

## MANSONELLA OZZARDI INFECTION IN BOLIVIA: PREVALENCE AND CLINICAL ASSOCIATIONS IN THE CHACO REGION

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**Abstract.** A cross-sectional survey carried out in the Chaco region of Bolivia showed that 26% (77 of 296) and 0.7% (2 of 298) of the rural population of the Camiri and Villa Montes areas, respectively, harbored *Mansonella ozzardi* microfilariae (mf). No significant differences were observed between sexes. The lowest prevalence (9%) was in the 0–14-year-old age group, with no children <11 months of age infected. The prevalence increased sharply in the 25–34-year-old age group (32%), and continued increasing in the older age classes. Microfilaremia, ranging from 1 to 305 mf/20  $\mu$ l of blood, was lowest in 0–14-year-old children (geometric mean concentration = 1.1 mf/20  $\mu$ l), and increased with age (>100 mf/20  $\mu$ l in people >44 years old). An expected increasing sensitivity with the blood volume examined was observed. No significant association between clinical symptoms (fever, skin rash, pruritus, headache, lymphedema, elephantiasis, and articular pain) and microfilaremia was observed.

*Mansonella ozzardi* is a filarial parasite whose distribution is limited to some Caribbean islands and tropical areas of Central and South America. Although experimental infection of animals has been possible, humans are considered the only significant reservoir of the nematode.<sup>1</sup> Biting midges of the genus *Culicoides* are believed to be the vector in the Caribbean islands, whereas *Simulium* blackflies have been incriminated in the Amazon basin. The parasite can also develop in the Liverpool strain of *Aedes aegypti*, but there is no evidence to suggest that this mosquito or any other mosquito species can act as vectors of *M. ozzardi* in nature.<sup>2,3</sup>

The pathogenicity of this filaria is not well defined. Most people infected are, in fact, symptomless, but a variety of clinical manifestations such as articular pain, pruritus, skin eruption, headache, and lymphadenitis have been associated with *M. ozzardi* infection in some reports.<sup>1,4–7</sup> Tissue invasion by *M. ozzardi* microfilariae (mf) has recently been observed in a patient with adenocarcinoma, and it has been suggested as a possible opportunistic infection in the immunocompromized host.<sup>8</sup>

The report in Bolivia of a symptomatic case of *M. ozzardi* from Santa Cruz city, and the occasional detection of the parasite both in thick blood smears collected during a routine malaria survey (Vallejos Y, Lagrava M, unpublished data) and from patients admitted to the Camiri District Hospital prompted us to study the prevalence of this filarial worm in the Chaco region of Bolivia.<sup>9</sup> A cross-sectional survey was therefore carried out in November 1997 with the agreement of the Bolivian National Department of Epidemiology of the Ministry of Social Welfare and Public Health and with the support of the Guaraní political organization (Asamblea del Pueblo Guaraní, APG).

### MATERIALS AND METHODS

The Chaco region lies in southeastern Bolivia between longitudes 64°30' and 58°50'E and latitudes 17°58' and 22°20'S, and includes 5 provinces: Cordillera, Luis Calvo, Hernando Siles, Gran Chaco, and O'Connor (Figure 1). The areas selected were located in the Cordillera and Gran Chaco provinces. The first province, with a population of 88,628

inhabitants, occupies the broad northern area (86,245 km<sup>2</sup>), whereas Gran Chaco is in the southern area (17,428 km<sup>2</sup>) and has a population of 17,612 inhabitants.<sup>10</sup>

The survey was carried out in 8 rural communities, 4 of them near Camiri (Cordillera province) and 4 near Villa Montes (Gran Chaco province). The communities near Camiri are distributed 5–10 km from the town on the Parapetí and Yuti Rivers at altitudes of approximately 800 m above sea level. The average yearly rainfall in the Camiri area fluctuates between 600 and 850 mm. The temperature generally ranges from 17.7°C to 26.3°C, but may decrease to 5–10°C from May to September due to the surazo winds from the Antarctic. The climate is described as sub-humid-dry. The rural communities near Villa Montes are located about 6 km from the town at an altitude of approximately 380 m. The Pilcomayo River is about 2 km from this area and the climate is defined as semidry.<sup>11</sup> The average yearly rainfall in this areas ranges from 400 to 600 mm, and the temperature is generally higher than that of the Camiri area even though recorded temperatures for this area are not available.

The study populations consisted of Guaraní Indians and mestizos. The majority of the population speak both Spanish and Guaraní, while a minority speak only Guaraní. They live in poor dwellings with walls of sticks, straw, and clay, and thatched roofs. The local economy is based on agriculture (mainly maize) and animal breeding (cattle, pigs, goats, chickens).

The number of inhabitants in the rural areas near Camiri and Villa Montes is about 3,300 each. The sample size was determined assuming an expected prevalence of microfilaremia of 20% with a worst acceptable error of 5% and a confidence interval of 95%.<sup>12</sup> A random cluster survey method was used, with each community constituting 1 cluster, and 4 communities in each area were selected at random. The study population of the rural area near Camiri included 301 individuals (165 females and 136 males). Their ages ranged from 4 months to 81 years (mean = 23.8 years, median = 14.0 years). The population studied in the rural area near Villa Montes consisted of 301 individuals (149 females and 152 males) with an age range of 3 months to 85 years



FIGURE 1. Administrative boundaries of the Bolivian Departments and locations of the studied areas (●).

(mean = 23.5 years, median = 14.0 years). The survey included all available inhabitants of the selected communities.

The field staff was composed of a physician, the health representative of the APG, and 8 paramedical workers who had received prior training.<sup>3</sup> A meeting was organized with the inhabitants of the communities to explain the purpose of the study, and its procedures. Informed consent was obtained from all adult participants and from parents or legal guardians of minors. The study design, including its ethical aspects, was reviewed and approved by the Bolivian Ministry of Social Welfare and Public Health and the local health authorities. Specially prepared forms were used to record personal and some clinical data, including body temperature, measured by axillary thermometer, and the presence of skin rash, pruritus, headache, lymphedema, elephantiasis, and articular pain.

Three 20 µl-thick blood films were obtained from each individual during the daytime (7:00 AM to 5:00 PM) using the fingerprick method. The blood sample was collected using a micropipette (Elkay XLDG 2-100 µl; Elkay Laboratory Products, Basingstoke, United Kingdom). The thick blood films were stained with a 5% Giemsa solution and examined with a 10× objective to screen for mf and a 100× objective under oil-immersion for their specific identification.

Analysis of prevalence was carried out using the C sample facility in Epi-Info version 6.04<sup>12</sup> to allow for both cluster and household aggregation. An association between reported symptoms and microfilaremia was assessed by logistic regression with adjustment for age (in strata) and sex, comparing microfilaremic and non-microfilaremic subjects from the Camiri area.

RESULTS

Of 602 subjects enrolled in the study (301 in the rural area of Camiri and 301 in that of Villa Montes), 8 (1.3%) were excluded from the analysis: 4 subjects (3 in the Camiri area and 1 in the Villa Montes area) refused the fingerprick, and 4 (2 in the Camiri area and 2 in the Villa Montes area) whose age was not recorded.

Examination of 1,782 thick films prepared from the 594 eligible subjects showed that all filarial infections were due to *M. ozzardi*. Of the 296 subjects screened in the rural area near Camiri, 77 (26%) harbored mf, while only 2 (0.7%) of the 298 examined in the rural area near Villa Montes were infected. These 2 individuals lived in the same household and had very low microfilaremias (3 and 32 mf/60 µl, respectively). Due to the low number of positive subjects found in the Villa Montes area, the subsequent analysis was limited to the Camiri rural population.

The distribution of microfilaremic people by age and sex is shown in Table 1. No significant differences were observed between sexes. The lowest prevalence of infection was in the 0-14-year-old age group, in which 9% of the subjects were positive. No children <11 months old were infected. Positivity increased sharply in the 25-34-year-old age group, in which 32% of the subjects examined had circulating mf. The prevalence continued increasing in the older age classes, except for some decrease (47%) in adults 65-74 years of age.

TABLE 1  
Microfilaria prevalence rates of *Mansonella ozzardi* by age and sex, in the human population of the rural area of Camiri, Bolivia

Age group (years)	Males		Females		Total	
	No. positive/No. examined	Prevalence % infected (95% CI)*	No. positive/No. examined	Prevalence % infected (95% CI)*	No. positive/No. examined	Prevalence % infected (95% CI)*
0-14	8/80	10 (3.5-16.4)	5/68	7 (0-15.1)	13/148	9 (3.7-13.9)
15-24	1/16	6 (0-16.7)	3/21	14 (0-28.2)	4/37	11 (2.0-19.6)
25-34	2/4	50 (0-100)	5/18	28 (5.9-49.7)	7/22	32 (12.4-51.2)
35-44	3/10	30 (2.5-57.5)	7/13	54 (26.5-81.2)	10/23	43 (25.8-61.2)
45-54	8/12	67 (38.1-95.2)	10/15	67 (42.8-90.6)	18/27	67 (46.6-86.8)
55-64	3/3	100 (0-100)	7/10	70 (41.1-99.0)	10/13	77 (53.2-100)
65-74	3/6	50 (1.9-98.0)	5/11	45 (18.0-72.9)	8/17	47 (18.9-75.2)
≥75	2/3	67 (12.0-100)	5/6	83 (53.5-100)	7/9	78 (50.0-100)
Total	30/134	22.4 (13.3-31.5)	47/162	29.0 (21.1-36.9)	77/296	26 (19.7-32.3)

\* Adjusted for clustering by household and community. CI = confidence interval.

TABLE 2

Parasite concentrations by age, in *Mansonella ozzardi*-infected people of the rural area of Camiri, Bolivia

Age group (years)	No. of microfilariae/20 µl of blood					GMC* 20 µl of blood	No. of infected individuals
	1-9	10-19	20-49	50-99	≥100		
0-14	12	1	0	0	0	1.1	13
15-24	1	3	0	0	0	10	4
25-34	3	1	2	1	0	6.1	7
35-44	7	2	0	1	0	2.5	10
45-54	9	4	3	1	1	5.5	18
55-64	4	0	2	3	1	18.8	10
65-74	4	1	1	0	2	13.1	8
≥75	3	0	2	1	1	17.6	7
Total	43	12	10	7	5	5.6	77

\* GMC = geometric mean concentration.

Microfilariaemia ranged from 1 to 305 mf/20 µl of blood, with mean and median values of 20.8 and 5.5 mf/20 µl, respectively. As shown in Table 2, microfilaria counts were lowest in 0-14-year-old children (range = 1-19 mf/20 µl; geometric mean concentration: 1.1 mf/20 µl), and increased with age (>100 mf/20 µl were observed in people >44 years old).

In detecting *M. ozzardi* infection, we observed an expected increasing sensitivity with the blood volume examined. The examination of three 20-µl blood samples detected 77 infected subjects, but only 73 and 67, respectively, if the procedure was limited to 40 µl and 20 µl.

With regard to the association between symptoms and presence of mf, a simple tabulation of data showed a strong positive relationship between microfilariaemia and articular pains, headache, or pruritus. These findings resulted from a very strong confounding effect of age, which was related to both increasing prevalence and increasing symptomatology. The results adjusted for age and sex are shown in Table 3. None of the symptoms was statistically significantly associated with infection. However, foot edema did show a strong positive relationship but with a wide confidence interval (odds ratio = 7.5, 95% confidence interval = 0.7-75.2).

#### DISCUSSION

Infection with *M. ozzardi* is known to be endemic in many regions of tropical Central and South America; however, to our knowledge, no data are available from Bolivia. The occasional reports of the parasite in people from the Chaco region (southeastern Bolivia) prompted us to systematically investigate *M. ozzardi* infection in the rural population of this area. Our findings confirmed the presence of *M. ozzardi* in the region, but a large difference in prevalence was observed between the 2 provinces surveyed. The communities in the rural area near Villa Montes showed a low prevalence (0.7%) compared with rural areas near Camiri (26%). The explanation for this may be related to differing microclimates, spatial and temporal distribution of the vector larval breeding sites, and/or to the feeding habits of the vectors. However, the local vector is not yet known.

The infection rate found in the Camiri area is similar to that detected in the subtropical northwestern region of Argentina (20.7%) near the border with Bolivia, and in the

TABLE 3

Association between symptoms and microfilariaemia adjusted for age and sex

Reported symptom	Overall % prevalence	Adjusted odds ratio of association with positive microfilariaemia (95% CI)*
Headache	45.6	1.4 (0.7-2.6)
Pruritus	15.2	1.3 (0.6-3.1)
Rash	10.2	1.9 (0.7-5.2)
Foot edema	3.4	7.5 (0.7-75.2)
Leg edema	2.7	0.8 (0.2-3.8)
Arm edema	0.7	-
Face edema	0.7	-
Articular pain	22.0	0.7 (0.3-1.5)

\* CI = confidence interval. - = odds ratios were not determined due to a low number of cases (n = 2).

Amazon Federal Territory of Venezuela (30%).<sup>13,14</sup> It is higher than those reported in the Federal Territory of Roraima, Brazil (3%), Trinidad (16.6%), and Haiti (16%).<sup>15-17</sup> In contrast, the prevalence found in Bolivia is lower than reported in the state of Bolivar, Venezuela (36.4%) (where in 1 area positivity increased to 94.4%), and in the Comisaría del Vaupes of Colombia (47%), where the adult Indians surveyed showed the highest infection rate (96%) recorded for *M. ozzardi* in the world.<sup>1,18</sup>

Our results confirm the observations of other investigators that the prevalence rate increases with age.<sup>1,17</sup> In contrast to Haiti and Trinidad, where less than 2% and 3%, respectively, of the subjects less than 20 years old were positive, our study found 9% of the children 0-14 years of age infected and the youngest positive individual was an 11-month-old child.<sup>16,17</sup> Since some cases may have gone undetected due to the low number of circulating mf characteristic of the youngest ages, it is likely that the people of the surveyed area are highly exposed even at a young age. The increasing prevalence with age is likely to be due to an increasing probability of exposure to an infectious bite.

The increase in microfilarial density with age is likely, based on animal data, to be due to increased frequency of exposure. Thus, the course of patency monitored in experimentally infected patas monkeys showed that after a mean prepatent period of 163 days, microfilariaemia increased steadily, peaking at about 20 weeks, and then decreased, stabilizing at low levels for up to 48 weeks.<sup>19</sup> Thereafter, mf disappeared from the peripheral blood and occasionally reappeared in scanty numbers. These observations suggest that the high microfilariaemia levels found in adults are probably due to a continuous succession of overlapping infections, although decreasing general immunity with age may play a role.

Even though the membrane filtration and Knott method are considered the most sensitive procedures in detecting *M. ozzardi* infections, they have several drawbacks that make their use less suitable in a large-scale survey. The venipuncture that is required to obtain more than 1 ml of blood is not well tolerated, especially among children. They are time-consuming and more expensive than the traditional methods of examining thick films of capillary blood. The fingerprick sampling method we used is considered to provide a sufficiently accurate estimation of the prevalence of *M. ozzardi* infection, and the combination of 3 fingerprick samples (60

$\mu\text{l}$  of blood) detected 95% of Knott-positive infections.<sup>20</sup> Examination of only the first 20- $\mu\text{l}$  blood sample would have missed 13% of the infected subjects; examining only 40  $\mu\text{l}$  would have missed 5%. On the basis of these results, we do not recommend the examination of only one 20  $\mu\text{l}$ -thick film for *M. ozzardi*.

Although the parasite is common in tropical America, little is known of its development and biology. It is generally regarded as a relatively innocuous parasite, yet several clinical symptoms including articular pain, headache, fever, pruritus, skin rash, and lymphadenopathies have been ascribed to *M. ozzardi* on the basis of studies performed in Brazil and Colombia.<sup>1,4-6</sup> In our survey, despite parasite densities of up to 15,250 mf/ml, we did not find any association between the presence of any of these symptoms and *M. ozzardi*. This is in accordance with another study conducted in Haiti.<sup>21</sup> However, this has to be viewed with some caution since for less prevalent symptoms the study had relatively little power. Nevertheless, studies in monkeys support these findings. In monkeys, the filaria prefers the subcutaneous tissues as its habitat rather than the body cavity, as suggested by earlier studies.<sup>22</sup> This location may account for the innocuous behavior of the parasite. Further work is planned to examine the relationship between age, immunity, and microfilaremia and to determine the predominant local vector.

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