



## Article/Artigo

# Investigation of the occurrence of *Mansonella ozzardi* in the State of Rondônia, Western Amazonia, Brazil

Investigação da ocorrência da *Mansonella ozzardi* no Estado de Rondônia, Amazônia Ocidental

Sergio de Almeida Basano<sup>1,2</sup>, Juliana de Souza Almeida Aranha Camargo<sup>1</sup>, Luana Janaína de Souza Vera<sup>1</sup>, Suzane Neves Velasques<sup>3</sup>, Guilherme Maerschner Ogawa<sup>3</sup>, Jansen Fernandes Medeiros<sup>4,5</sup>, Gilberto Fontes<sup>6</sup> and Luís Marcelo Aranha Camargo<sup>1,3</sup>

### ABSTRACT

**Introduction:** *Mansonella ozzardi*, a human filarial nematode transmitted by Diptera Simuliidae flies (black flies), is widely spread in the Amazon, while the occurrence of mansonellosis in the State of Rondônia (Western Amazonia) has not been reported thus far. The existence of this parasitic disease in this state is investigated in this study. **Methods:** A total of 4,452 people who lived alongside the Madeira, Mamore, Guapore, Machado and Preto Rivers, in the State of Rondônia, were examined using the thick blood smear method. Alongside the Machado and Guapore rivers, 2,112 adult simuliid specimens were captured. **Results:** Infected people were not reported, although simuliids with vector potential such as *Cerqueirellum pydanielli*, *C. argentiscutum* and *C. amazonicum* were found. **Conclusions:** This study registered no infected people. This finding may be explained by the individuals' low level of microfilaremia that is below the limit of detection of the diagnostic method used in the study or insufficient migration of people infected with *M. ozzardi* from endemic areas, making it impossible to introduce the disease into Rondônia. Further studies using more sensitive diagnostic techniques could shed light on this question.

**Keywords:** *Mansonella ozzardi*. Microfilariae. Simuliidae. State of Rondônia.

### RESUMO

**Introdução:** *Mansonella ozzardi* é uma filária humana que tem como vetor dípteros simulídeos e é amplamente distribuída na Amazônia. Não há informações sobre a ocorrência de casos de mansonelose no Estado de Rondônia, e neste trabalho procurou-se investigar a situação epidemiológica desta parasitose em áreas urbanas e ribeirinhas na Amazônia Ocidental. **Métodos:** Foram examinados 4.452 moradores às margens dos rios Madeira, Mamoré, Guaporé, Machado e Preto em Rondônia, através da técnica da gota espessa de sangue. Capturou-se às margens do rio Machado e Guaporé 2.112 simulídeos adultos das espécies *Cerqueirellum pydanielli* e *Chirostibia pertinax*. **Resultados:** Não foram encontradas pessoas infectadas com *M. ozzardi* e foram encontradas diferentes espécies de simulídeos com potencial de transmissão da mansonelose. **Conclusões:** Não foram encontrados indivíduos infectados com *M. ozzardi* neste estudo. Este fato pode ser explicado pela existência de pacientes com baixas microfílemias, associado à técnica diagnóstica de baixa sensibilidade utilizada no estudo. Pode-se ainda aventar a possibilidade de ter ocorrido migração insuficiente de pacientes infectados de áreas endêmicas, impossibilitando a implantação da endemia. Estudos posteriores com técnicas diagnósticas mais sensíveis podem ajudar a esclarecer esta questão.

**Palavras-chaves:** *Mansonella ozzardi*. Microfílarias. Simuliidae. Rondônia.

### INTRODUCTION

*Mansonella ozzardi* microfilariae are found in the peripheral blood of human hosts and are one of the etiologic agents of mansonellosis. In some countries, this parasite appears simultaneously with other filarial parasites, such as *Onchocerca volvulus* and/or *Mansonella perstans*<sup>1,2</sup>. In Brazil, this parasite was first observed in a nationwide survey of the occurrence of lymphatic filariasis in the City of Manaus in the Amazon region in 1949<sup>3</sup>. Three and a half percent (3.5%) of the population was examined by the thick blood smear method with positive results found in 0.6% (15 patients) for *M. ozzardi*. Among these patients, seven had never left the city of Manaus, which showed that *M. ozzardi* was autochthonous<sup>3</sup>. Later, some researchers observed individuals infected with *M. ozzardi* alongside the Solimoes, Purus and Negro Rivers in the State of Amazonas<sup>4-6</sup>. Recent data have indicated that this parasite is still found with high prevalence rates in the Amazonian territory<sup>7-9</sup>.

Outside of the State of Amazonas, this parasite has already been found in the State of Mato Grosso and Roraima<sup>10,11</sup>. In Brazil, only insects of the Simuliidae family transmit *M. ozzardi*: *Cerqueirellum amazonicum*, *C. argentiscutum* and *C. oyapockense*<sup>12-14</sup> and more recently *C. pydanielli* are potential vectors for mansonellosis in the State of Amazonas<sup>15</sup>.

A survey on filariasis was conducted in the State of Rondônia in the 1950s as part of the national survey, and *M. ozzardi* hosts were not found. Only lymphatic filariasis caused by *Wuchereria bancrofti* was detected in this State<sup>16</sup>. Since then, no other national survey has been performed in the region. However, a few local studies of *M. ozzardi* have been performed on the population in the Amazon region. No studies have been conducted in the State of Rondônia despite the fact that some cases of mansonellosis have been reported in the state, mainly in individuals that migrate from the State of Amazonas, especially from the municipalities of

1. Departamento de Medicina, Faculdade São Lucas, Porto Velho, RO. 2. Hospital Cemeton, Secretaria de Saúde de Rondônia, Porto Velho, RO. 3. Unidade de Pesquisa de Monte Negro, Instituto de Ciências Biomédicas, Monte Negro, RO. 4. Departamento de Entomologia, Instituto Nacional de Pesquisa da Amazônia, Manaus, AM. 5. Departamento de Entomologia, Universidade Estadual do Amazonas, Manaus, AM. 6. Campus Centro Oeste (CCO), Universidade Federal de São João Del Rei, Divinópolis, MG.

**Address to:** Dr. Luis Marcelo Aranha Camargo. Rua Carlos Prestes 1234, 76888-000 Monte Negro, RO, Brasil.

Phone: + 55 69 9219-8714.

e-mail: spider@icbusp.org

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Labrea and Humaita (LMA Camargo: personal information), which are endemic areas for mansonelliasis<sup>8,17</sup>. Thus, further studies to find possible foci of mansonelliasis in the region are necessary.

## METHODS

This work included a parasite microscopic survey in the State of Rondônia Brazil, more specifically in the urban areas and alongside the rivers of the City: Guajara-Mirim (Mamore River, neighborhood City of Triangulo sectors at 10° 47' 35.01" S and 62° 20' 03.90" W), and the urban area City of Porto Velho (Madeira River, neighborhoods of Cai N'agua, Uniao, and City of Candelaria sectors at 08° 45' 42.57" S and 63° 54' 07.06" W), in the inhabitants living alongside the middle Madeira River, in the communities of São Carlos (08° 26' 16.90" S and 63° 29' 52.33" W), Nazare (08° 09' 51.88" S and 62° 19' 13.29" W), Calama (8° 02' 53.59" S and 62° 51' 46.40" W), Rio Preto (8° 26' 16.90" S and 62° 55' 03.10" W), middle Machado River (Tabajara 8° 10' 14.37" S and 62° 46' 47.53" W, *Dois de Novembro* 8° 55' 29.68" S and 62° 04' 48.15" W), and Guapore River, in the municipality of São Francisco do Guapore (12° 31' 43.53" S and 63° 32' 10.89" W) (Figure 1).

The sampling of the population was non-random, and individuals older than five years of age (population at risk) who are exposed daily to simuliids stings were invited to voluntarily participate in the study. These patients were not only informed orally but also informed in written form of the low risks and procedures of the study. The hematologic survey was conducted using the thick blood smear method. Two to three blood drops (60µL) were obtained by

finger prick and were then placed on a slide for microscopy, dried at room temperature, and washed with distilled water (12h after being prepared). The slides were then stained with Giemsa and examined with an optical microscope (10x and 40x magnification) in a blind, controlled trial performed by two microscopists.

In addition to the hematologic study, adult and immature (larvae and pupas) simuliids were collected along the Machado River in the communities of Tabajara on the middle of Machado River (8° 55' 29,68" S and 62° 04' 48,15" W) and *Dois de Novembro* (8° 54' 54.90" S and 62° 07' 21.11" W) and Guaporé River at São Francisco do Guaporé (12° 31' 43.53" S and 63° 32' 10.89" W). The adult simuliids were captured by suction traps upon landing in the morning (8 to 10 am), and in the afternoon (2 to 4 pm). The immature simuliids (larvae and pupas) were captured on the leaves and branches that had fallen into the rivers and water channels of the region using tweezers and scissors. All the collected simuliids (2,112 specimens) were soaked in 70% alcohol and taken to the laboratory of the National Institute of Research of Amazonia in the City of Manaus (State of Amazonas) for identification, according to Coscarón & Coscarón-Arias<sup>18</sup>. Larvae and pupas were identified for species, while 90% (ninety percent) of the adult simuliids were dissected into three parts (head, thorax and abdomen) using a stylet under a light microscope to determine infection with *M. ozzardi* microfilariae and/or larval stages (L<sub>1</sub>, L<sub>2</sub> and/or L<sub>3</sub>).

### Ethical considerations

The project was submitted to the Ethical Research Committee of the São Lucas College in Porto Velho (State of Rondônia), approved and recorded under the registry number 344/09.

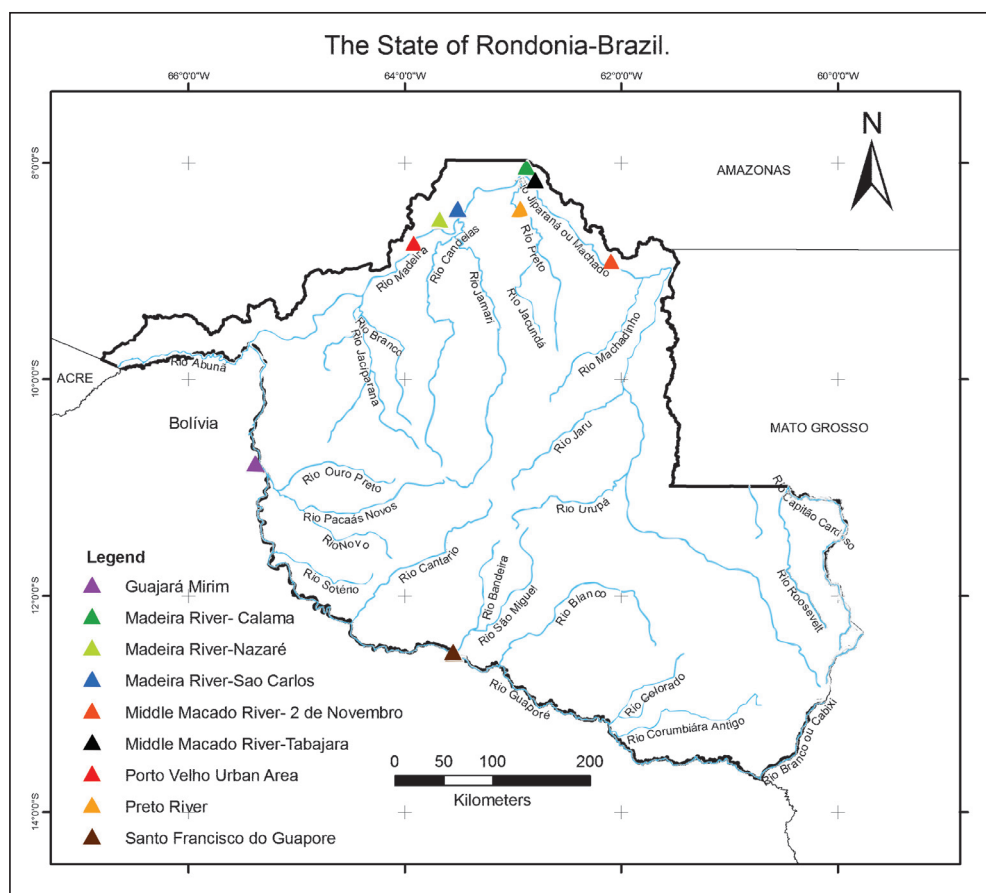


FIGURE 1 - Collection area of *Simuliidae* and blood samples.

## RESULTS

Altogether 4,452 individuals were examined alongside the major rivers of Rondônia, and all of these individuals were found to be negative for *M. ozzardi* microfilariae by hematologic examination (Table 1).

Only two simuliid species were found to have hematophagic activity, and *Cerqueirellum pydanielli* was more abundant in comparison to *Chirostilbia pertinax* (Table 2).

Ninety percent of the adult specimens were dissected, and none of them was found to be positive for *M. ozzardi*. The following species of larvae and pupas were collected: *Kempfsimulium simplicicolor*, *Inequalium subnigrum*, *Ectmnaspis rorotaense*, *E. perflava*, *Coscaroniellum cauchense*, *C. quadrifidum*, *C. ulyssesi*, *C. goeldii*, *Psaroniocompsa minuscula*, *Cerqueirellum argentiscutum*, *C. amazonicum*, *C. pydanielli*, *Chirostilbia pertinax*, *Notolepria exigua*, and *Shelleyllum siolli*.

TABLE 1 - Hematologic survey employing the thick blood smear method assessing *Mansonella ozzardi* microfilariae in individuals older than 5 years of age, State of Rondônia, 2008 to 2010.

Place	River	People examined*	Total of the population older than 5 years of age who were submitted to the survey in relation to the local population (%)
Guajara Mirim	Mamore River	441	56.0
Porto Velho Urbano	Madeira River	2,352	76.0
Porto Velho	Middle Madeira River	1,052	52.0
Tabajara e Dois de Novembro	Middle Machado River	358	88.0
Rio Preto	Preto River	226	92.0
Sao Francisco do Guapore	Guapore River	23	72.0
<b>Total</b>		<b>4,452</b>	<b>67.6</b>

\*All negative for *Mansonella ozzardi*.

TABLE 2 - Species of Simuliidae with hematophagic activity that were collected in the communities of Tabajara and Dois de Novembro, middle Machado River, State of Rondônia, 2008 to 2010.

Local	Collecting period	Number of insects collected					
		<i>Chirostilbia pertinax</i>		<i>Cerqueirellum pydanielli</i>		Total	
		n	%	n	%	n	%
Tabajara	morning	56	56.6	741	36.8	797	37.7
	afternoon	30	30.3	598	29.7	628	29.7
Dois de Novembro	morning	13	13.1	339	16.8	352	16.7
	afternoon	0	-	135	6.7	135	6.4
Santo Antonio do Guapore	morning	-	-	200	9.9	200	9.5
	afternoon	-	-	-	-	-	-
<b>Total</b>		<b>99</b>	<b>5.2</b>	<b>2,013</b>	<b>94.8</b>	<b>2,112</b>	<b>100.0</b>

## DISCUSSION

The literature indicates that the Amazonian region is the only region in which *M. ozzardi* has been found in Brazil, and this region includes the States of Amazonas, Roraima, and Mato Grosso<sup>10,11</sup>. Rondônia is in the Amazonian region and was the subject of a study on the occurrence of mansonelliasis in the 1950s; however, there was no evidence of this parasitic disease<sup>3,4</sup>. In the present study, a large number of individuals were examined in several areas in the State of Rondônia and all of them tested negative for *M. ozzardi* microfilariae, confirming the results found in the previous decades. There is a question of whether the diagnosis method used in this study was not sensitive enough to detect low rates of microfilaremia. However, many studies carried out in the Amazon use the same method and show prevalence of up to 60% of microfilaremia<sup>7-9</sup>. Another hypothesis may be that there are not enough individuals infected with a high enough parasite load to disseminate this endemic disease in areas not affected

by the infection. People with microfilaremia are a source of infection for insects that are vectors for the parasite and are responsible for perpetuating this endemic disease. Studies have shown that, in places with favorable conditions for other filarial diseases caused by *W. bancrofti*, the migration of individuals suffering from microfilaremia may be responsible for establishing new parasitic vector foci. In Sri Lanka, infected migrants introduced lymphatic filariasis into areas in which there were no previous parasitic diseases<sup>19</sup>. A similar situation was noted in areas previously free of filariasis such as the metropolitan area of the City of Recife (Brazil) that has since had cases of autochthonous lymphatic filariasis<sup>20</sup>.

In this work, no focus of mansonelliasis was found despite the presence of potential parasite vectors and environmental conditions similar to those existing in mansonelliasis endemic areas in the Amazon region that borders Rondônia to the north. Among the factors that influence the capability of the vector to be infected and transmit the infection is the level of microfilaremia of the vertebrate host. The infection rate of mosquitoes is proportional to the density

of the microfilariae in the peripheral blood of the patients<sup>21</sup>. With regard to lymphatic filariasis caused by *W. bancrofti*, areas where the microfilariae density is kept under 5 microfilariae/60µl of blood are considered free of transmission risk<sup>22</sup>. Studies have shown that an individual with *W. bancrofti* microfilariaemia who has migrated from an endemic area and lived for over 10 years in a non-endemic area was not able to induce the formation of a new focus of bancroftosis although the areas have similar environmental conditions<sup>23</sup>. One assumption was that the low level of the microfilariaemia of the infected individual (4 microfilariae/mL of blood) was not sufficient to introduce parasitosis in a new area<sup>23</sup>. In the 1950s, individuals with *W. bancrofti* microfilariaemia found in the Cities of Porto Velho and Guajara Mirim were allochthonous cases and had very mild microfilariaemia (out of the seven individuals infected by parasites, five had less than 5 microfilariae per slide)<sup>4,16</sup>. It is probable that the small number of infected individuals and the mild microfilariaemia was not enough to infect and sustain the transmission of the lymphatic filariasis caused by *W. bancrofti* in parasite-free areas. A similar phenomenon may have occurred with regard to current *M. ozzardi* infection in terms of the lack of occurrence of mansonelliasis foci in this region. The existence of competent vectors and the proximity of a hyper-endemic area with regard to the occurrence of *M. ozzardi* (Lábrea, AM)<sup>8</sup> as well as the absence of this parasitic disease in Rondônia has been noted.

The 2,112 simuliid samples captured in the area of Rondônia represented a diversified fauna that has not been extensively studied. This study reports new species occurring in Rondônia: *C. pydanielli*, *E. rorotaense*, *C. cauchense*, *C. ulyssesi*, and *N. exigua* and also fauna with potential for *M. ozzardi* transmission (*C. pydanielli*, *C. argentiscutum*, *C. amazonicum*)<sup>14,15</sup>. Among these species, *C. pydanielli* was frequently captured and found to be exerting hematophagous activity.

We conclude that human infections caused by *M. ozzardi* are not been found in the evaluated areas. This finding could be explained by the fact that there are individuals with low-level microfilariaemia not detected by the diagnostic method used in the study or there is insufficient migration of people infected with *M. ozzardi* from endemic areas, making it impossible to introduce the disease into Rondônia. Further studies using more sensitive diagnostic techniques may address this question.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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