Urban and suburban malaria in Rondônia (Brazilian Western Amazon) II. Perennial transmissions with high anopheline densities are associated with human environmental changes

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Longitudinal entomological surveys were performed in Vila Candelária and adjacent rural locality of Bate Estaca concomitantly with a clinical epidemiologic malaria survey. Vila Candelária is a riverside periurban neighborhood of Porto Velho, capital of the state of Rondônia in the Brazilian Amazon. High anopheline densities were found accompanying the peak of rainfall, as reported in rural areas of the region. Moreover, several minor peaks of anophelines were recorded between the end of the dry season and the beginning of the next rainy season. These secondary peaks were related to permanent anopheline breeding sites resulting from human activities. Malaria transmission is, therefore, observed all over the year. In Vila Candelária, the risk of malaria infection both indoors and outdoors was calculated as being 2 and 10/infecting bites per year per inhabitant respectively. Urban malaria in riverside areas was associated with two factors: (1) high prevalence of asymptomatic carriers in a stable human population and (2) high anopheline densities related to human environmental changes. This association is probably found in other Amazonian urban and suburban communities. The implementation of control measures should include environmental sanitation and better characterization of the role of asymptomatic carriers in malaria transmission.

Key words: anopheline - urban malaria - Brazilian Amazon

High incidence of malaria, with annual parasite index (API) of 150 to 300 was previously described in the urban locality of Vila Candelária in Porto Velho (Tada et al. this issue). Vila Candelária is a riverside district of the city, less than 2 km far from the city center. In the longitudinal follow up, from 2001 to 2004, it was observed a period of high malaria incidence in the rainy season, peaking in April-May, and a season of lower incidence in the dry season (June to August) characteristic of flooding areas of the rural riverine profile model described for Amazonian localities (Alves et al. 2002, 2005). However, other high incidence periods were irregularly observed in the dry season, as described in dry land areas of Rondônia (Camargo et al. 1994, 1996). This second period of higher incidence may persist up to the beginning of the following rainy season in November/December. Therefore malaria is found year round, in contrast with seasonal variation in typical dry land areas and typical riverine rural areas (Gil et al. 2003). In 2001 an active search and treatment of clinical malaria cases started in Vila Candelária. However, this did not change the total malaria incidence and its monthly distribution. This finding suggested a central role of asymptomatic carriers as source of infections to maintain malaria transmission. The prevalence of asymptomatic carriers, in various surveys, was indeed between 10 to more than 20% of the adult population (Tada et al. this issue).

In the present study we present entomological evidence supporting an additional explanation for the maintenance of the high malaria transmission rate in Vila Candelária: the high anopheline densities observed throughout the year associated with seasonal variation in rainfall and deep environment changes in riverside areas caused by human activity.

MATERIALS AND METHODS

Study area - The entomological studies took place alongside the right riverside areas of the Madeira River including Vila Candelária (8º47'08" S, 63º55'04" W), at the south limit of the urban area of Porto Velho and the neighbor suburban community of Bate Estaca (8º47'55" S to 63º55'48" W). Vila Candelária was described in detail in the previous paper (Tada et al. this issue).

Bate Estaca is a rural community situated at the south of Vila Candelária, extending over 4 km long and a few hundred meters large, along the right Madeira riverside following the old Madeira Mamoré railroad. Inhabitants of Bate Estaca live in relatively primitive scattered houses, provided with electricity but no water supply or sanitation. A total of 126 inhabitants living in 37 family houses have been identified in the demographic survey

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performed at the end of 2004. Houses are isolated, distant from each other by hundreds of meters; human activity is essentially subsistence agriculture and fishing. The population of Bate Estaca is unstable and many inhabitants have settled in the area during the last few years. Various sites along the river banks are frequented for fishing by local and Vila Candelária inhabitants as well as by fishermen from Porto Velho.

Mosquito collection - Mosquito collections were performed during one night each month from January 2001 to December 2004 in the sites identified by numbers, from January 2002 to December 2004 in the sites 1, 2, and 3 (Fig. 1). Simultaneous indoor and outdoor 12 h captures from 6 pm to 6 am were achieved by trained volunteers supervised by the authors of this paper. Outdoor sites were chosen at distances of no more than 6 m from the indoor sites. Six volunteers were working in each section divided in pairs where one person captured indoors and the other at the outdoor site. Each capturer worked for 1 h and remaining part in the second hour. Ethic clearance of the procedure was given by the Ethic Committee of the Cepem which included written and signed information about the risk of acquiring malaria during captures. No accidental infection, however, were observed in these 4 years of activity. Mosquitoes were captured over the capturers' legs with hand-held aspirators. The average hourly number of mosquitoes captured per person was scored as Hour Biting Rates "HBR" (Forattini 2002).

Identification, dissection and determination of mosquito's infection with malaria parasites - The Consoli and Lourenço de Oliveira (1994) standard key for Brazilian *Anopheles* mosquitoes was used for identification. Mosquitoes were then dissected and their salivary glands, midgut, and ovaries were removed for microscopic examination for the presence of oocysts (midgut) and sporozoites (salivary glands). Ovary tracheoles were examined for the determination of parity. Samples of mosquitoes of each capture were stored: carcasses were directly frozen and stored at –20°C while the salivary glands and midgut was ressuspended in 0.5 ml of 0.9% NaCl solution and then frozen at –20ºC for future genetic studies. Samples included mosquitoes captured in different sites, indoors and outdoors and on different hours of the night.

RESULTS

Anopheline mosquitoes species captured in the localities - From 2001-2004 captures in Vila Candelária 2257 female anopheline mosquitoes were captured, 2131 (94.4%) were *An. darlingi* and only 126 (5.6%) other species. The most frequent non-*darlingi* species were *An. nuneztovari*, *An. kondori*, *An. evansae*, *An. triannulatus*, and *An. mattogrossensis* (Table I). In Bate

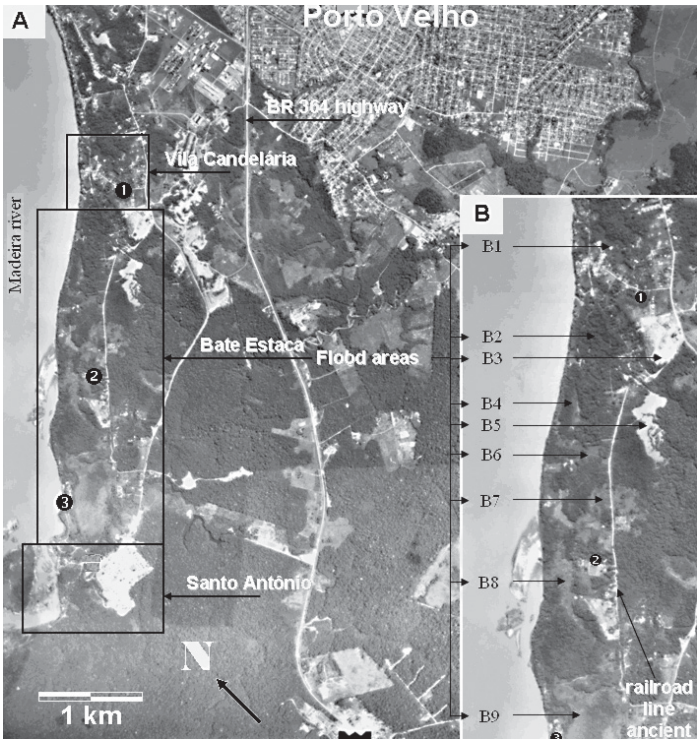

Fig. 1A: general sigth of the area corresponding to urban Vila Candelária, suburban Bate Estaca and Santo Antonio localities, Porto Velho, Rondônia. The photo was taken in dry season. Sites A1, A2, A3 correspond to vectors' collection point; B: enlargement of the flooded areas showing the Madeira-Mamoré railroad ancient line (EFMM); B1 and B5: artificial water collection created by barriers in igarapés courses; B4, B6, B7, B8: flooded areas corresponding to original fish breeding sites or agriculture work land; B3 water supply treatment government complex unit; B5 dam for collecting water to the government complex water treatment unit; B2: flooded area covered by vegetation. Site A1 have 0.33 km from Vila Candelária. Site A2 have 0.48 km from Bate Estaca. Site A3 have 0.49 km from Bate Estaca.

TABLE I

Anopheles species distribution in Vila Candelária, Rondônia, Brazil during 2001-2004. *An. darlingi* is the main vector collected

	Vila Candelária									
	Outdoor					Indoor				
	2001	2002	2003	2004		2001	2002	2003	2004	
Anopheles species	n	n	n	n	T	n	n	n	n	T
An. darlingi	501	378	342	509	1730	92	161	58	90	401
An. nuneztovari	70	1	4	2	77	8	2	0	0	10
An. kondori	9	1	4	2	16	0	0	0	3	3
An. triannulatus	0	0	0	3	3	0	0	0	0	0
An. evansae	11	0	0	0	11	0	0	0	0	0
An. rangeli	1	0	0	0	1	0	0	0	0	0
An. mattogrossensis	3	0	0	0	3	0	0	0	0	0
An. peryassui	0	0	0	2	2	0	0	0	0	0
Total	595	380	350	518	1843	100	163	58	93	414

TABLE II

Anopheles species distribution in Bate Estaca, Rondônia, Brazil during 2002-2004. *An. darlingi* is the main vector collected

	Bate Estaca							
	Indoor				Outdoor			
	2002	2003	2004		2002	2003	2004	
Anopheles species	n	n	n	T	n	n	n	T
An. darlingi	729	1765	1314	3808	145	592	362	1099
An. nuneztovari	8	21	10	39	0	1	2	3
An. kondori	17	48	6	71	2	0	1	3
An. triannulatus	17	16	27	60	0	0	1	1
An. peryassui	0	0	2	2	0	0	0	0
An. mediopunctatus	1	1	1	3	0	0	0	0
Total	772	1851	1360	3983	147	593	366	1106

Estaca, from 2002-2004, 5089 female anopheline mosquitoes were captured, 4907 (96.4%) were *An. darlingi* and only 182 (3.6%) other species. The most frequent non-*darlingi* species were *An. kondori, An. triannulatus, An. Nuneztovari,* and *An. mediopunctatus* (Table II).

Plasmodium infection index of captured mosquitoes - Among 957 mosquitoes dissected from Vila Candelária from 2001-2003, being 923 *An. darlingi* (96.4%), 11 *An. nuneztovari* (1.4%), 10 *An. kondori* (1.1%), 9 *An. evansae* (0.9%), 3 *An. matogrossensis* (0.3%), and 1 *An. rangeli* (0.1%). Only 3 were found with oocysts in the midgut (0.3%). One of them had sporozoites in the salivary glands (0.1%). In 2004, 306 mosquitoes were dissected, being 302 *An. darlingi* (98.6%), 3 *An. kondori* (0.9%), and 1 *An. nuneztovari* (0.3%). One was oocyst infected (0.3%) and one has sporozoites in the salivary glands (0.3%). In Bate Estaca, from 2002-2003, 917 mosquitoes were dissected but only one was found infected with sporozoites (0.1%), being 853 *An. darlingi* (93.1%), 32 *An. kondori* (3.4%), 16 *An. triannulatus* (1.7%), 14 *An. nuneztovari* (1.5%), and 2 *An. mediopunctatus* (0.2%). In 2004, among 656 dissected mosquitoes, being 632 *An. darlingi* (96.3%), 11 *An. kondori* (1.6%), 9 *An. triannulatus* (1.3%), 3 *An. nuneztovari* (0.4%), and 1 *An. mediopunctatus* (0.1%). One had oocysts in the midgut (0.1%). All infected mosquitoes were *An. darlingi*. For technical reasons, were not dissected 100% captured mosquitoes in both localities.

Seasonal dynamics of anopheline population and malaria transmission - Monthly distribution of anopheline mosquitoes captured from 2001 to 2004 in Vila Candelária and 2002 to 2004 in Bate Estaca, in simultaneously indoors and outdoor captures, are shown in Figs 2 and 3 expressed in hourly biting rates (HBR). In both localities the number of mosquitoes captured outdoors are always 3 to 5 higher than indoors. Time and procedures employed were always the same, figures from both localities can be compared. Overall anopheline density was much higher in Bate Estaca. Considering the average HBR observed in both localities (Figs 2 and 3) a rough comparative calculation can be made as follows: in Vila Candelária an individual outside the house would suffer from 10 thousand to 16 thousand anopheline bites during one year period, while a exposed individual inside the house would suffer 3 to 5 times fewer bites. In Bate Estaca these figures would be 20 to 50 thousand outside and 10 to 16 thousand inside the houses. Con-

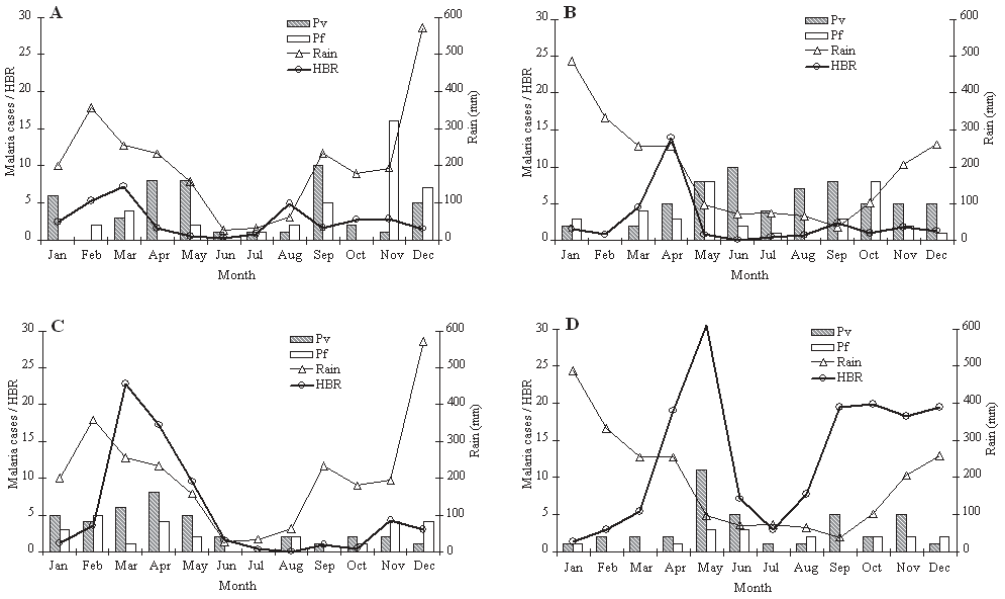

Fig. 2A, B: monthly fluctuations of rain precipitation (in mm), hour bite rates (HBR) outdoors values: fluctuation and monthly incidence of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) malaria in Vila Candelária in 2002 and 2003; C, D: monthly fluctuations of rain precipitation (in mm), HBR outdoors values: fluctuation and monthly incidence Pf and Pv malaria in Bate Estaca in 2002 and 2003.

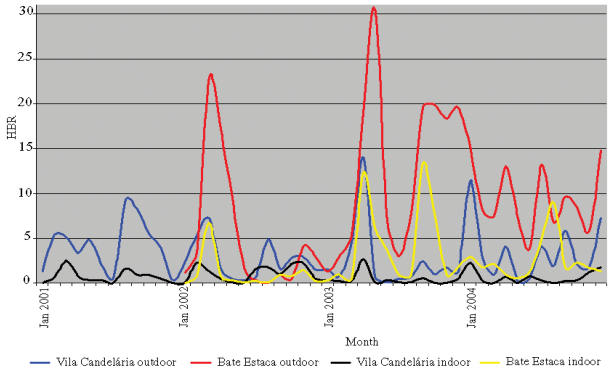

Fig. 3: monthly distribution of hour bite rates (HBR) in Vila Candelária and Bate Estaca from 2001-2004.

sidering the observed mosquito's infection's index, the risk for getting infective bites per year is approximately 10 outside and 2 inside the house in Vila Candelária and about 3 to 5 times more in Bate Estaca.

In both sites, peaks of high density of mosquitoes were registered from March to May, following the peak of rain precipitation (Fig. 2) and a decrease in mosquito density in the peak of the dry season (June/July). However, new peaks of high anopheline densities were registered sporadically in the end of the dry season (August/September) that may persist up to the beginning of the following rainy season (October/November).

General survey of anopheline breeding sites - Sample collection of larvae has been frequently done, and, as expected, more than 500 larvae and pupae captured in different breeding sites were almost all *An. darlingi*. The land area of Vila Candelária and Bate Estaca communities is of irregular relief and comprises riverside areas, close to the Madeira banks, inundated in the rainy season.

The residential area of Vila Candelária is located in a hillside and anopheline breeding sites usually are formed only in the rainy season when, at its climax in January/February, the level of the Madeira River increases by 10 m or more and floods areas in the lower riverside area of Vila Candelária and in large areas of Bate Estaca originating some "igapó-like" flooded areas. The distance between breeding sites is a 0.33 km (Fig. 1, - 1).

In the Bate Estaca, the houses are located at lower altitudes along the Madeira-Mamoré railroad line. Permanent anopheline breeding sites are present all over the year and most of them result from human activity (Fig. 1B). The primary event responsible for creating artificial breeding sites was the construction in the beginning of the XX century of the Madeira-Mamoré Railroad that established barriers for the free course of waters from local small streams and rivers (Fig. 1, B5). Secondary events were caused by the inhabitant's activities that eliminated the primary forest to implement agriculture and/or fish breeding sites. Later on, these structures were abandoned, exposing the area to flooding during the rainy season that is not completely drained at the end of the rainfalls (Fig 1, B6, B7, B8, B9). All these flooded areas are exposed to sun and plenty of organic matter from the aquatic vegetation and are permanent or semi permanent excellent breeding sites for *An. darlingi*. The distance between breeding site is a 0.48 km (Fig. 1, 2) and 0.49 km (Fig 1, 3).

DISCUSSION

The contrast between the two epidemiological models of malaria transmission (riverside malaria and dry land malaria), responsible for residual malaria in Rondônia, results primarily from differences in the nature and timing of the establishment of mosquito breeding sites by flooding. *An. darlingi* is the main vector in all Amazon areas (Deane 1988, Lourenço-de-Oliveira 1988,

Tadei & Dutary-Thatcher 2000) and it is the dominant species in both model areas (Gil et al. 2003). However, while its favorable breeding sites are always large collections of water rich in aquatic vegetation and exposed to sun, they drastically differ in origin and development in different areas.

In dry land north and northeast of Rondônia, malaria is observed in agro-industrial settlements and peripheral urban localities. Breeding sites originate after rainy season. Rainfall (up to two thousand millimeters from October to April or May) leads at each downpour episode, to an important increase in volume of secondary tributary rivers which flood the margins. The rapid flow rate of the water is inappropriate for in situ mosquitoes breeding, since larvae and eggs are removed. However, by the end of the rainy season, when rivers recover their original courses, large stable water collections are formed in areas previously inundated along their borders (Marques 1986, Camargo et al. 1994). These stagnated water collections comprise excellent breeding sites for *An. darlingi*. Therefore, high anopheline densities are observed at the end of rainy season and beginning of the dry season explaining the malaria outbreaks in the dry season (Camargo et al. 1994, 1996, Tadei & Dutary-Thatcher 2000).

In contrast, in the riverine situation, the large main rivers such as Madeira and Machado in Rondônia, water draining from all tributaries rivers promotes progressive increase from 10 to 14 m in water level, flooding large border areas and inundating the forest creating what is known locally as "igapós". The flow rate of water in the "igapós" area is usually low, and therefore they are favorable breeding sites, resulting in high HBR levels for the riverine population during the rainy season. However, with the end of rainfall, "igapós" progressively disappear during the dry season and, with them, also the breeding sites. Such characteristics explains the HBR variation recorded for local populations varying from 20 to 30 in April and May and decreasing to less than 1 in August to September (Alves et al. 2002, Gil et al. 2003) in a typical rural area where there are no serious modifications of the environment by human activities.

Human interventions changing original environmental situations are observed in every human community and are particularly intense in urban and suburban areas. The situation observed in Vila Candelária and Bate Estaca was therefore not surprising and can be considered typical. Riverside areas of these residential areas have been deeply modified and changed without any measure to prevent formation of mosquito breeding sites.

One aspect of malaria transmission observed in Vila Candelária and Bate Estaca needs to be highlighted, namely the low infection index found in captured anopheline mosquitoes. The 0.1-0.3% infection index found is in contrast with the high API and high prevalence of asymptomatic infections observed in Vila Candelária and in Bate Estaca (data not shown). Similar low infection indexes were found in previous studies in dry land areas and riverine rural areas of Rondônia (Gil et al. 2003). In a recent review, Tadei and Dutary-Thatcher (2000) registered infection index of 0.2 to 2.0 for *An. darlingi* captured in various endemic areas of Brazilian Amazon, including sites close to those of the present study. Santos et al. (2005) also found high infection indexes (6% by ELISA and 2.7% by microscopy) in an epidemic malaria outbreak in the city of Anajás (state of Pará, East Brazilian Amazon).

Several factors, however, can explain these contrasting findings. Under laboratory conditions, Klein et al. (1991) documented a high vector capability and susceptibility of *An. darlingi* from Rondônia. The high infection indexes found by Tadei and Dutary-Thatcher (2000) and Santos et al. (2005) is probably a result of the use of ELISA methods to identify infected mosquitoes (Zavala et al. 1982), known to be much more sensitive than microscopy. In addition, the studies by Tadei and Dutary-Thatcher (2000) were performed in "highways and towns where rapid and uncontrolled (malaria) growth had occurred" and Santos et al. (2005) describe an urban epidemic outbreak. In contrast, the present and previous studies by our group were developed in areas of low density populations and stable residual malaria. Finally, the low infection index observed among captured mosquitoes of Vila Candelária and Bate Estaca, could have been influenced by the constant presence of physicians, technicians, and entomologists, who actively searched and treated malaria infection, and in addition periodically sprayed piretroid insecticides in the residential areas. Nevertheless even with a low infection index of 0.1, the risk of malaria in Vila Candelária and Bate Estaca was 2-5 to 10-30 infecting bites per year, a level corresponding to mesoendemic areas of Africa.

In conclusion, the results of the present study taken together with those of Tada et al. (this issue) show that there are two related factors for the high prevalence and incidence of malaria in the urban neighborhood of Porto Velho: (1) the high prevalence of asymptomatic carriers in the human population; (2) the high anopheline density persisting during practically the whole year, resulting from deep environmental changes originated from human activities. This situation that is probably found in other areas of the Amazon requires innovative control measures that include implementation of control measures should include environmental sanitation and better characterization of the role of asymptomatic carriers in malaria transmission, as already emphasized by Coura et al. (2006).

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