groups from other endemic areas. In addition, they welcome Indians migrating from Suriname and travel to the city's urban area, where the reserve's border is. This movement enables the frequent entry of infected people in the area and the occurrence of cases in the villages. An example of this was the outbreak of nine cases of malaria notified in 2002, in the Placa village, five hours away from the Mapuera village by river, introduced by Indians who migrated from Suriname. This outbreak resulted in four cases detected by active search in the Mapuera village, all of them originated from the Placa village. In Cuminapanema, in 2001, a total of 171 malaria cases were recorded, approximately one case/person/year. After May 2002, the area was considered free from malaria, with constant laboratorial surveillance of suspected disease cases.

In view of the direction of malaria vector control actions in Indian reserves of the area, the present study aimed to describe aspects related to the composition, ecological and behavioral characteristics and infectivity of *Anopheles* species.

METHODS

The present study was performed in the Nhamundá-Mapuera and Cuminapanema Indian reserves, in the state of Pará, Northern Brazil. The Nhamundá-Mapuera reserve has an area of 1,049,520 ha and is located in the city of Oriximiná. This study was performed in the main village, Mapuera (0°41'46.14''S, 57°58'23.87''W), located on the left bank of the Mapuera river, and approximately 220 km from the seat of the municipal government, in a straight line, accessible by river or air. This village is one hour and 45 minutes away from the city of Santarém by air, from where field teams would depart. The predominant ethnic group were the Wai-Wai and the population was 1,135 inhabitants, in 2002. In the Mapuera village, there was electricity and a telephone system. This population speaks Portuguese and has been in contact with the non-indigenous population for over 50 years. The village had 227 typical indigenous dwellings of Brazil (made of wood, bamboo, straw and palm leaves, without windows and with only one entrance for entry and exit) and eight wooden houses, belonging to non-indigenous people, totaling 235 dwellings. The non-governmental organization (NGO) *Instituto de Desenvolvimento de Atividades de Auto-Sustentação das Populações Indígenas* (Indaspi – Institute for the Development of Self-Sustainable Activities of Indigenous Populations) was responsible for health care; provided medical and outpatient, dental and laboratorial services; and conducted vaccination campaigns. Indians maintained subsistence agricultural practices, in addition to hunting and fishing.

Some performed paid activities through Indaspi, the Oriximiná City Hall and the *Fundação Nacional do Índio* (Funai – Brazilian Indian Foundation) and went to the city every month to receive their salaries and sell handicrafts.

The second reserve, Cuminapanema (0°19'42.89"S, 55°50'7.61"W), has an area of 624,000 ha and is located in the city of Óbidos, 212 km from the seat of the municipal government, in a straight line, and was managed by the *Frente de Proteção Etno-Ambiental Cuminapanema* (Cuminapanema Organization for Ethnic-Environmental Protection). Access is by air, in a one-hour flight, departing from Santarém. In 2002, this reserve had 203 inhabitants belonging to the Zo'é ethnic group. The FUNAI considered this population to be isolated, due to their recent and not frequent contact with non-indigenous population in the last ten years or so. Indians were not fluent in Portuguese. Their diet was based on hunting, fishing and fruits gathered in the forest. They had semi-nomadic habits, traveling between the sub-villages built in different areas of the forest. They are fixed points where Indians stay for a while, migrating to different points, in turns. These sub-villages were comprised of shelters consisting of frames with straw roofs, without walls and resembling sheds.

A total of three *Anopheles* collections, each lasting two weeks, were made in each reserve, planned according to the tides of the Amazon rivers: the first between April and May, in the beginning of the high tide; the second in August, at the peak of the high tide; and the third in November, in the low tide. In each collection, adults were captured from 6.00 pm to 9.00 pm and from 6.00 pm to 6.00 am. In the mornings, between 7.00 am and 10.00 am, immature forms were collected. The afternoons were reserved for identification, counting, dissection and storing of material.

All captures of adults were conducted in indoor and outdoor environments. In Cuminapanema, due to the dwellings not having walls, indoor captures were made in the central sheltered area where Indians slept, while outdoor captures were conducted 20 meters away from this point. Mosquitoes were captured using the human landing trap method with a Castro sucking tube. Professionals were protected by long-sleeved shirts, long trousers and long black socks. Captures of three hours were conducted by two professionals and those of 12 hours by four professionals who took turns every three hours.

In the Mapuera village, five points were selected for three-hour captures: one in a central location, and the others in diametrically opposite positions. A total of three captures were conducted per point. A total of two 12-hour captures were conducted in the most productive point; this point was maintained in the three collections. In all, 69 hours of capture in the first and third collections were spent. In the second collection, three-hour captures were reduced to two per point and the 12-hour capture to one, due to the need to perform control activities in another village of the reserve, totaling 42 hours.

In the Cuminapanema village, the eight main sub-villages where Indians passed were included: Kuruaty, Nareth, Ovytxãteary, Zawarakiãem, Pirity, Tawaribururupá, Pururuty and Keijã. In each survey, captures from 6.00 pm to 6.00 am were conducted in the points with higher number of mosquitoes in the three-hour captures. In the first collection, the five most frequently sub-villages used by Indians were surveyed: Nareth, Ovytxãteary, Zawarakiãem, Pirity and Keijã, with 12-hour captures in Nareth, Pirity and Ovytxãteary, thus totaling 66 hours. In the second, the sub-villages surveyed were extended to eight, including the Kuruaty, Tawaribururupá and Pururuty. Captures of 12 hours were conducted in the Nareth, Kuruaty and Pururuty sub-villages, totaling 84 hours. In the third collection, the Kuruaty and Pirity sub-villages were excluded due to lack of water and shelter and the absence of Indians in these locations. Captures of 12 hours were conducted in the Pururuty, Ovytxãteary and Keijã sub-villages, totaling 72 hours.

The number of mosquitoes collected was converted into a mosquitoes/collector/hour index (MCHI) and *An. darlingi*/collector/hour index (DCHI), dividing the total number of mosquitoes captured by the number of collectors and then by the total number of hours worked.

Breeding places around the villages were surveyed to detect and collect larvae, using 500 ml ladles. Every 10 m included 20 ladlefuls. Extensive breeding places were assessed as far as 100 m from the starting point, on both river banks. Each breeding ground was assessed for up to two consecutive days. In the Mapuera village, breeding sites were either permanent or temporary, in the backwater of streams or lakes, all of which had lush vegetation comprised of grasses and water hyacinths. There were also a hollow area and depressions in the rocks, formed by the tides of the Mapuera river. In Cuminapanema, there were different types of breeding places such as the backwater of rivers, streams, permanent and temporary lakes and swamps rich in aquatic vegetation.

The identification of immature forms and adults was performed using Consoli & Lourenço-de-Oliveira,[3] Gorham et al[a] and Faran & Linthicum[6] keys.

[a] Gorham JR, Stojanovich CJ, Scott HG. Clave ilustrada para los mosquitos anofelinos de Sudamerica Oriental. Atlanta: United States Department of Health, Education and Welfare; 1967.

Parity and infectivity

On the days following the captures, mosquitoes were identified and one sample with at least 10% of *An. darlingi* females, depending on the operational capacity, had their salivary glands, stomachs and ovaries dissected. Mosquitoes were classified according to parity, based on the aspect of tracheal filaments, following Detinova's technique.[5] The head and thorax of all *Anopheles* dissected and not dissected were placed in eppendorf tubes with silica gel and stored in thermal boxes, so that enzyme-linked immunosorbent assays (ELISA) could be performed to detect infection by plasmodiums at the *Laboratório de Parasitologia do Instituto Evandro Chagas* (Evandro Chagas Institute Laboratory of Parasitology). Mosquitoes were tested for the following species: *P. falciparum, P. vivax* 210*, P. vivax* VK 247 and *P. malariae*; individually for *An. darlingi*; and in groups of up to ten mosquitoes for the remaining species. Difficulties in logistics prevented dissections from being performed in the first survey, in the Mapuera reserve.

The research was developed by the *Departamento de Saúde Indígena* (Department of Indigenous Health)*,* the *Gerência Técnica de Malária da Fundação Nacional de Saúde* (Brazilian Health Foundation Technical Management of Malaria) and the *9ª Regional de Proteção Social da Secretaria de Saúde do Estado do Pará* (State of Pará Department of Health 9th Regional Social Protection Office), *Instituto Evandro Chagas* (Evandro Chagas Institute) and *Fundação Nacional do Índio*. Mosquito captures were conducted by the researchers themselves and by the technicians of the *9ª Regional de Proteção Social da Secretaria de Saúde do Estado do Pará* and the *Instituto Evandro Chagas*, who performed this activity as part of their work routine. All collectors signed a Work Agreement Form.

RESULTS

During 2002, 8,668 mosquitoes were captured, when all three collections in the two reserves were added up.

In the Mapuera village, 828 mosquitoes were collected. The species captured were *An. darlingi* (n=487), *An. nuneztovari* (n=289), *An. triannulatus* (n=37), *An. intermedius* (n=13) and *An. albitarsis* l.s. (n=2) (Table 1). Captures with the highest MCHI (2.8) were those from the first (April/May/2002) and third (November/2002) collections. Species captured indoors were *An. darlingi*, *An. nuneztovari* and *An. triannulatus*, all with percentages below 10% of the total number collected.

The total number of breeding sites per collection varied from five to seven. The highest number of breeding sites was observed in the third collection (November/2002), in the low tide of the Mapuera river, when temporary lakes were dry, even though all other breeding sites

were active and positive for *Anopheles* species. *An. nuneztovari* was the most frequent species, present in all collections and particularly abundant in the third one (Table 2). Immature *An. darlingi* forms were not found in Mapuera.

In the Cuminapanema reserve, 7,840 mosquitoes were collected. The species captured were *An. darlingi* (n=6,392), *An. minor* (n=1.129)*, An. intermedius* (n=148), *An. oswaldoi* (n=101), *An. triannulatus* (n=33), *An. mediopunctatus* (n=16)*, An. peryassui* (n=10)*, An. mattogrossensis* (n=4) *An. nuneztovari* (n=3), *An. squamifemur* (n=3) and *An. albitarsis l.s.* (n=1). The capture with the highest DCHI occurred in the second collection (August/2002) (Table 1). In this collection, 6,061 *An. darlingi* specimens were captured and the DCHI varied among sub-villages, from no specimens collected, in Tawaribururupá, to up to 106.3, in Nareth.

The total number of breeding sites analyzed per survey ranged between five and ten. The highest number of breeding sites was observed in the second collection (August/2002), during the high tide. Among immature forms, the most frequent species was *Chagasia bonnae*, followed by *An. triannulatus*. Specimens of *An. darlingi* were collected in a second survey (August/2002) (Table 2), in breeding grounds near Nareth (n=1)*,* Ovytxãteary (n=8), Keijã (n=1) and Tawaribururupá (n=16).

The activity of *An. darlingi* was recorded in the 12 hours of capture, in both reserves. In the Mapuera reserve, the peak of activity was concentrated between 8.00 pm and 12.00 am. In Cuminapanema, the number of *An. darlingi* remained high until 12.00 am, subsequently decreasing, and finally increasing again in the early morning (Figure).

An. nuneztovari and *An. minor*, the second most frequent species in each of the villages, showed activity that predominated at dusk. Considering only the 12-hour captures, 72.7% of all the *An. nuneztovari* specimens and 79.0% of *An. minor* specimens were captured in the first hour, between 6.00 pm and 7.00 pm.

All the 7,800 *Anopheles* specimens were tested for infectivity by ELISA. Of all the 6,350 *An. darlingi* specimens analyzed, 18 were detected with infection by plasmodiums. The most frequent infection was by *P. vivax,* followed by *P. falciparum* (Table 3). In the Mapuera reserve, the VK 247 variant and *P. malariae* were detected. In Cuminapanema, the variant VK 247 and *P. falciparum* were detected with low frequency and there was no infection by *P. malariae.* Of all the 1,450 specimens of other species analyzed by ELISA, none was found infected.

There were no sporozoites in the dissected salivary glands, but stomachs showed infection by oocysts. All

the six mosquitoes with infected stomachs in the first collection in Cuminapanema were not positive when tested by ELISA (three *An. darlingi,* two *An. intermedius* and one *An. oswaldoi*) (Table 3). The percentage of gravid *An. darlingi* females was always high in both reserves, except for the second collection (August/2002) in the Cuminapanema reserve (Table 4).

DISCUSSION

In the period of study, 13 species of adult Anopheles mosquitoes and immature forms were collected, including *Ch. bonnae.* Adult forms of *An. darlingi* were the most frequent species found in both villages. The number of species in the Mapuera village was smaller than that in the Cuminapanema village, where the

dimension of anthropic impact is lower, and villages are small and located in the forest, enabling species with wild behavior to approach. However, the importance of these species in the epidemiology of malaria has not been shown yet, once only *An. darlingi* specimens were found infected with human plasmodiums by ELISA.

In the Mapuera village, captures with higher MCHI (2.8) were the first (April/2002) and third (November/2002) collections, corresponding to the beginning of the high tide and low tide of the Mapuera river, respectively. However, in the first collection, the greatest contribution to the MCHI was *An. nuneztovari*, while, in the third, it was *An. darlingi* almost exclusively. The abundance of Culicidae is associated with the availability of breeding grounds, which, in their turn,

Table 1. Adult *Anopheles* captured in the Mapuera and Cuminapanema Indian reserves, Northern Brazil, 2002.

Species	April/May	August	November	Total
Mapuera				
Anopheles darlingi	58	53	376	487
Anopheles nuneztovari	278	8	3	289
Anopheles triannulatus	34	2	1	37
Anopheles intermedius	13	0	0	13
Anopheles albitarsis l.s.	0	2	0	2
Subtotal	383	65	380	828
Mosquito/collector/hour				
Hours worked	69	42	69	180
DCHI*	0.4	0.6	2.7	1.4
MCHI**	2.8	0.8	2.8	2.3
Cuminapanema				
Anopheles darlingi	15	6,061	316	6,392
Anopheles minor	447	532	150	1,129
Anopheles intermedius	147	1	0	148
Anopheles oswaldoi	53	31	17	101
Anopheles triannulatus	18	5	10	33
Anopheles mediopunctatus	12	4	0	16
Anopheles peryassui	6	4	0	10
Anopheles mattogrossensis	3	1	0	4
Anopheles nuneztovari	0	3	0	3
Anopheles squamifemur	1	2	0	3
Anopheles albitarsis l.s.	0	1	0	1
Anopheles braziliensis	0	0	0	0
Chagasia bonnae	0	0	0	0
Subtotal	702	6.645	493	7.840
Mosquito/collector/hour				
Hours worked	66	84	72	222
DCHI*	0.1	36.1	2.2	14.4
MCHI**	5.3	39.6	3.4	17.7
Total	1,085	6,710	873	8,668

* DCHI: *Anopheles darlingi* per collector per hour index

** MCHI: Mosquitoes per collector per hour index

Table 2. Immature *Anopheles* forms collected in the Mapuera and Cuminapanema Indian reserves, Northern Brazil, 2002.

| Location/Species	April/May	August	November	Total
Mapuera				
Anopheles nuneztovari	190	336	777	1,303
Anopheles triannulatus	4	83	186	273
Anopheles (Anopheles) sp.	0	32	6	38
Anopheles albitarsis l.s.	0	8	6	14
Subtotal	194	459	975	1,628
Breeding sites surveyed	5	6	7	18
Positive breeding sites	4	6	7	17
Cuminapanema				
Chagasia bonnae	58	123	36	217
Anopheles triannulatus	4	10	83	97
Anopheles oswaldoi	0	21	51	72
Anopheles mattogrossensis	0	0	29	29
Anopheles darlingi	0	26	0	26
Anopheles minor	5	1	0	6
Anopheles intermedius	0	0	3	3
Anopheles nuneztovari	0	1	0	1
Anopheles braziliensis	1	0	0	1
Subtotal	68	182	202	452
Breeding sites surveyed	5	10	7	22
Positive breeding sites	4	9	6	19
Total	262	641	1,177	2,080

depend on several local factors such as precipitation, variation of tides of rivers and use of soil.[1,8,22] The highest density of *An. darlingi* was recorded in the low tide of the Mapuera river, during the transition from the dry to the wet season. The DCHI was low, when compared to what was observed in other areas of the Amazon.[7,9,24] However, even with the low capture rates, transmissions have been recorded,[10,22] probably as a result of *An. darlingi*'s high susceptibility to plasmodiums.[4] This susceptibility has also been observed in the present study, given the high rate of infection in the first survey. Another factor that contributes to the species' susceptibility is its longevity, exemplified by the high parity rates found.

An. nuneztovari, the second most abundant species, is not considered to be a vector of human malaria in Brazil,[4] even though it is frequently found to be infected by plasmodiums.[7,16,21,22] It is possible that this species has an adaptive advantage in impacted areas of the Amazon, becoming very abundant in these environments.[22,23] This could be observed in the present study, where this species was the second most captured in the Mapuera village, and represented less than 0.1% of the total number collected in Cuminapanema.

In the Cuminapanema reserve, the amount of *Anopheles* found varied, increasing substantially in the second collection, with a MCHI of 39.6. DCHI values also

showed major variations in the sub-villages. The reserve's breeding sites seem to show a different dynamics than that observed in Mapuera. The area of the forest where the reserve sub-villages are established is mountainous, the breeding sites are extensive and there is no dominance by a main river. Thus, the density of adult mosquitoes in each sub-village seems to be conditioned by the nature of the breeding sites that surround it and, probably, its association with precipitation. *An. minor*, the second most abundant species in the reserve, seems not to influence malaria transmission.[4] This species was present in the first hours of the night, Indians were greatly annoyed during this period, and the mosquito was rarely captured after 9.00 pm.

An. darlingi's behavior was significantly exophylic, according to what was observed in other inhabited areas of the Amazon forest.[1,10,18] In the case of the Mapuera village, the exophylic behavior of *An. darlingi*, associated with the dwellings' architecture itself, which show a small, single entrance, may be a protective factor for dwellers, after they return home. This same characteristic was observed among the Xavantes tribe, in the Central-West region of Brazil, as they have a similar type of dwelling.[10] Yet, in Cuminapanema, cultural habits do not include building dwellings with walls, there is no barrier against mosquitoes, and the human-vector contact is facilitated. The high number of mosquitoes annoys Indians and the difference
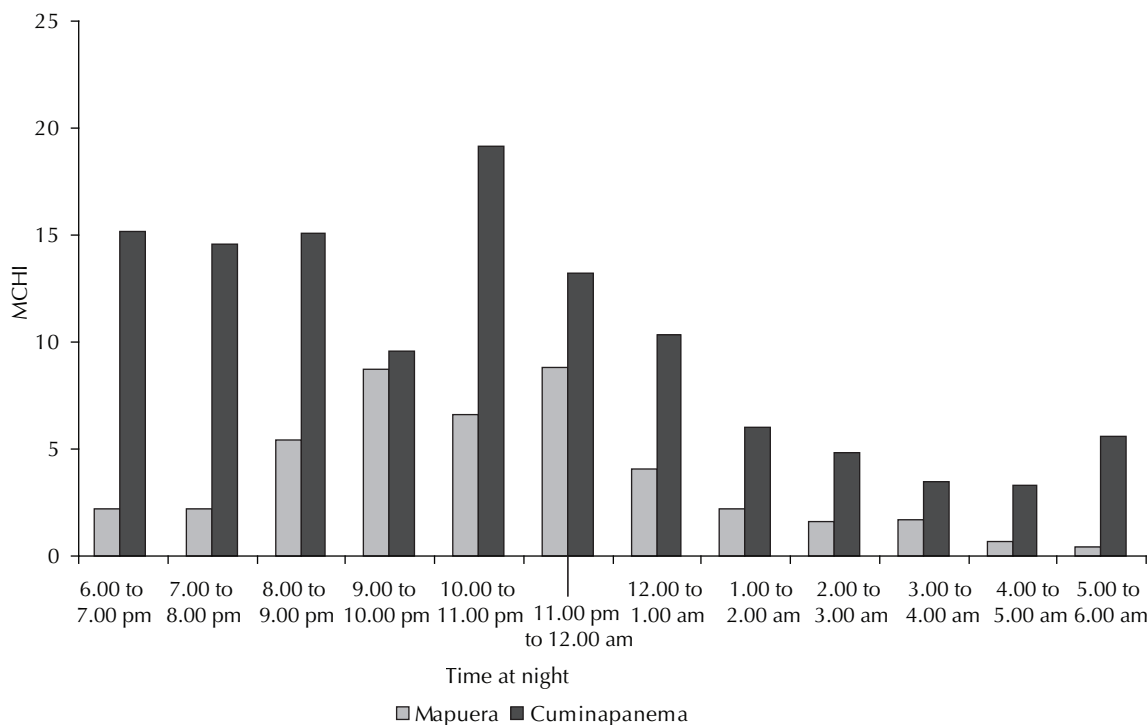
Figure. *Anopheles darlingi* blood feeding activity in the Mapuera and Cuminapanema reserves, Northern Brazil, 2002. MCHI: *Anopheles darlingi* per collector per hour index

observed among sub-villages stimulates migration of the population, who seek those which are more peaceful for rest.

An. darlingi's activity was recorded in the 12 hours of capture in both reserves, with high nocturnal activity. In the Mapuera reserve, the peak of activity was concentrated between 8.00 pm and 12.00 am, and activity at dawn/dusk was very small. A similar pattern was recorded in Anajás, on Marajó island (PA)[20] and in the state of Amapá.[24] In Cuminapanema, the environment is little affected by anthropic action and the number of *An. darlingi* remained high until midnight, subsequently decreasing, and finally increasing again early in the morning (bimodal pattern), as previously observed by Barros et al[1] in Roraima. The blood feeding activity observed in both reserves indicates that studies limited to the dusk period may underestimate the size of the *An. darlingi* population. Moreover, there may be variations in the activity pattern of the same population, at different times of the year,[24] thus increasing the complexity of vector control activities. Measures such as thermal fogging – widely used in the Amazon for malaria control –, may be little effective if performed not according to the mosquitoes' blood feeding activity.

Despite the reduction or absence of malaria cases in the Mapuera and Cuminapanema villages, in 2002, *Anopheles* infected with the three species of plasmodium were found in this study. The proportion of mosquitoes infected with *Plasmodium* species reflected the proportion of human cases in the Amazon, with about 20% of infections caused by *P. falciparum*.[a] These observations emphasize the fact that these parasites are introduced into the villages by several sources, due to the interaction with other populations. These findings concern the Cuminapanema reserve in particular, because the Zo'é people are considered isolated by the Funai. Evidence shows that other sources of infection are present in the reserve, perhaps other indigenous populations or gold miners who pass through this area and contribute to the flow of parasites. Oocysts observed in stomachs, not confirmed by ELISA, suggest that parasitic forms do not develop into sporozoites or infection by other *Plasmodium* species present in local animals. In addition, other *Anopheles* species, with lower susceptibility to human plasmodiums,[11] had their stomachs infected. The fact that the natural environment is close enables the exchange of microorganisms in the vector population. The indigenous population, according to their own nature, is exposed to several pathogens present in the natural environment, such as arboviruses, many

[a] Ministério da Saúde. Secretaria de Vigilância em Saúde. Situação epidemiológica da malária. Brasília; 2008[cited 2008 Oct 31]. Available from: http://portal.saude.gov.br/portal/arquivos/pdf/folder_malaria_2008_final.pdf

Table 3. *Anopheles* infected with plasmodiums, diagnosed with ELISA and stomach dissection, in the Mapuera and Cuminapanema Indian reserves, Northern Brazil, 2002.

Location/Month	Total analyzed	An. darlingi	P. vivax	P. vivax VK 47	P. falciparum	P. malariae	Total infectivity (%)	An. dalingi infectivity (%)	Dissected stomachs	Positive n	Positive %
Mapuera											
April/May	221	48	6	1	2	0	4.1	18.8	-	-	-
August	63	53	0	0	0	0	0.0	0.0	27	0	0.0
November	312	311	0	1	0	1	0.6	0.6	31	1	3.2
Subtotal	596	412	6	2	2	1	1.8	2.7	58	1	1.7
Cuminapanema											
May	560	5	0	0	0	0	0.0	0.0	73	6	8.2
August	6,177	5,633	4	0	0	0	0.1	0.1	67	4	6.0
November	467	300	1	1	1	0	0.6	1.0	64	1	1.6
Subtotal	7,204	5,938	5	1	1	0	0.1	0.1	204	11	5.4
Total	7,800	6,350	11	3	3	1	0.2	0.3	262	12	4.6

Table 4. *Anopheles darlingi* parity in the Mapuera and Cuminapanema Indian reserves, Northern Brazil, 2002.

Location/Month	Dissected	Nulliparity	Parity	Parity (%)
Mapuera			–	
Abril	–	–	27	–
Agosto	27	0	27	100.0
Novembro	35	8	54	77.1
Sub total	62	8		80.2
Cuminapanema			11	
Maio	11	0	23	100.0
Agosto	67	44	51	34.3
Novemrbo	64	13	85	79.7
Sub total	142	57	139	59.9
Total	204	65		67.5

of which have not been identified yet. Protection against human malaria vectors may also enable populations to be protected against other vector-borne diseases.

An. darlingi larvae were rarely collected, as breeding sites are many and diverse. According to Deane,[4] in the dry season, *An. darlingi* breeding sites are great water collections, in the sun or partly shaded; however, in the rainy season, these can be found in distinct bodies of water.[14,21,22] However, the occurrence of high densities has been recorded near urban areas.[8] The difficulty in accurately locating *An. darlingi* breeding sites, associated with the dimension of bodies of water in the Amazon, does not favor the use of biological larvicides. These products have been recommended to control *Anopheles* species in urban areas, provided the number of breeding sites is limited.[25] In addition, there is the need to successively apply them in a short period of time,[15,17] which would imply technicians frequently traveling to reserves.

The characteristics of the *An. darlingi* populations observed in both reserves, such as exophilic behavior, intense nocturnal activity and low larval density, did not favor usual vector control activities, such as indoor residual spraying, thermal fogging, and the use of biolarvicides. On the other hand, detection of *Anopheles* infection shows that Indians are constantly at risk of contracting malaria, which leads to the need to investigate alternative health protection measures in these populations.

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