

# Epidemiological studies of *Mansonella ozzardi* (Nematoda, Onchocercidae) in indigenous communities of Pauini municipality, Amazonas, Brazil<sup>1</sup>

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## ABSTRACT

The *Mansonella ozzardi* has a widespread distribution among the indigenous and riverine communities of Amazonas, Brazil. We estimated the prevalence of *Mansonella ozzardi* in indigenous communities of the Pauini municipality, Amazonas state, Brazil and the rate of parasitic infection in vectors. We collected thick blood smears from individuals from six Apurinã indigenous communities along the Purus River and its tributaries. Collections of simuliids were made and dissected, and the larval instars of *M. ozzardi* identified. The overall prevalence of *M. ozzardi* was 28.40%, with the highest incidence among males and agricultural workers. Among age groups, children 2-9 years of age had the lowest incidence, while individuals older than 58 exhibited the highest rates of infection. We found infected simuliids in three communities, with Parasitic Infection Rates (PIR) of 0.34-6.58%. The prevalence of *M. ozzardi* among the Apurinã people is high, possibly related to the diary activities of the riparian people, where a high abundance of the vectors exists.

## KEY WORDS

*Mansonella ozzardi*, Mansonelliasis, Simuliidae, Amazonas, Brazil.

## *Estudos Epidemiológicos da Mansonella ozzardi (Nematoda, Onchocercidae) em Comunidades Indígenas do Município de Pauini, Amazonas, Brasil*

## RESUMO

Esse trabalho tem como objetivos estimar a prevalência de *Mansonella ozzardi* em comunidades indígenas do município de Pauini, Amazonas, Brasil e estimar a Taxa de Infecção Parasitária nos vetores. Foram examinados indígenas da etnia Apurinã, pelo método de gota espessa, em seis comunidades localizadas as margens e afluentes do rio Purus. A prevalência geral para *M. ozzardi* foi de 28,40%, com maior incidência para o sexo masculino e nos indivíduos com atividade no campo (agricultores). Em relação à faixa etária, as menores prevalências foram obtidas nos indivíduos mais jovens (2-9 anos), enquanto as maiores nos indivíduos acima de 58 anos. Foram encontrados simuliídeos parasitados em três comunidades, com uma Taxa de Infecção Parasitária que variou de 0,34 a 6,58%. A prevalência de *M. ozzardi* entre os Apurinã é elevada, possivelmente relacionada às suas atividades diárias as margens dos rios, onde existe uma grande abundância dos vetores.

## PALAVRAS-CHAVE

*Mansonella ozzardi*, Mansonelose, Simuliidae, Amazonas, Brasil.

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## INTRODUCTION

*Mansonella ozzardi* is a filarial worm with an exclusively American distribution, and in Brazil, is found in the states of Amazonas, Mato Grosso (high River Xingu), and Roraima (Deane et al., 1954; Oliveira, 1963; D'Andretta et al., 1969). In Amazonas, this filaria has an ample distribution and infects riverine and indigenous communities of the Solimões, Purus, and Negro Rivers and their tributaries (Lacerda & Rachou, 1956; Moraes et al., 1978; Lawrence et al., 1980, Tavares, 1981).

Mansonelliasis is a relatively little-studied filarial disease, with doubtful pathology. The people with mansonelliasis and high microfilaremia present moderate fever, joint pains, adenitis, headaches, and coldness of the legs (Batista et al., 1960). A new symptom is the presence of visual lesions, which may lead to blindness (Branco et al., 1998; Garrido & Campos, 2000).

The *M. ozzardi* is transmitted by dipterian insects of the families Ceratopogonidae and Simuliidae. In Brazil, only simuliids are recognized as vectors, and were first recorded by Cerqueira (1959) who found *Simulium amazonicum* (= *Cerqueirellum argentiscutum*) infected with larvae of *M. ozzardi*. Shelley & Shelley (1976) found *S. amazonicum* (= *C. amazonicum*) incriminated as a vector in the Purus river, Amazonas; and Shelley et al. (1980) found *S. amazonicum* (= *C. amazonicum*) and *S. argentiscutum* (= *C. argentiscutum*) to be vectors in the high Solimões river. Moraes et al. (1985) demonstrated that *S. oyapockense* (= *C. oyapockense*) was a vector in the state of Roraima.

In the Pauini region of Amazonas, a possible link between corneal lesions and mansonelliasis was discovered while Branco et al. (1998) were working in riverside communities. Our study is the first epidemiological work on mansonelliasis in the Pauini municipality. The objectives of this paper were to estimate human prevalence of mansonelliasis, to incriminate the simuliid species involved in transmission of *M. ozzardi*, and estimate the Rate of Parasitic Infection in the vectors.

## MATERIAL AND METHODS

### STUDY AREA

We studied people from indigenous communities in the municipality of Pauini (07°42' S/ 67°01' W), Amazonas state, Brazil. Pauini is located along the Purus River, in the southeast of Amazonas and is accessible only by water or air. We visited the communities along the Purus and Pauini rivers by boat.

### ESTIMATES OF HUMAN PREVALENCE

We examined individuals from six indigenous communities Apuriná (Caciriqui, Mapiri, Makiri, Peneri, Afogados and Jagunço) considering sex, age and occupation (farmer, housewives, students, children, and adults without formal occupations), in November 2004 and May 2005. The sample

was done in individuals of both sexes ( $\geq 2$  years of age) and at least 50% or more of the populations of all visited communities were examined. We estimated *M. ozzardi* prevalence using thick blood smears obtained by digital punctures. We collected two drops of blood from each individual and placed them on a glass slide, covering approximately a 1.5 x 1.5 cm area (about 0.02 ml). After air-drying, the blood was dehemoglobinized in methylene blue, stained with Giemsa solution, and then washed with distilled water. We examined the slides under light microscopes, using the 10x and 20x objectives, and when positive slides were found, the microfilariae were identified following Orihel et al. (1997). We then counted the number of microfilariae per smear. We obtained permission from each participating individual adult, or in the case of children, their parents.

### SIMULIID CAPTURE AND DISSECTION

Simuliids black flies were caught in several communities using a rapid entomological assessment technique using manual suction collectors. Simuliids were placed in test tubes containing 70% ethanol and identified as to species in our laboratory.

The black flies were stained with acid hematoxylin during a period of 48 hours and dissected using a stereomicroscope. We divided each collected simuliid into three parts (head, thorax, and abdomen) using a stylet. We placed the three parts on a slide, each in their own drop of glycerin and covered with a cover slip, and the slides were examined under a light microscope. When filarial worms were found, we identified and classified them as to developmental stage (Mf, L1, L2, or L3), and then quantified them following Yarzabal et al. (1985).

We calculated the Parasitic Infection Rate (PIR) as the number of black flies parasited by *M. ozzardi* of any stage divided by the total number of females collected, multiplied by 100.

### DATA ANALYSIS

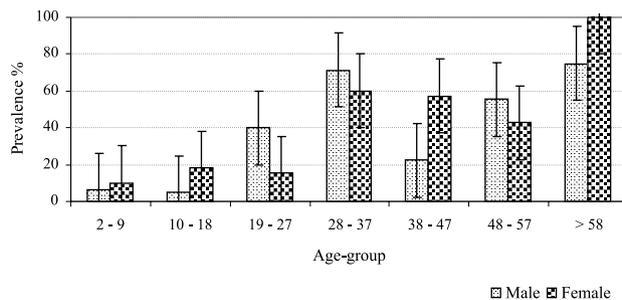
We estimated human prevalence among different sex, age, and occupation classes. Male and female prevalence was compared using the non-parametric "G-test". We used "Spearman rank-correlation" considering the prevalence and age groups and among the number of microfilaria parasitizing individuals in different age groups.

## RESULTS

We examined a total of 169 individuals (90 men and 79 women) between the ages of 2 and 71 and found 48 individuals parasitized (28.40%), with significantly higher prevalence in men (28 individuals, 31.33%) than women (20 individuals, 25.32%) ( $G = 34.44$ , d.f. 5,  $P < 0.001$ ). The community of Makiri had the lowest infection rates (18.57%), while Afogados had the highest (43.48%). The mean number of microfilariae found in blood smears varied among communities, with the

highest numbers in the communities of Afogados (83.40±166.93) and Caciriqui (61.66±106.80) (Table 1).

Among age groups, we found the lowest infection rates in individuals in their first decades of life (2-9 years, 8.33% individuals infected; 10-18 years, 11.63% individuals infected). Prevalence was highest among individuals older than 58 (80.00%). These trends were the same in both men and women and we found a positive correlation between age and prevalence ( $r_m = 0.78$ ,  $r_f = 0.82$   $P < 0.05$ ) (Table 2, Figure 1).



**Figure 1** - Prevalence of *Mansonella ozzardi* by age in indigenous communities in November/2004 and May/2005, Pauini municipality, Amazon state, Brazil.

**Table 1** - Number of individuals examined/positive for *Mansonella ozzardi* by sex in indigenous communities in November/2004 and May/2005, Pauini municipality, Amazon state, Brazil.

Communities	Positive/ Examined			Prevalence %			Mean of mf./individual
	Males	Females	Total	Males	Females	Total	
Caciriqui	5/10	1/9	6/19	50.00	11.11	31.58	61.66±106.80
Mapiri	3/12	2/7	5/19	25.00	28.57	26.31	28.40±30.90
Makiri	4/12	2/20	6/32	33.33	10.00	18.57	48.33±69.78
Peneri	9/42	10/28	19/70	21.43	32.14	27.14	30.26±50.06
Afogados	6/11	4/12	10/23	54.54	33.33	43.48	83.40±166.93
Jagunço	1/3	1/3	2/6	33.33	33.33	33.33	12.00±5.06
Total	28/90	20/79	48/169	31.11	25.32	28.40	46.56±92.85

mf. = microfilariae

The mean number of parasites in blood samples was greater in males (68.50±116.12) than females (15.85±21.51). Overall, individuals in age groups 2-9 and 8-10 years had the lowest mean number of microfilariae per sample. In males, we found the highest microfilaria numbers in individuals aged 48-57 (74.20±62.80) and above 58 (151.83±201.7). We found a significant correlation between mean number of microfilariae and age ( $r = 0.52$ ,  $P < 0.05$ ). In females, we found the highest number of microfilariae per blood smear in women aged 28-37 (36.00±52.94) (Table 2). A 60-year old man from the community of Afogados had the highest number of microfilariae per slide in the study with 549 microfilariae in two drops of blood.

Among occupation categories, individuals who work outdoors (farmers) had the highest prevalence (47.73%), as well as mean microfilaria (78.89±131.87). Housewives had the next highest prevalence (35.48%), and a mean number of parasites of 21.00±27.81 (Table 3).

In order to know the Parasitic Infection Rate (PIR) of mansonelliasis vectors, we collected specimens of the simuliid species *C. amazonicum* biting humans in three communities. In Caciriqui, of the 171 simuliids collected, three were infected with a PIR of 1.75%. The community of Mapiri had the highest PIR, 6.38%, six of the 94 simuliids collected. We collected 185

**Table 3** - Prevalence of *Mansonella ozzardi* in relation to occupation of individuals in indigenous communities in November/2004 and May/2005, Pauini municipality, Amazon state, Brazil.

Occupation	Positive/ Examined	Prevalence (%)	Mean of mf./occupation
Farmer	18/38	47.37	78.89±131.87
Housewife	11/31	35.48	21.00±27.81
Student	4/39	10.26	10.00±8.70
Without a formal occupation	2/20	10.00	1.50±0.70
Other*	2/2	100.00	13.5±10.60

\*blind person, teacher.

**Table 2** - Prevalence of *Mansonella ozzardi* by age and sex in indigenous communities in November/2004 and May/2005, Pauini municipality, Amazon state, Brazil.

Age group	Examined			Infected (Prevalence %)			Mean of mf./sex	
	Males	Females	Total	Males	Females	Total	Males	Females
2 - 9	16	20	36	1(6.25)	2(10.00)	3(8.33)	2.0	11.00±14.14
10 - 18	21	22	43	1(4.76)	4(18.18)	5(11.63)	7.00	7.00±4.89
19 - 27	20	13	33	8(40.00)	2(15.38)	10(30.30)	43.75±95.76	12.00±5.65
28 - 37	7	8	15	5(71.42)	3(60.00)	8(53.33)	47.80±68.09	36.00±52.94
38 - 47	9	7	16	2(22.22)	4(57.14)	6(37.50)	19.00±2.82	13.75±13.67
48 - 57	9	7	16	5(55.55)	3(42.86)	8(50.00)	74.20±62.80	19.00±16.82
≥ 58	8	2	10	6(75.00)	2(100.00)	8(80.00)	151.83±201.7	11.50±7.77
Total	90	79	169	28(31.11)	20(25.32)	48(28.40)	68.50±116.12	15.85±21.51

simuliids in Peneri and found only one infected, for a PIR of 0.54%. All parasites found were L1 or L2 larval stages in the thorax of the simuliids (Table 4).

**Table 4** - Number of *Cerqueirellum amazonicum* collected and infected; Parasitic Infection Rate (PIR); larval stages of *Mansonella ozzardi*, during November/2004 and May/2005 in indigenous Pauini municipality, Amazon state, Brazil.

Communities	Total number of simuliids collected	Simuliids Infected	PIR%	Larval stages
Caciriqui	171	03	1.75	2L1, 2L2
Mapiri	94	06	6.38	8L1, 8L2
Peneri	185	01	0.54	1L1

PIR = Parasitic Infection Rate

## DISCUSSION

The overall mansonelliasis prevalence shows that *M. ozzardi* is widespread across Apurinã populations of the Pauini municipality. All communities had an elevated rate of mansonelliasis, ranging from 18.57% to 43.48%. Moraes *et al.* (1978) found high prevalence among the Ticunas people, with an average infection rate of 45.7% (although in some communities he found more than 50.0% of the people examined infected) and a mean microfilaremia count per person of 37.1. In indigenous people of the Baniwa, Yanomami, Kanamari, Jaminawa, Marubo, Kashinawa, and Katukina tribes were found to have a mean prevalence of 12.0% with the highest prevalences in a Baniwa (63.0%) and a Tikuna (35.0%) community (Lawrence *et al.*, 1979). They estimated mean microfilarial count per individual at 22.0, varying between 11 and 82 (Lawrence *et al.*, 1979). In surveys in Venezuela, a mean prevalence of 16.1% in indigenous communities of the middle Orinoco and the Parima mountains was found; and a prevalence of 58.0% among indigenous people of the Caura River basin, in southwest Bolivar state was recorded (Godoy *et al.*, 1980; Botto *et al.*, 1983). In the Pakaraima mountains, Guyana, a prevalence of *M. ozzardi* of 10.7% in indigenous communities was observed (Nathan *et al.*, 1982).

Moraes *et al.* (1978) found a non-significant difference in male-female infection rates, with slightly higher prevalence in males, and concluded that the risk of infection was the same for both sexes. In this work, we found a significantly higher prevalence in males, especially in farmers, which we believe is directly related to the degree of exposure to vectors of *M. ozzardi*. Due to diary activities, the men spent more time in the fields or along river margins than women; these areas have high densities of simuliids exhibiting hematophagic activity.

*M. ozzardi* infection can occur in the first decade of life, although we encountered low prevalence among this age group (1.77%). Infection becomes more common in adults in the third

decade of life with the highest prevalence in older individuals. Possibly due to the cumulative characteristic of this disease and because the elder people had been more exposed to the vectors, can explain these high prevalence. Other studies have also found that the probability of microfilaremia increases with age (Moraes *et al.*, 1978; Lawrence *et al.*, 1979; Nathan *et al.*, 1982). In one study, approximately 90% of men over 60 were infected. Women in their third decade of life had infection rates of 60% and maintained this rate until they were 60 years old (Moraes *et al.*, 1978).

In the Pauini region, we collected *C. amazonicum* biting humans, and some of them were infected with *M. ozzardi*. Although we captured fewer simuliids than we expected, we found high Parasitic Infection Rates (PIR) in the specimens collected. For example, in the community of Mapiri, we found a PIR of 6,38%, while Shelley & Shelley (1976) recorded a PIR of 0.99% in black flies captured along the Purus River, in the municipality of Lábrea. Shelley *et al.* (1980) found an infection rate of 3.1% in simuliids collected among the Ticunas along higher Solimões river. The difference of PIR between the studied communities just can be explained with a systematic collections of black flies during at least a year because the fluctuation of seasonality (e.g. Medeiros & Py-Daniel, 2004). Also the population habits may influence the PIR.

We observed *M. ozzardi* directly affecting the health of the indigenous populations of Pauini. We observed that persons with mansonelliasis may have fever, headaches, and general pains in parts of the body, those symptoms may confuse with malaria (Medeiros *et al.*, 2006). Since some government agencies still classify mansonelliasis as apathogenic, there exists no program to treat and control the transmission of this disease in affected communities.

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