



**Research in Progress**

## Mansonellosis at Medium Purus River (Brazilian Amazon)

DOI: 10.3395/reciis.v2i1.113en



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### Abstract

*Mansonella ozzardi* is a filarial autochthonous parasite of the American continent that is frequently found infecting human beings in the Brazilian Amazon. The human infection by *M. ozzardi* is still considered as non pathogenic despite the variety of symptoms related to it such as fever, articular pain, headache, lymphadenopathy, eosinophily, and pruritic skins eruptions. During an expedition in the state of Acre, we were able to visit the Kamikuã, an Indian village located along the Purus river, close to the city of Boca do Acre (Amazonas). The inhabitants had been diagnosed as harboring dog's worms (*Dirofilaria immitis*) but our blood samples collections from some individuals evidenced *M. ozzardi* infections. In fact, human populations from endemic areas are always complaining about symptoms of *M. ozzardi* infection and remain living together with high parasitic loads in the expectation of correct diagnostic and treatment. Studies on mansonellosis chemotherapy and control should be carried out in order to add knowledge about the infection and to provide hope and answers for those who live in endemic areas.

### Keywords

Mansonellosis, infection, treatment, Ivermectin, Mansonella

*Mansonella ozzardi*, one of the eight filarial parasites that commonly infect humans, is endemic in many regions of tropical Central and South America.

In Brazil, however, only *Onchocerca volvulus* and *Wuchereria bancrofti* are seen as agents of filariasis and public health problems by health organizations.

*M. ozzardi* is native to the American Continent, frequently found among indigenous populations and was first described in Brazil by Dr M. Deane in Manaus, State of Amazonas, in 1949.

In Brazil its presence seems to be confined to some geographic areas as Alto Amazonas (Solimões river) and along the Purus and Negro rivers (MORAES et al., 1985). In these regions, two potential vectors which share the same distribution - *Simulium amazonicum* and *S. argenticutum* - are apparently responsible for the transmission of the parasite (SHELLEY et al., 1980; MEDEIROS et al., 2004).

In the Caribbean Islands and also in Argentina, differently from Brazil, diptera from the *Ceratopogonidae*

family, *Culicoides* genus are incriminated in the transmission (SHELLEY et al., 2001).

Besides Amazonas, this filarial parasite can be found - even in limited extension - in the states of Acre, Mato Grosso (north region), and Roraima (DEANE et al., 1953; OLIVEIRA, 1963; MORAES et al., 1985).

Most of the studies on *M. ozzardi* infections were carried out long ago and their goal was to show the epidemiological situation about human infection by filarial parasites in Brazil. Indeed, most of them did not contribute with much data about symptomatology, and thus knowledge related to this issue is still poor. Perhaps this may be explained because human infection by *M. ozzardi* is still considered as non pathogenic despite the variety of symptoms related to it such as fever, articular pain, headache, lymphadenopathy, eosinophily, and pruritic skins eruptions.

In this work, we describe an expedition to an Indian Village, whose inhabitants showed both - high prevalence and parasitic load - of *M. ozzardi* infections and the difficult questions related to the treatment our group faced in the area.

In July 2006, a preliminary study about filariasis infection among riparian populations of the Acre and Purus rivers was organized by our laboratory with logistical support by Funtac (Fundação de Tecnologia do Acre) and the Health Institution from the State of Acre (Sesacre). The team consisted of multidisciplinary professionals such as a social assistant, one anthropologist, one physician, three biologists, and a chemist.

During this expedition, we visited the Kamikuã Indian Village located along the Purus river close to the city of Boca do Acre (S 08°45'07'' O 67°23'52'') in the state of Amazonas in the boundary of the State of Acre (Figure 1). The inhabitants had been diagnosed by local physicians as harboring dog's worms (*D. immitis*) in their blood and most of them presented articular pain, undefined skin disease, and general uneasiness. Indeed, previous data obtained through ELISA essays carried out with Ov10, Ov11, and Ov16 recombinant proteins from *O. volvulus* in our laboratory showed that some of them were reactive to the parasite antigens (personal communication).



**Figure 1 - Geographical localization of Boca do Acre, State of Amazonas, Brazil. Source: adapted from Google Maps.**

Our group achieved an authorization with the local government responsible for Indian's Health (DESEI/Boca do Acre) whose responsible was Mrs Maria de Nazareth Pinheiro and the Kamikuã's main leader. Thus, there was an explanation about our parasitological study and the approaches involved to the inhabitants and those who agreed to be volunteers signed a Consent Form previously approved by an Ethical Comitee from Fiocruz (Parecer 281/05). The informed consent was prepared to record personal and clinical data such as presence/absence of any kind of skin disease, pruritus, headache, coldness

in the legs, lymphadenopathy, and articular pain, which may be present in mansonellosis patients.

Some of the Kamikuã inhabitants were very receptive but some were suspicious and refused to be volunteers; only seven individuals agreed to be examined.

We performed blood collection as described below for further analysis in our laboratory, as conditions in the field were very precarious.

Venous blood samples collections were performed in 10 ml sterile and descartable syringe and thick blood

films were prepared at the moment of collection followed by dehemoglobinization, fixation in metanol and staining with Giemsa. In order to minimize false negative results, 1 ml of venous blood was deposited in a polystyrene tube with 10 ml of a 2% formaline solution for Knott's Method and after a period of 12 hours, thin films were prepared with the deposited sediment, fixed with metanol and stained with Giemsa.

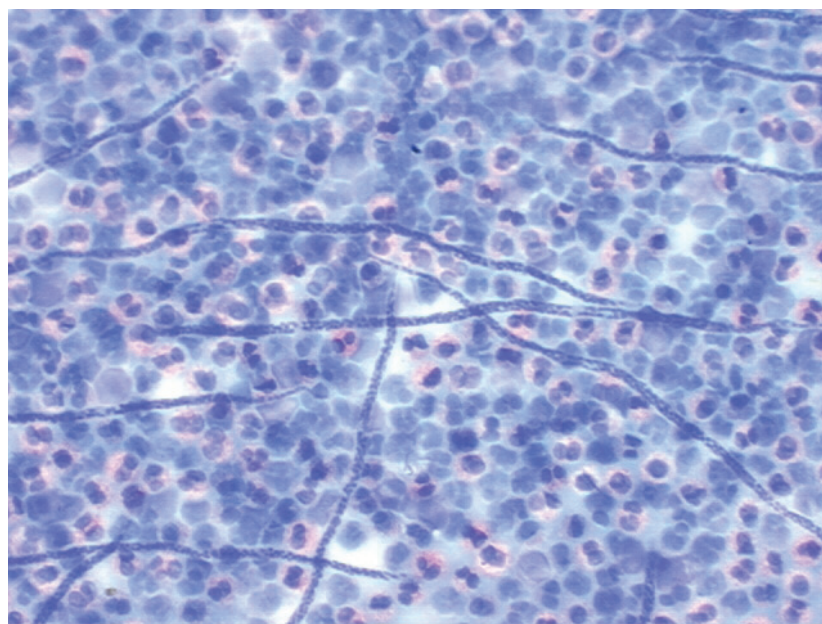
"Fischer's Exact Test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software San Diego California USA, www.graphpad.com."

Blood specimen examinations obtained through thick films and Knott's Method showed that 6 out of 7 volunteers were infected by *M. ozzardi* (Table). Additionally, in 4 infected volunteers (66.7%) it was possible to note high parasitic loads (Figure 2), some with more than 100 mf/20µl of blood and most of them complained of general uneasiness, articular pain, and headache.

Although Knott's Method had been capable of detecting one thick film false negative infection, there was no statistical significance when comparison of the results of the two methods was performed ( $p > 0.05$ ).

**Table - *Mansonella ozzardi* infections at Kamikuã Village detected through two sampling methods**

Method	Infected (total)	Uninfected (total)	Infection rate (%)
Thick Film	5 (7)	2 (7)	71.4
Knott's Test	6 (7)	1 (7)	85.7



**Figure 2 - Giemsa-stained microfilarias of *Mansonella ozzardi* isolated from a Kamikuã volunteer. 100X. Source: photomicrography of the author.**

Our preliminary results at Kamikuã Village showed that 85.7% of volunteers were positive for *M. ozzardi* infections as detected through Knott's Test and that this might be a subestimate of the local village because of our small sample. Indeed, we found that the Kamikuã's were very concerned about etiology of their infection and questions related to treatment.

These results highlighted a critical ethical situation: how to explain to the village people that there is no established official treatment policy to mansonellosis?

Ivermectin is the drug of choice in programs of Onchocerciasis treatment and control (by its known microfilaricida activity) and has also been demonstrated to be useful in the blood clearance of microfilarias from *W. bancrofti*.

In this last case, some microfilaremic patients show slight systemic reactions such as fever, headache, and muscular pain.

Those systemic reactions are proportional to microfilaremia, being triggered by higher parasitic loads, but

showed an autolimited spectrum and disappeared after 48 hours of treatment (DREYER et al., 1997).

In the same way as with *W. bancrofti*, Ivermectin has activity against *M. ozzardi* microfilarias and induced clearance of these blood forms has already been attained (NUTMAN et al., 1987).

In areas under onchocerciasis control programs, the drug is employed in mass treatment and inhabitants receive – without any cost – the drug twice a year. In these regions, detected cases of *M. ozzardi* infection are common and technicians from the Brazilian Ministry of Health have delivered the medication to some infected individuals which in its turn presented systemic reactions (personal communication) as described above for bancroftosis, which may be due to the fast destruction of these parasite blood forms in the blood vessels. As these results are non official, since then, the recommendation is caution with relation to mansonellosis patients.

Recently, new perspectives are represented by the intracellular symbiotic bacteria from the genus *Wolbachia*. They are found in filarial nematodes of medical importance like *O. volvulus* and *W. bancrofti*, and its presence in *M. ozzardi* microfilarias has already been demonstrated (CASIRAGHI, 2001) supporting additional evidence that the bacteria could be a target to chemotherapy. In fact, these findings could mean an argument for the use of antibacterial drugs such as tetracycline and doxycycline to cure filarial disease (FENN et al., 2004).

Our epidemiological researches on *M. ozzardi* prevalence into the Kamikuã village are subject of another expedition to Boca do Acre, but we conclude that studies concerning mansonellosis epidemiology in the Brazilian Amazon should be carried out more seriously in order to provide supporting evidence for its pathology and the basis for chemotherapeutic studies and safe drug administration schemes.

## Acknowledgement

To the Departamento de Ações Básicas de Saúde/Secretaria Estadual de Saúde do Acre, and Fundação de Tecnologia do Acre for logistical support. To Dr Arlindo Serpa Filho for suggesting topics of the manuscript. To Dr Sixto Coscarón and Dr Guilherme Herzog for critical review of the article, and Ana Carolina dos Santos Valente for technical assistance. This work was supported by Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), Faperj (Fundação de Amparo à Pesquisa do Rio de Janeiro), and Coordenação de Pós Graduação em Biologia Parasitária do Instituto Oswaldo Cruz (PGBP/IOC/Fiocruz/RJ).

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