Am. J. Trop. Med. Hyg., 90(6), 2014, pp. 1170–1175
doi:10.4269/ajtmh.13-0410

Sustained Clearance of *Mansonella ozzardi* Infection after Treatment with Ivermectin in the Brazilian Amazon

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Abstract. Therapy for mansonelliasis is challenging because there is no standard drug recommended for its treatment. This non-randomized study was conducted to evaluate the effectiveness of a single dose of 0.15 mg/kg of ivermectin to reduce *Mansonella ozzardi* microfilaraemia in infected persons. A total of 74 patients were studied within the municipality of Lábrea, which is located in Amazonas State, Brazil. The patients were treated with ivermectin after detection of the parasite by blood examination. Significant microfilaraemia reduction was observed and its residual effect was maintained for at least 12 months. There was no significant change in the laboratory blood count, hepatic metabolites, and nitrogen-bounding compound excreta dosage values that could compromise the use of this drug, demonstrating that ivermectin has a low toxicity level.

INTRODUCTION

The filarial worm *Mansonella ozzardi* is the etiologic agent of mansonelliasis. The geographic distribution of this helminth is limited to Central and South America and ranges from Mexico to Argentina, excluding Chile, Uruguay, and Paraguay.[1] This parasite is transmitted by two Diptera families (i.e., Ceratopogonidae and Simuliidae[2,3]) and was first described by Deane[4] in 1949 in the municipality of Manaus in the state of Amazonas, Brazil. Subsequently, Lacerda and Rachou[5] detected persons infected with *M. ozzardi* along the Solimões, Purus, and Negro Rivers in the Brazilian Amazon. These findings were confirmed by Moraes,[6,7] who reported that *M. ozzardi* was common in the Amazon region. Within the study area, there were no cases of onchocerciasis or bancroftiasis or any other *Mansonella* species.[8–11]

The symptoms of mansonelliasis in humans have been extensively studied and persons with mansonelliasis have moderate fever, cold legs, arthralgia, and adenitis with dizziness and headache.[8,12,13] Currently, researchers are trying to correlate the occurrence of ocular lesions on the cornea with mansonelliasis.[14–16] However, Bartoloni and others[17] described infections as asymptomatic.

There have been are few studies that focused on the treatment of *M. ozzardi* infection. Tavares and Fraiha Neto[1] treated the patients using a single dose of 0.2 mg/kg of ivermectin to eliminate the microfilariae from the peripheral blood within 24 hours, and persons remained parasite negative for a month. No adverse reaction was reported in response to the drug, although no patient was followed-up for more than 30 days. Gonzalez and others[18] found that the administration of a single dose of ivermectin (6 mg) reduced the parasitemia by 82% for a four-year period after treatment. Nutman and others[19] successfully treated one female patient by using 0.14 mg/kg ivermectin and reported that the patient showed symptoms compatible with an allergic reaction.

Because no controlled trials have been conducted to measure the effectiveness and/or the occurrence of adverse reactions of ivermectin,[18,19] studies conducted on the use of ivermectin have not been able to clarify how long it takes to eliminate parasitemia or the possibility of the microfilaraemia recrudescence. Ivermectin has already been demonstrated to be a safe and effective drug for the treatment of other helminthes[20–23] that occur in the studied area.[24,25]

This study characterized the efficacy of ivermectin to treat infections by *M. ozzardi* up to 360 days after treatment and subsequent side effects of the drug. These findings may contribute to the development of clinical assays to test other drugs and aid in the control of mansonelliasis.

MATERIALS AND METHODS

Study area. The study was conducted during 2009–2010 in the Lábrea Municipality (western Amazon, State of Amazonas, Brazil: 7°15′34″S, 64°47′59″W) (Figure 1), which has an estimated population of 38,000 inhabitants, of whom 5,0000 live along rivers in 112 small communities. Seven communities were chosen for the study: Cassiana, Bacural, Jucuri, Buraco, Santa Rosa, Jurucua, and Samauma, within a surrounding area located up to 200 km far from the municipal center of Lábrea. The main economic activities within the communities are the exploitation of natural resources, agriculture, and fishing.[26]

Sample size. To determine the minimum sampling size, 30 *M. ozzardi*-infected persons from Lábrea were randomly selected and had their microfilaraemia quantified by measuring the amount of microfilariae/milliliter of blood (filtered through a polycarbonate membrane). Based on the pilot sampling, the microfilaraemia was estimated by dispersion and a central measure trend that supported a sampling size calculation that considered a two-tailed alpha test = 0.05 (type I error), and β test = 0.20 (type II error). Based on these criteria, 40 persons were determined to be the minimum sample size for this study. However, as a preventive measure against the expected high drop-out rate during the 12-month

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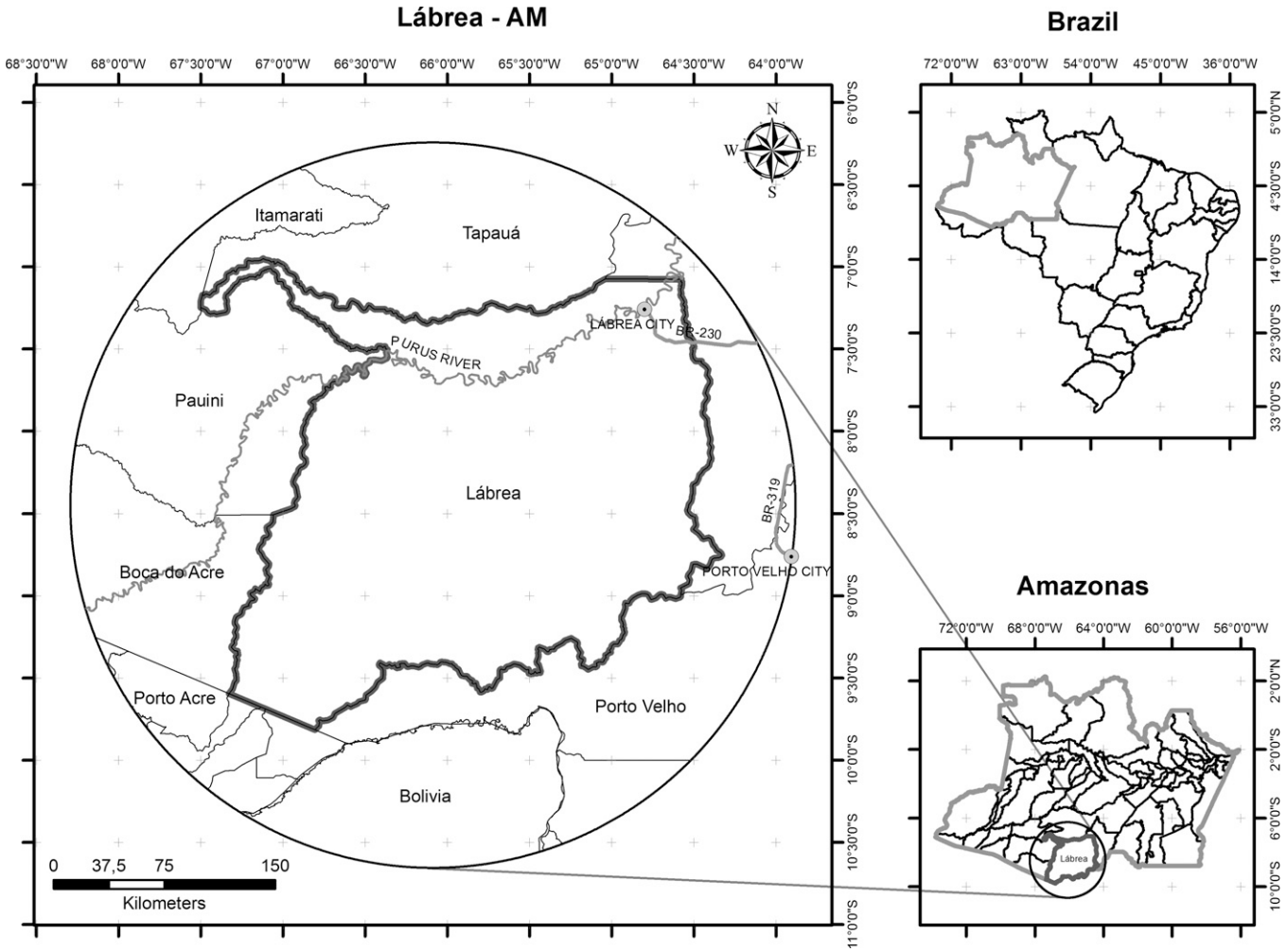

FIGURE 1. Location of the study area, municipality of Lábrea, Amazonas state, Brazil.

follow-up period, 74 microfilariae-positive cases were included in the study.

Population. Three hundred persons were estimated to live within the areas appraised, 171 of whom were present by the time of the study, met the inclusion criteria, and agreed to participate. Of these potential participants, 74 (43.3%) were infected with *M. ozzardi* microfilariae and were voluntarily engaged in the study. Of these persons, 53 remained in the study for one year after treatment.

Inclusion and exclusion criteria. Only *M. ozzardi*-infected persons 5–60 years of age and women who were not pregnant (women with a positive result for serum chorionic gonadotropin) or breastfeeding were included in this study. All persons had to consent to participate in the study, and persons less than 18 years of age needed the consent of a legal guardian. In addition, persons were excluded if they had cardiac, renal, neurologic, and/or hepatic pathologies; malnutrition and biochemical dosage alteration caused by hepatic or renal function impairments; a drug allergy history (Mazzotti's reaction); a record of any drug intake with anthelmintic effect over the past 30 days (albendazole, mebendazole, ivermectin, piperazine, pyrantel, pyrvinium, levamisole, and diethylcarbamazine); or were currently using central nervous system suppressing medications. Persons who did not consent to participate in the study were also excluded.

Ethics. The patients received written and oral communication about the risks and procedures involved and signed a specific consent agreement. The study was approved by the Ethical Committee of Sao Lucas College (registration no. 344/09), and has been registered at the Australian and New Zealand Clinical Trial Registry under the registry no. 12609000005257.

Intervention. The therapeutic regimen adopted was oral administration of 3-mg ivermectin tablets, registry no. NFN27660/0832210, supplied by the Ministry of Health of Brazil. A single 0.15-mg/kg dose was given to each of the 74 patients. Because this was a single-arm study, the treatment was not blinded. The medication was given under direct medical supervision and the use of all medications was recorded in the clinical epidemiologic file. For three days after ivermectin administration and a one-year period after treatment, treated patients were followed-up by the research team for possible adverse reactions. Complementary laboratory examinations, such as complete blood count, hematocrit, serum hemoglobin, aminotransferases, and γ-glutamyl transferase (GGT), were performed before treatment and on the third day after treatment by using commercial kits are widely used at the Brazilian Unified Public Health Service. The microfilariae count was assessed before treatment (D0) and on days 3 (D3), 30 (D30), 90 (D90), 180 (D180), and 360 (D360) after drug administration. Reference values obtained from manuals of commercial

TABLE 1

Biochemical and hematologic profiles of all patients who began and remained in the study until the third day, municipality of Lábrea, Amazonas state, Brazil*

Examination (no.)†	Reference values		Normal results‡		Paired McNemar test *P* value	Median	(Q1; Q3)	Median	(Q1; Q3)	Paired Wilcoxon test *P* value
	Males	Females	D0	D3		D0		D3		
Leukocytes (59)	4,000–10,000 mm^3		58	59	1.000	7,200	6,600; 7,980	7,600	6,700; 8,150	0.763
Lymphocytes (59)	800–4,000 mm^3		59	59	NA	1,933	1,761; 2,175	1,914	1,789; 2,185	0.443
Monocytes (59)	80–1,000 mm^3		59	59	NA	355	315; 410	316	272; 390	< 0.001§
Neutrophils (58)	1,800–7,400 mm^3		57	58	1.000	4,345	4,066; 4,955	4,964	4,187; 5,305	0.105
Eosinophils (59)	40–500 mm^3		44	55	0.013§	390	276; 498	261	210; 343	< 0.001§
Hematocrit (59)	42–54%	36–46%	31	30	1.000	38.0	37.0; 40.0	39.0	37.5; 40.0	0.437
Hemoglobin (59)	14–8 g/dL	12–16 g/dL	31	31	1.000	12.7	12.3; 13.3	13.1	12.45; 13.3	0.523
GGT (58)	7–45 U/L	5–27 U/L	51	46	0.063	19	15; 24	20	15; 30	0.004§
AST (58)	11–39 U/L	6–27 U/L	39	41	0.804	28	21; 33	27	22; 33	0.870
ALT (58)	11–33 U/L	7–27 U/L	42	44	0.791	24	18; 32	22	18; 28	0.152
Bilirubin (58)	≤ 0.8 mg/dL		53	58	0.063	0.5	0.4; 0.6	0.5	0.4; 0.5	0.117
Urea (58)	10–40 mg/dL		56	56	1.000	26	20; 31	26	21; 29	0.736
Creatinine (58)	0.5–1.4 mg/dL		56	54	0.625	0.8	0.7; 0.9	0.8	0.7; 1.0	0.868

*Reference values, median and quartiles used for the comparison of the results between pre- and post-treatment (D0 and D3) with ivermectin (0.15 mg/kg) are shown. The non-parametric paired Wilcoxon signed-rank test was used to compare the nominal exam results, and the McNemar test was used to compare the normal and abnormal examination result frequencies between D0 and D3. Because of technical issues, some examination data are missing,. Thus, only data from patients who had valid results at D0 and D3 were included in the analysis. NA = *P* values could not be calculated because there were no abnormal results; GCT = γ-glutamyl transferase; AST, aspartate aminotransferase; ALT = alanine aminotransferase.

†No. persons who had valid examination results at D0 and D3.

‡Persons with examination results within the range of reference values at each time point. Abnormal results can be calculated as N minus the number of normal results.

§Statistically significant.

kits used for the biochemical and hematologic examinations are shown in Table 1.

The absence of a control group was justified by the ethical issues of not treating patients and that there is no report regarding any other drug that would be effective against this parasite. No other intervention was implemented during the defined study period.

Although it has known limitations,[27] the before-and-after non-randomized study design was chosen because of the recommendations of the ethical committee against leaving a group of patients untreated.

Diagnosis and blood microfilariae quantification. Ten milliliters of blood was collected by venous puncture from each patient to detect and quantify microfilariae using the polycarbonate membrane filtering method.[28] One milliliter of the collected venous blood was diluted in 0.9% physiological saline solution and then filtered through a 3-micra pore size polycarbonate membrane (Nucleopore Corporation (Pleasanton, CA). Membranes were placed on microscope slides, fixed in methanol, stained with Giemsa, and examined. The number of microfilariae (mf) per membrane was determined by two technicians who used an optical microscope and was calculated based on the median number of mf/mL of blood.

Outcomes. The primary outcome was parasitologic clearance on D360. The secondary outcome was adverse reactions and clinical cure (absence of symptoms) on D30.

Adverse events (AEs). Clinical epidemiologic questionnaires were used before giving the medication on D0 and after giving the medication on D3 to measure the signs and symptoms. Signs and symptoms that were absent before treatment and were present or increased after drug administration were considered AEs. Because the medication half-life was 22–28 hours,[29] patients were examined for three days after treatment for AEs.

Statistical methods. Statistical analysis was performed by using Statistica 8.0 software and SPSS 13.0 software (IBM, Armonk, NY). The square root of the number of microfilariae per membrane observed were depicted graphically by using a box plot (Figure 2). Square root transformation was applied because of the high range of values observed at D0. For non-parametric related samples (paired), Friedman test was performed to compare cure rate evaluation (i.e., parasitemia clearance with no recrudescence on the 30th, 60th, 90th, 180th, and 360th days after the treatment) at each time point by taking a significance level of 0.05. Because non-parametric statistics were used, the square root transformation of the data does not influence the tests results and further conclusions. The biochemical and hematologic profiles were described by their median and first and third quartiles. Nominal examination results for D0 and D3 were compared by using a non-parametric paired Wilcoxon signed-rank test. Normal examination result frequencies were also presented. The McNemar test, which is useful for related samples (paired), was applied to compare the frequency of normal and abnormal results of biochemical and hematologic tests.

RESULTS

Of the 171 persons invited to participate in the study, 74 (43.3%) were positive for *M. ozzardi* microfilariae and met the inclusion criteria. In this non-random sample, an average microfilaraemia concentration of 7.2 mf/mL of blood was found for 74 persons before the treatment (D0) (median value = 1.0 mf/mL, minimum value = 1.0 mf/mL, Q_1 = 1.0 mf/mL, Q_3 = 3.25 mf/mL, maximum value = 250.0 mf/mL, and SD = 29.6 mf/mL). All volunteers were negative by the polycarbonate membrane technique when examined and analyzed after the following time points: D3 (n = 74), D30 (n = 74), D90 (n = 74), D180 (n = 66), and up to D360 (n = 53) after the treatment (Friedman $\chi^2 = 159.00$, $P < 0.0001$) (Figure 2).

General symptoms were quantified during D0–D3 (hemoptysis, abdominal pain, back or neck pain, arthralgia, asthenia, leg pain, arm pain, chest pain, dyspnea, dizziness, headache, fever, itchiness, nausea, vomiting, cold legs, adenomegaly, and blurred vision) in the 74 patients. Symptoms decreased in 75.7% of patients at D3. The health condition of 68 persons (91.9%) was improved by D30; five patients (6.7%) still had

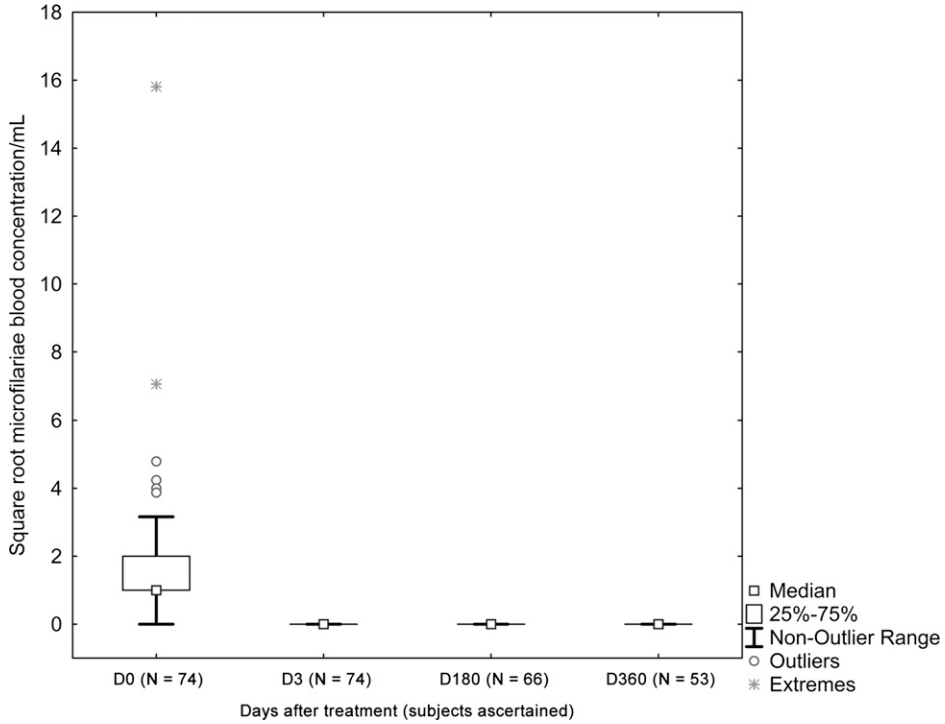

FIGURE 2. Square root of microfilariae density in patients studied up to 360 days after administration of a single dose (0.15 mg/kg) of ivermectin, municipality of Lábrea, Amazonas state, Brazil.

arthralgia and one person (1.4%) had one of the eight symptoms reported before treatment was alleviated.

The AEs (symptoms that occur or are aggravated after treatment) showed a significant occurrence in 64.9% of the 74 patients who were selected for the study. The average number of AEs reported per patient was 1.3 (range = 0–4 AEs). Of the patients investigated, 27.0% had one AE, 16.2% had two AEs, 18.9% had three AEs, and 2.7% had four AEs.

The most prevalent AEs were hyperthermia (28.7%), headache (26.6%), arthralgia (10.6%), and dizziness (7.4%). These AEs appeared after a mean ± SD of 16.2 ± 8.4 hours after medication intake and ended 13.9 ± 8.2 hours after symptom appearance; 100% relief was observed. Only one medical intervention was required for hyperthermia, vomiting, and myalgia, using symptomatic medications orally. No severe AEs occurred.

A significant increase in the level of GGT ($P = 0.004$) was observed in pre- and post-treatment biochemical parameters (Table 1). Normal and abnormal biochemical test results comparing D0 and D3 by using the McNemar related (matched) samples test are shown in Table 1. No significant changes were observed.

The following hematologic alterations were observed during D0–D3: a decrease in monocytes ($P < 0.001$) and eosinophilic granulocytes ($P < 0.001$) (Table 1). Normal and abnormal hematologic test results comparing D0 and D3 by using the McNemar related (matched) samples test are shown in Table 1. A significant improvement of eosinophilic granulocytes to normal levels was observed.

DISCUSSION

The Amazon region shows a high *M. ozzardi* prevalence with infection rates ranging from 0.4% to 70% depending on the locality studied and the diagnostic methods used.[4,9–12] In this study, the *M. ozzardi* prevalence was 43.3% (74/171) by using the polycarbonate membrane method, which was found to be more sensitive than the diagnostic methods used in a previous study.[30] Mansonelliasis can thus be considered a neglected disease mainly in the Brazilian Amazon and throughout the Americas and in the Caribbean.

Although inevitable, the study dropout rate was high. Anticipating this fact, we increased the original sample (40 patients) to 74 patients. The main cause of the high dropout rate was emigration or patient absence from the study area during visits by the research team.

The use of ivermectin in this study was determined by the extensive use of this medication in anthelmintic human treatments. Since 1987, this drug has been used in the treatment of filariae of medical significance such those of *Onchocerca volvulus*, *M. perstans*, and *M. streptocerca* in Africa.[31–37] However, there are few studies on the specific treatment of *M. ozzardi* with this drug.[1,18,19,38]

This study confirmed the effectiveness of ivermectin in parasitologic blood clearance of previous studies.[1,18,19] This study also showed that the microfilariae of *M. ozzardi* blood clearance persisted after a year, thus providing more detailed clearance information than obtained in other studies.[1,18,19]

Regarding the biochemical parameters, GGT levels showed a significant increase ($P = 0.004$, by Wilcoxon signed-rank test), although no statistically significant alterations were observed in for bilirubin, aminotransferases, urea, and serum creatinine levels. Few studies have focused on these parameters and this was the first such study conducted in Brazil. It is worth pointing out that the increase in GGT levels had no clinical repercussions. The Wilcoxon signed-rank test showed a significant decrease in monocytes ($P < 0.001$) and eosinophilic

granulocytes ($P < 0.0001$) between D0 (after treatment) and D3 (72 hours after treatment), as expected. In addition, the results suggested an improvement in normal levels of eosinophilic granulocytes. Unlike previous studies, this study showed leukocyte alterations in only 1.3% of the patients compared with leukocyte alterations in 16.2% of the patients reported by Batista and others,[12] and 6.5% of the patients reported by Tavares.[8]

The only consistent finding between this study and previous studies was the alteration of eosinophilia (64.4%). The same alterations were described by Batista and others[12] and Tavares,[8] and an extremely high value was reported by Nutman and others,[19] who reported 20% eosinophilia in one patient. However, values of eosinophil counts returned to normal levels after treatment ($P = 0.013$, by McNemar test and $P < 0.001$, Wilcoxon signed-rank test).

Another objective of the present study was to evaluate and compare the actual effectiveness of ivermectin and adverse reactions associated with treatment. To achieve this goal, treatment was supervised and patients were followed-up for 72 hours to evaluate signs and symptoms. The adverse reactions were not restrictive, although they occurred in a significant number (64.9%) of the patients. However, by D3, no adverse reactions remained, which was consistent with results of previous studies.[19,38]

Up to 30 days after the treatment, 68 (91.9%) of 74 persons reported relief from all symptoms that were present before treatment (hemoptysis, abdominal pain, back and neck pain, arthralgia, asthenia, leg pain, arm pain, chest pain, dyspnea, dizziness, headache, fever, body itching, nausea, vomiting, cold legs, adenomegaly, and blurred vision). It is also plausible that symptom alleviation could be caused by the treatment of other helminthes besides *M. ozzardi.*.

Because this was a non-randomized single-arm study, these results may be biased and have a natural limitation, as described by Deeks and others.[27] We are aware that non-randomized study limitations are more remarkable when the intervention outcome is difficult to measure and that non-randomized designs should be undertaken only when randomized controlled trials are infeasible or unethical. In the absence of another treatment design, the ethics committee consulted reported that a randomized controlled trial would be unethical. Considering the dimension and the homogeneity of the results (in all 53 followed-up patients, the *M. ozzardi* clearance that was observed three days after the intervention was sustained for one year), we could not determine any other factor that could be related to such simultaneous, sustained clearance. Also, considering that no restrictive adverse reactions occurred and no adverse reactions remained at D3, corroborating the findings of previous studies,[19,38] it could be stated that ivermectin was safe.

Prichard and others reported the importance of establishing a research agenda for human helminthic diseases.[39] Considering the results from this study, we suggest that mansonelliasis should be included on that agenda. Moreover, the use of ivermectin may also contribute to the treatment of other helminthic diseases that also occur at this region, and its use could be a relevant strategy for public health programs in areas to which more than one disease is co-endemic.

Future controlled clinical assays based on these findings should evaluate the use of ivermectin in relation to other anti-helminthic drugs and/or a placebo to better understand clinical symptoms in a blind and randomized design over a two-year period. The prolonged suppression of microfilaraemia (12 months) and no severe AEs suggest the possibility of using this medication to control mansonelliasis.

Received July 14, 2013. Accepted for publication February 16, 2014.

Published online April 7, 2014.

Acknowledgments: We thank Professor Marisis Camargo, Professor Erney Camargo, and Flavia Fontes for English review of the manuscript; Marcelo Zagonel (Instituto Nacional de Genética Médica Populacional of the Conselho Nacional de Desenvolvimento Científico e Tecnológico) for drawing the map; and people of Lábrea Municipality, Brazilian Amazon, for participating in the study.

Financial support: This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (grant no. 2008/10817-6).

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