

## ***Mansonella ozzardi* in Amazonas, Brazil: prevalence and distribution in the municipality of Coari, in the middle Solimões River**

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*This study investigated some epidemiological aspects of the *Mansonella ozzardi* in municipality of Coari, Amazonas. Clinical symptoms were correlated with the filarial infection and the parasitic infection rates (PIR) were estimated in simuliid vectors. The general *M. ozzardi* human prevalence rate was 13.3% (231/1733), of which 10.2% (109/1069) were from the urban area and 18.4% (122/664) from the rural area. The prevalence rates were higher in men (14.5% urban and 19.7% rural) than in women (6.7% urban and 17.2% rural) and occurred in most age groups. The indices of microfilaremics were higher in people  $\geq 51$  years old (26.9% urban and 61.5% rural). High prevalence rates were observed in retired people (27.1% urban area), housewives and farmer (41.6% and 25%, respectively, in rural area). The main clinical symptoms were joint pains and sensation of leg coldness. Only *Cerqueirellum argentiscutum* (Simuliidae) transmits *M. ozzardi* in this municipality (PIR = 5.6% urban and 7.1% rural). *M. ozzardi* is a widely distributed parasitic disease in Coari. Thus, temporary residency in the region of people from other localities involved with the local gas exploitation might be a contributing factor in spreading the disease.*

Key words: filariasis - mansonelliasis - black flies - Amazonia

*Mansonella ozzardi* is a nematode parasite whose microfilariae circulate in peripheral blood of infected humans. The parasite is identified by its morphological characteristics such as an unsheathed, long slender tail without nuclei at the tip (Orihel & Eberhard 1982). The nematode is one of the causative agents of mansonelliasis and its pathogenicity remains unclear. A variety of symptoms such as headache, vertigo, moderate fever, joint pains in the knee and ankle and the sensation of coldness in legs are implicated in *M. ozzardi* infection (Batista et al. 1960a, Tavares 1981, Bartoloni et al. 1999), which sometimes may be confused with malaria symptoms (Medeiros et al. 2008). In addition, ocular lesions have also been reported as possibly associated with the parasite infection (Branco et al. 1998, Garrido & Campos 2000, Cohen et al. 2008). Also, a large percentage of people are infected but lack clinical symptoms (Bartoloni et al. 1999).

In Brazil, *M. ozzardi* has been reported in the states of Amazonas (AM), Mato Grosso and Roraima (Deane et al. 1954, Oliveira 1963, D'Andretta et al. 1969). The filarial parasite has a wide distribution in AM with high prevalence rates along the Solimões-Amazon River (the upper Amazon River), Purus and Negro (Deane et al. 1954, Moraes et al. 1978). To date, only simuliid vector species have been known to transmit the disease in Brazil and particularly in AM, two other vector species are im-

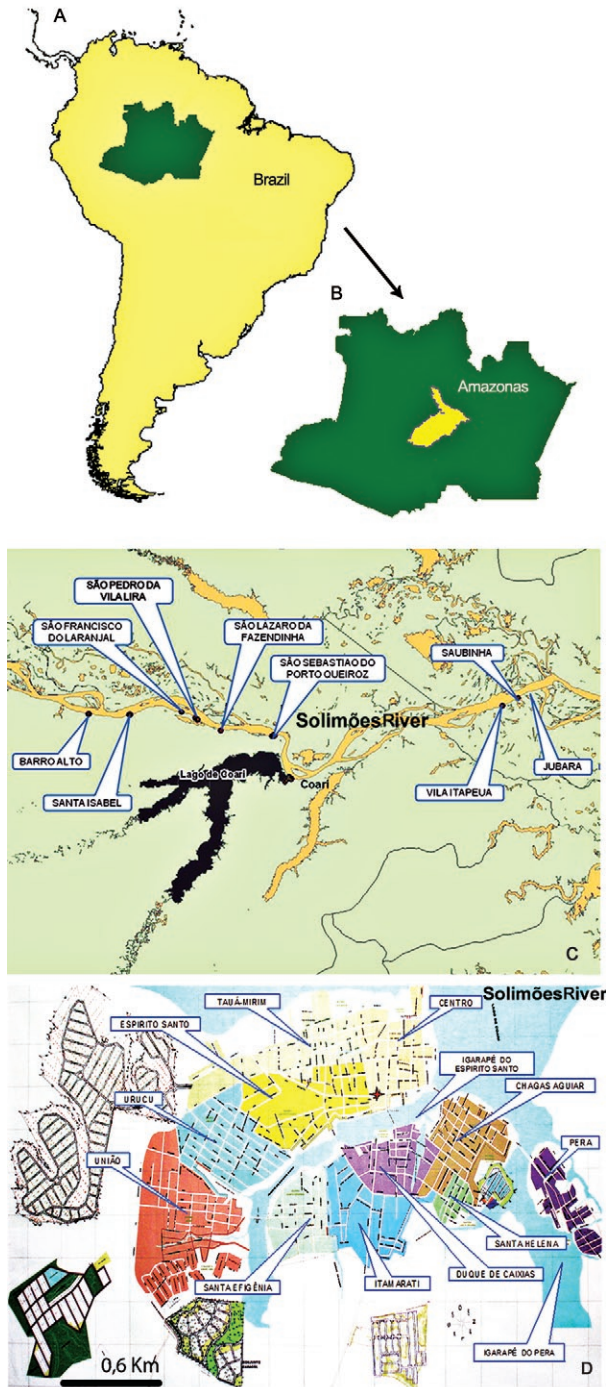
plicated in the infection, *Cerqueirellum amazonicum* and *Cerqueirellum argentiscutum* (Cerqueira 1959, Shelley & Shelley 1976, Shelley et al. 1980). Recently, Pessoa et al. (2008) have suggested another species, *Cerqueirellum pydanielli*, as a potential vector of the disease in AM.

Since the first reports on the occurrence of *M. ozzardi* in Brazil (Deane 1949, Rachou 1954), its prevalence rates have not decreased and the presence of filariasis have been recorded in new localities in the past years (Branco et al. 1998, Cohen et al. 2008, Medeiros et al. 2008, 2009a, b). The first epidemiological survey for mansonelliasis in the municipality of Coari, AM, Brazil, was conducted by Rachou (1957), who found a prevalence rate of 10% for *M. ozzardi*; therefore, the present investigation is aimed at estimating the prevalence of *M. ozzardi* in both rural and urban areas of this municipality, assessing clinical manifestations of the disease and identifying the vector of *M. ozzardi* and its parasitic infection rate (PIR). Although this filariasis is a very neglected disease, since the 1950s, researchers have been calling attention to the high rates of infection (Rachou 1954, Lacerda & Rachou 1956, Oliveira 1961, Shelley 1975, Moraes et al. 1978, Tavares 1981, Adami et al. 2008, Medeiros et al. 2008, 2009b). The controversy over symptoms and the absence of a permanent health assistance as well as orientation for treatment and prophylaxis is continuing.

### **PATIENTS, MATERIALS AND METHODS**

Our investigation was conducted along the middle Solimões River in the municipality of Coari, AM, Brazil (4°05'S 63°08'W) (Figure). Coari has over 65,000 inhabitants [urban population = 39.133 (60.20%), rural = 26.089 (40.14%)] (IBGE 2007). The climate is warm and humid and temperatures may rise above 36°C with relative air

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Location of the study area, municipality of Coari, Amazonas (AM), Brazil. A: Brazil; green area, the position of AM; B: AM, showing the Coari region; C: rural area of Coari; D: urban area of Coari.

humidity near 100%. Economic activity is based on agriculture, livestock farming and natural gas exploitation.

The human infection by *M. ozzardi* microfilariae were investigated in both rural communities and the urban area of the municipality's main town. Sample size was determined by assuming expected prevalence of 18.9%

(Cohen et al. 2008), error of 20%, with a confidence interval established at 95%. The rural survey included 664 residents of 10 small communities along the white-water Solimões River. For the urban survey, a random cluster survey method was used, with some streets (1-3 clusters) within each of the 13 districts of the Coari main town were selected at random and 1,069 residents surveyed. In each rural community and street all residents were invited to participate voluntarily in the study.

The infection by *M. ozzardi* was determined by light-microscopy examination (200X and 400X) of Giemsa-stained thick smears of peripheral blood obtained by digital puncture with a sterile disposable lancet (Orihel & Eberhard 1982, Post et al. 2003). The haemoscope survey took place between March 2006-August 2008. All the subjects under investigation were grouped according to gender, age (groups: 1-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50 and  $\geq 51$ ) and occupation (included farmer, retired, housewife, student, civil servant, other general services such as temporary farm workers, sewing activity, fishermen, builders, teachers and unemployed).

In addition, we conducted preliminary studies to explore the possible association of *M. ozzardi* infection with clinical disease among our study subjects. To this end, all infected individuals, plus a group of 58 uninfected individuals, were clinically examined by an experienced physician; particular attention was paid to signs and symptoms potentially related with filarial infections, such as joint pain, presence of red, itching skin plaques, leg coldness, headache, fever and lymph node swelling.

Finally, a rapid entomological assessment was conducted in the rural and urban areas under investigation. Potential *M. ozzardi* vectors (Simuliidae) were collected with manual suction devices for nearly 8 h in each study community and street. Flies were identified by species, stained with haematoxylin and dissected into three parts (head, thorax and abdomen) under a stereoscopic microscope (Medeiros et al. 2007). Individual flies were then examined under a light microscope to determine *M. ozzardi* infection and larval stage (L1, L2 and/or L3) and derive the PIR (Yazarbal et al. 1985). We adopted the blackfly nomenclature proposed by Py-Daniel and Sampaio (1994).

Epidemiological data were analysed with the EPI-INFO program (CDC 2000). We used  $\chi^2$  tests (Chi-square analysis) to compare prevalence in urban vs. rural areas and in male vs. female subjects. The relationships between age and prevalence were explored using Spearman correlation. Finally, the clinical manifestations related by individuals (microfilariae positive and negative) were analysed by comparing two proportions, non-balanced data, that is, with different numbered samples. In all tests,  $p$  values  $< 0.05$  were considered as indicative of statistically significant differences.

The present study was approved by the Research Ethical Committee of the Fundação de Medicina Tropical do Amazonas (2283/04/FMTAM), Manaus, AM, Brazil. Informed consent was obtained from all adults as well as from the parents of all children  $\geq 18$  years old included in the present study.

TABLE I  
Prevalence of *Mansonella ozzardi* infections in urban and rural areas,  
in municipality of Coari, Amazonas, Brazil, 2006-2008

Urban area	Positive/examined			Prevalence %		
	Males	Females	Total	Males	Females	Total
Espírito Santo	2/34	2/48	4/82	5.9	4.2	4.9
Centro	12/35	3/30	15/65	34.3	10	23.1
Chagas	6/91	5/120	11/211	6.6	4.2	5.2
Duque de Caxias	2/18	0/31	2/49	11.1	0	4.1
Igarapé do Espírito Santo	3/9	1/13	4/22	33.3	7.7	18.2
Igarapé do Pera	4/12	5/12	9/24	33.3	41.7	37.5
Pera I	17/84	10/101	27/185	20.2	9.9	14.6
Pera II	10/53	8/48	18/101	18.9	16.7	17.8
Pera III	6/60	3/53	9/113	10	5.7	8
Santa Efigênia	0/10	1/19	1/29	0	5.3	3.4
Tauá-Mirim	5/39	1/55	6/94	12.8	1.8	6.4
União	0/16	1/30	1/46	0	3.3	2.2
Urucu	2/15	0/33	2/48	13.3	0	4.2
<b>Total</b>	<b>69/476</b>	<b>40/593</b>	<b>109/1069</b>	<b>14.5</b>	<b>6.7</b>	<b>10.2<sup>b</sup></b>
<b>Rural area</b>						
Barro Alto	9/35	13/39	22/74	25.7	33.3	29.7
Barro Novo	0/1	1/4	1/5	0	25	20
Jubara <sup>a</sup>	1/1	0/0	1/1	100	0	100
Santa Izabel	3/7	1/15	4/22	42.9	6.7	18.2
São Francisco do Laranjal	5/22	6/28	11/50	22.7	21.4	22
São Lázaro da Fazendinha	3/15	3/13	6/28	18	23.1	21.4
São Sebastião do Porto						
Queiroz	11/41	6/53	17/94	26.8	11.3	18.1
São Pedro do Lira	13/70	15/65	28/135	18.6	23.1	20.7
Saubinha	6/60	1/55	7/115	10	1.8	6.1
Vila Itapeua	9/52	16/88	25/140	17.3	18.2	17.9
<b>Total</b>	<b>60/304</b>	<b>62/360</b>	<b>122/664</b>	<b>19.7</b>	<b>17.2</b>	<b>18.4<sup>b</sup></b>

a: only evaluated resident; b: X<sup>2</sup> test, p < 0.001.

## RESULTS

A total of 1,733 local inhabitants were analysed, out of which 231 (13.3%) were *M. ozzardi* positive. The rural area (18.4%) showed significantly higher prevalence rates than the urban area (10.2%) (X<sup>2</sup> = 17.50; p < 0.001). In the rural area, out of 664 people studied, 122 (18.4%) tested positive for *M. ozzardi*. The highest prevalence rates were in the following communities: Barro Alto (29.7%), São Francisco do Laranjal (22%) and São Lázaro da Fazendinha (21.4%). In the urban area, out of 1,069 inhabitants, 109 (10.2%) were positive for *M. ozzardi* and the highest prevalence rates were in the Igarapé do Pera (37.5%), Centro (23.1%) and Igarapé Espírito Santo (18.2%) (Table I).

Regarding gender in the urban area, men showed significantly higher prevalence rates than women (X<sup>2</sup> =

17.09; p < 0.001). Out of the 476 men and 593 women, 69 (14.5%) and 40 (6.7%) were *M. ozzardi* positive, respectively. In the rural area, out of 304 men, 60 (19.7%) tested positive for *M. ozzardi*, while 62 (17.2%) women out of 360 tested positive.

In both genders, the disease prevalence was found to be positively correlated with age (r = 0.882 p < 0.001; r = 0.843 p < 0.001) in both areas under investigation. Lower prevalence rates were found in younger people of both genders as follows (male and female percentages per area): 1-5 (3.03% and 3.2% urban area; 2.1% and 2.2% rural area), 6-10 (3.4% and 0% urban area; 3.3% and 1.3% rural area) and 11-15 (1.9% and 6.4% urban area; 1.9% and 10.3% rural area). In contrast, higher prevalence rates were found in inhabitants as follows (male and female percentages per area): aged 31-35 (37.5% and 10.8% urban area) 46-50 (66.7% and 50% rural area) and

TABLE II  
Prevalence of *Mansonella ozzardi* infections by gender and age, from urban and rural areas, in municipality Coari, Amazonas, Brazil, 2006-2008

Age groups	Urban area						Rural area					
	Positive/examined			Prevalence %			Positive/examined			Prevalence %		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
0-5	2/66	2/63	4/129	3	3.2	3.1	1/48	1/46	2/94	2.1	2.2	2.1
6-10	3/89	0/75	3/164	3.4	0	1.8	2/60	1/79	3/139	3.3	1.3	2.2
11-15	1/53	5/78	6/131	1.9	6.4	4.6	1/52	6/58	7/110	1.9	10.3	6.4
16-20	3/45	3/69	6/114	6.7	4.3	5.3	6/34	2/21	8/55	17.6	9.5	14.5
21-25	4/30	0/58	4/88	13.3	0	4.5	2/11	2/18	4/29	18.2	11.1	13.8
26-30	6/30	2/39	8/69	20	5.1	11.6	1/19	9/32	10/51	5.3	28.1	19.6
31-35	9/24	4/37	13/61	37.5	10.8	21.3	6/14	10/33	16/47	42.9	30.3	34
36-40	4/23	3/31	7/54	17.4	9.7	13	15/24	7/27	22/51	62.5	25.9	43.1
41-45	5/18	2/31	7/49	27.8	6.5	14.3	6/14	4/8	10/22	42.9	50	45.5
46-50	4/21	2/22	6/43	19	9.1	14	4/6	4/8	8/14	66.7	50	57.1
> = 51	29/78	16/89	45/167	37.2	18	26.9	16/22	16/30	32/52	72.7	53.3	61.5
Total	70/447	39/592	109/1069	14.7 <sup>a</sup>	6.6 <sup>a</sup>	10.2	60/304	62/360	122/664	19.7	17.2	18.4

a: x2 test, p < 0.001.



people  $\geq 51$  years old (37.2% and 18% urban area; 72.7% and 53.3% rural area) (Table II).

Concerning correlations between prevalence rates and occupation, retired local people from urban areas were more affected (30% men and 25% women), followed by civil servants (23.7% men). In the rural area, the highest prevalence was found in temporary farm workers (46.7% men), followed by housewives (41.6%) (Table III).

Overall, of the 289 individual were examined (individuals positive = 231, negative = 58), 127 (43.9%) had any one of the observed clinical signs and 162 (56.1%) showed no clinical symptoms related with mansonelliasis (Table IV). Individuals infected with *M. ozzardi*, 94 (40.7%) had at least one symptom related to the disease. The most common symptoms were joint pains [57 patients (24.7%)], sensation of coldness in legs [55 patients (23.8%)], headache [52 patients (22.5%)] and fever [49 patients (21.2%)]. Lymphadenopathy [10 patients (4.3%)] was the least common symptom. Of individuals without infection, 33 (56.9%) also had clinical signs, the most prevalent being headaches [12 patients (20.7%)], joint pains [7 (12.1%)] and the least prevalent being lymphadenopathy [2 patients (3.5%)]. Joint pains, coldness in legs and fever was statically different in both groups (Table IV).

Only the *C. argentiscutum* species was collected during this study. In the urban area, 498 flies were caught, of which 28 were *M. ozzardi* positive (PIR = 5.6%) with different larval developmental stages: L1 (16 individuals - 57.1%), L2 (11 individuals - 39.3%) and L3 (1 individual - 3.6%). The L1, L2 and/or L3 mean per infected fly was 13.4 (range 1-34). In the rural area, 170 simuliids were captured, of which 12 were infected (PIR = 7.1%) with different larval developmental stages: L1 (8 individual - 66.7%), L2 (3 individual - 25%) and L3 (1 individual - 8.3%). The L1, L2 and/or L3 mean per infected fly was 3.25 (range 1-10) (Table V). Only *M. ozzardi* was found in humans and flies in this study, confirmed by a new identification protocol using the polymerase chain reaction (M Martins et al., unpublished observations).

## DISCUSSION

The present study showed that *M. ozzardi* is widely distributed in the municipality of Coari because it was found in every district of the urban area (prevalence: 10.2%; variance between 2.2-37.5%) and in every community in the rural area (prevalence: 18.4%; varied between 6.1-29.7%). Our results showed a total index of 13.3% in the region studied, which is higher than the last recorded survey (10%) that included other localities such as Manacapuru (0.3%), Tefê (5.8%), Benjamin Constant (4.6%) and São Paulo de Olivença (12.6%) (Rachou 1954, Lacerda & Rachou 1956). In addition, by comparison with recent data found in Lábrea and Boca do Acre, Purus River (Medeiros et al. 2008, 2009b), our study indicates that microfilaremia rates have risen in the region of Coari in recent years. We observed a higher percentage of microfilaremic-positive people from neighborhoods near streams and the Solimões River, which was probably due to their proximity of the breeding place of the vectors (D in Figure).

TABLE III  
Prevalence of *Mansonella ozzardi* infections by occupation and gender, from urban and rural areas, in municipality of Coari, Amazonas, Brazil, 2006-2008

Occupation	Urban area			Rural area		
	Males	Females	Total	Males	Females	Total
	Positive/examined (prevalence %)			Positive/examined (prevalence %)		
Farmer	2/10 (20)	1/49 (2)	3/59 (5.1)	26/102 (25.5)	2/47 (4.3)	28/149 (25)
Retireds	6/20 (30)	7/28 (25)	13/48 (27.1)	3/40 (7.5)	3/21 (14.3)	6/61 (9.8)
Housewife	0/15 (0)	10/200 (5)	10/215 (4.7)	-	32/77 (41.6)	32/77 (41.6)
Student	2/31 (6.5)	4/48 (8.3)	6/79 (7.6)	10/40 (25)	10/55 (18.2)	20/95 (21.1)
Civil servant	14/59 (23.7)	6/67 (9)	20/126 (15.9)	2/35 (5.7)	4/12 (33.3)	6/47 (12.8)
Otherservices <sup>a</sup>	31/181 (17.1)	6/84 (7.1)	37/265 (14)	14/30 (46.7)	7/90 (7.8)	21/120 (17.5)
Unemployed	15/103 (14.5)	5/37 (13.5)	20/140 (14.3)	5/25 (20)	4/28 (14.3)	9/53 (17)
Without information	0/58 (0)	0/79 (0)	0/137 (0)	0/32 (0)	0/30 (0)	0/62 (0)
Total	70/477 (14.7)	39/592 (6.6)	109/1,069 (10.2)	60/304 (19.7)	62/360 (17.2)	122/664 (18.4)

a: other general services, temporary farm workers, sewing activity, fishermen, builders, teachers.

TABLE IV  
Comparison between clinical symptoms in inhabitants infected and not infected with *Mansonella ozzardi* in municipality of Coari, Amazonas, Brazil, 2006-2008

Clinical symptoms	Individual sampled		p
	Individual positive for microfilarie (n = 231)	Individual negative for microfilarie (n = 58)	
Articular pains	57 (24.7)	7 (12.1)	< 0.01
Coldness in legs	55 (23.8)	3 (5.2)	< 0.01
Headache	52 (22.5)	12 (20.7)	0.4
Fever	49 (21.2)	5 (8.6)	< 0.01
Red itching cutaneous plaques	13 (5.6)	4 (6.8)	0.36
Lymphadenopathy	10 (4.3)	2 (3.5)	0.38
Without clinical symptoms	137 (59.3)	25 (43.1)	< 0.01

TABLE V  
Simuliid black flies collected, parasitic infection rate (PIR) and larval stages of *Mansonella ozzardi* from urban and rural areas in the municipality of Coari, Amazonas, Brazil

Communities	Simuliids collected	Total parasitized	PIR (%)	Simuliids with L1	Simuliids with L2	Simuliids with L3	Larval stages of <i>M. ozzardi</i>
Urban area							
Pera	498	28	5.6	16	11	01	173L1, 197L2, 4L3
Rural area							
Pedro da Vila Lira	37	02	5.4	01	-	01	1L1, 10L3
Porto Queiroz	32	06	18.7	06	-	-	16L1
Barro Alto	79	03	3.8	01	02	-	3L1, 8L2
São Francisco do Laranjal	22	01	4.5	-	01	-	1L2
Rural total	170	12	7.1	08	03	01	20L1, 9L2, 10L3
Total	668	40	6	24	14	02	193L1, 206L2, 14L3

Furthermore, mansonelliasis prevalence rates were higher in men than women, mainly in the urban area, which corroborates data obtained in other studies (Batista et al. 1960b, Lawrence et al. 1980, Kozek et al. 1983, Medeiros et al. 2009a). This difference might be due to the fact that men are more exposed to vectors when working outdoors. However, in rural areas, probably women have a similar degree of exposure, which may explain the small difference between gender found here (Table I). Further evidence of such exposure to transmission was the high prevalence of disease in relation to professional activity. In the rural area, housewives and farmers represented the most microfilaremic groups, indicating that individuals working along river

margins and in agricultural fields are more exposed to simuliids and therefore are at a greater risk of infection than other occupations. However, in the urban area, the highest prevalence was found in the retired, suggesting that these individuals could have suffered continuous succession of overlapping infections that remained untreated during their life. Other investigations carried out in AM have correlated higher *M. ozzardi* infection rates with occupations linked to high exposure (Batista et al. 1960b, Shelley 1975, Tavares 1981, Medeiros et al. 2009a), such as in farmers and fishermen.

In contrast, the lowest prevalence rates found were in younger individuals (ages 1-5; 6-10; 11-15) and were increasingly higher from 31 years old on, mainly

among men, which suggest that they become infected when they work in the field due to exposure to the vectors, in both urban and rural areas. In the rural area, the older the individuals, the higher were the prevalence rates, while in the urban area, rates varied according to the age ranges under study. Possibly, such variations, according to age between rural and urban areas, might be due to the degree of exposure to vectors, since in the rural area, local workers are more exposed to continuous transmission. Other investigations have also shown higher infection rates correlated with older individuals (Batista et al. 1960b, Moraes et al. 1978, Medeiros et al. 2007, 2008, 2009a).

*M. ozzardi* has been viewed by some authors as an insignificant filarial pathogen, although a causal association between this parasite and various clinical illnesses has been made. Through clinical examinations, we observed that the main symptoms in microfilaremic individuals were joint pains, sensation of coldness and fever. Such data are in accordance with those evidenced by Batista et al. (1960a), Tavares (1981) and Bartoloni et al. (1999). Coldness sensation is related to peripheral vasoconstriction caused by toxins or metabolic substances produced by microfilaria and joint pains might be related to joint capsule or tendon edema (Tavares 1981). To other authors (Oliveira 1961, Undiano 1971), mansonelliasis pathogenesis is related to reactions caused by repeated infections of *M. ozzardi*. Therefore, additional studies are required to define clearly the pathogenesis of this parasite in relation to its manifestation. We have also observed that the symptoms correlated with *M. ozzardi* infection are frequently evidenced in the local people infected with malaria and other serious endemic disease of the area (Braga et al. 2006), which makes it crucial that differential diagnosis through identification of the parasite be made using blood smear examinations (Medeiros et al. 2008). Due to the high percentage of people found without apparent symptoms, more accurate studies must be made to evaluate the life quality impact of this disease.

The municipality of Coari has experienced an increase in population. It may be related with the fact that an oil company has recently settled in the region, bringing more service providers. This sort of company usually hires temporary workers, who are then infected with *M. ozzardi*. Upon returning to their regions, they are thought to spread the disease into new areas because the vector species of the genus *Cerqueirellum* have a wide geographic distribution in Brazil (Shelley et al. 2006).

According to our results, *C. argentiscutum* is the vector of *M. ozzardi* on the Solimões River, which corroborates data provided by other authors as Cerqueira (1959), Shelley et al. (1980) and Medeiros and Py-Daniel (2004). Furthermore, PIRs showed an increase (urban = 5.6%, rural = 7.1%) when compared with results by Medeiros and Py-Daniel (2004) in the downstream region of the Solimões River (0.08%), which suggest that both urban and rural areas are under high transmission risk of *M. ozzardi*, as the PIRs showed (193 L1, 206 L2 and 14 L3) in captured simuliid flies. The presence

of vectors in urban areas should be taken into account when control measures are designed, similar to what has been done for other diseases such as malaria, dengue and Chagas.

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