



Interruption of lymphatic filariasis transmission in Manaus, a former focus of *Wuchereria bancrofti* in the Western Brazilian Amazon

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ABSTRACT

Objective. To confirm the absence of *Wuchereria bancrofti* autochthonous cases in Manaus, a former focus of lymphatic filariasis in the Western Brazilian Amazon.

Methods. A field survey was carried out in 2016 using immunochromatographic rapid tests (ICT card) for the detection of circulating filarial antigens in blood. The sample included a group of 3 000 schoolchildren aged 6 to 10 years enrolled in schools from different urban areas of Manaus (including the former lymphatic filariasis focus in the city) and a group of 709 adolescents and adults, between the ages of 11 and 85 years, born and raised in different areas of Manaus.

Results. All of the individuals tested negative for *W. bancrofti* antigen.

Conclusions. Although Manaus was once considered endemic, this focus no longer seems to be active for lymphatic filariasis transmission. The results of this study could support the certification by the World Health Organization of the lymphatic filariasis transmission elimination exercise in Brazil.

Keywords

Wuchereria bancrofti; elephantiasis, filarial; Brazil.

Lymphatic filariasis (LF) is a vector-borne neglected tropical disease with serious social and economic impact. It is caused by three nematode species, *Brugia malayi*, *B. timori*, and *Wuchereria bancrofti*, the last of which is the main etiological agent, responsible for more than 90% of cases (1, 2).

The disease impairs the lymphatic system, leading to chronic disability such as lymphedema, hydrocele, and elephantiasis, and is considered a global public health problem by the World Health Organization (WHO) (1, 2). Currently, LF is found in 49 countries in Asia, Africa, and the Americas, affecting approximately 100 million people, and putting around 893 million people at risk of acquiring it (1, 2). The two most recent countries to receive validation from WHO for the elimination of LF are Yemen and Kiribati, a Pacific island nation (3).

In the Americas, the disease is caused exclusively by *W. bancrofti*. It is believed to have been introduced as a result of the slave trade from Africa, the most unfortunate and tragic legacy of colonialism (4). *Culex quinquefasciatus* mosquitoes are considered to be the only competent vector species in this continent (5). The parasite microfilariae found in patients circulate in the peripheral blood mainly at night (nocturnal periodicity) (6), and can be detected in thick blood smear samples taken for diagnostic purposes.

In 1997, the World Health Assembly (Resolution WHA 50.29) set the year 2020 as the target for the elimination of LF as a global public health problem (7). In 2000, WHO launched the Global Programme to Eliminate Lymphatic Filariasis to achieve this goal (8). As per WHO recommendations, the main strategy to interrupt LF transmission is mass drug administration. This

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should be followed by a Transmission Assessment Survey and monitoring (9).

LF foci persist in four countries of the Americas: Brazil, the Dominican Republic, Guyana, and Haiti (10, 11). Endemic areas of LF along the northeastern coast of Brazil are limited to Recife, in Pernambuco State, and three cities within the Recife metropolitan region (Olinda, Jaboatão dos Guararapes, and Paulista) (11, 12). These foci are currently being reassessed through a Transmission Assessment Survey, in order for the Brazilian LF Elimination Program (PEFL) to certify the absence of active *W. bancrofti* transmission.

The cities of Belém (Pará State) and Manaus (Amazonas State), situated in the north of Brazil, were also known to be endemic, yet they are now considered nonendemic (11). Interruption of transmission in Belém was confirmed in 2001. No microfilaremic subjects and no infected mosquitoes were found as a result of extremely thorough study (11, 13), attesting to the effectiveness of the measures adopted in that city. No study has been conducted to assess interruption of LF transmission in Manaus.

Results of a study conducted in Manaus in 1949 revealed LF positive findings in 2% (48/2 405) of the study population (autochthonous cases) (14). The last LF epidemiological study in the city was carried out in 1956, the findings of which revealed 0.2% positivity (microfilariae positive for *W. bancrofti*) among the 10 889 individuals examined (15). There is no further information relative to the distribution and prevalence of autochthonous cases of *W. bancrofti* in Manaus since these two earlier studies. Thus, the aim of this study was to reassess the occurrence of LF in Manaus to support the country's dossier in the certification of interruption of LF transmission.

MATERIALS AND METHODS

Study area

The study was conducted in the urban area of Manaus (03°06'07" S, 60°01'30" W), capital of Amazonas State, located in the Western Amazon, Brazil (Figure 1). The city has a total area of 11 401 092 km², encompassing 63 neighborhoods with an estimated population of 2 182 763 inhabitants (population density of around 158.1/km²) (16). Manaus is a city with areas of poverty, per capita income of 790.27 Brazilian reals, and a Municipal Human Development Index of 0.737 (16), with high population density.

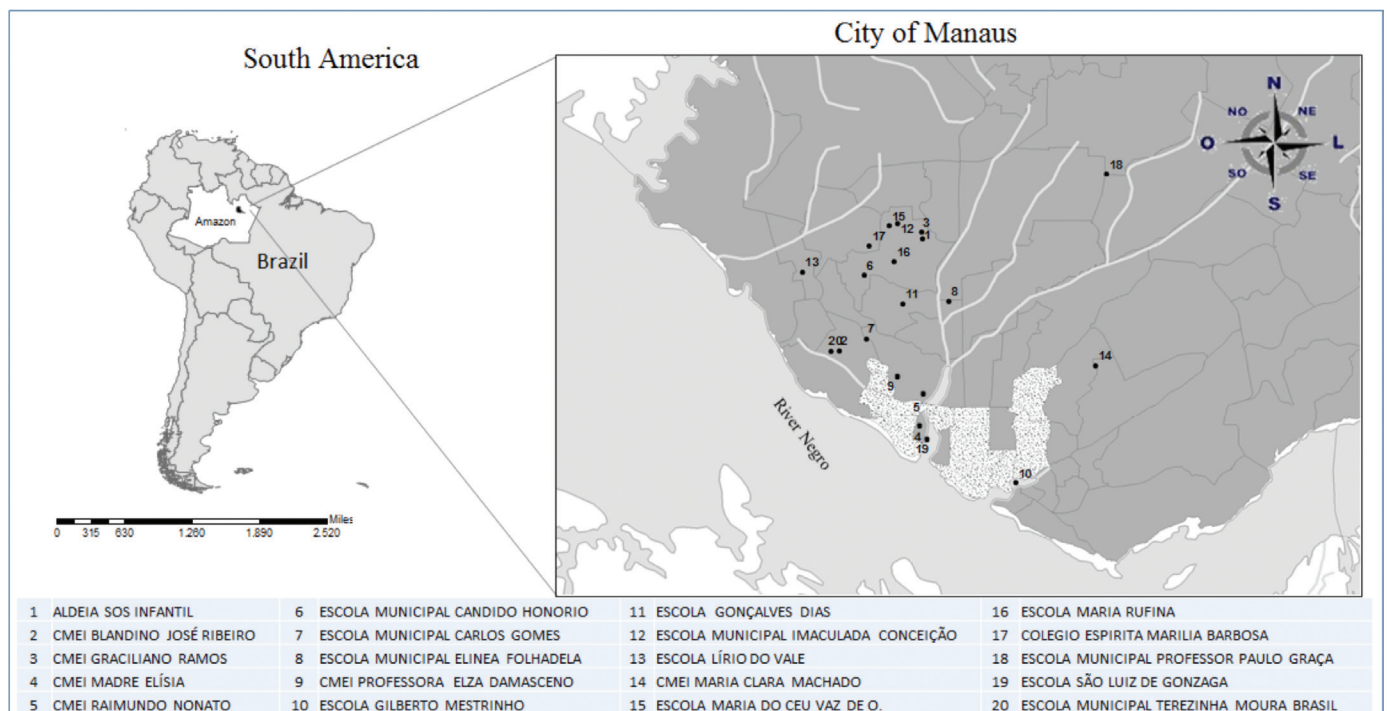
Sampling and collection

A cross-sectional randomized survey was conducted from September 2015 to July 2016. The study samples were composed of school-age children and general population.

School-age children. Students aged 6 to 10 years from urban area municipal public elementary schools were investigated as per guidance of WHO (8) and the Transmission Assessment Survey of the Global Programme to Eliminate Lymphatic Filariasis (9). WHO calls for a sample of 3 000 children to be tested following the rapid survey methodology based on rapid immunochromatographic test (ICT) cards to verify LF transmission in a suspected area.

The municipal education department provided an updated list with the names of the students enrolled in the elementary schools, to allow the survey to be carried out. In order to come

FIGURE 1. Map of Manaus, Amazonas State, Brazil, showing the geographic location of the 20 schools (1–20) where a sample of schoolchildren was examined for circulating *Wuchereria bancrofti* antigen, 2016



up with the population to be surveyed among the city's different neighborhoods, 20 (34.5%) out of 58 urban municipal public elementary schools (comprising a total of 18 990 school-age children) were randomly selected. The total population of the 20 selected schools was 7 562 children. Five of these schools were selected among those neighborhoods of Manaus where microfilariae carriers had been detected in the past (14, 15). The other 15 schools were randomly chosen, representing the overall urban area of the city.

To reach the required total sample size of 3 000 children to be tested, the sample size withdrawal was proportional to the number of students enrolled in each school, in relation to the total students from the 20 chosen schools (7 562). Schoolchildren were randomly selected from the list of students provided by the schools. The children's data (name, age, sex, and address) were obtained from the school's administrative records.

Lectures about the disease and the survey were given to teachers, parents/legal guardians, and students at each school. The inclusion criterion was enrolled students aged 6 to 10 years. Consent forms were signed by the parents or legally authorized representatives of each child.

General population. Adolescents (>10 years old) and adults were randomly selected from within the same areas as the sampled schoolchildren. The authors included this group in order to assess the status of people who were born, raised, and had lived for many years in one of the former LF-endemic neighborhoods. All participants were informed about the study and those ≥18 years old were asked to sign a consent form. Minors had the form signed by their parents or legal guardians.

Diagnostic tool and blood collection

Participants were tested using rapid ICT (BinaxNOW Filariasis, Alere Scarborough, Orlando, United States of America) for the detection of *W. bancrofti* circulating antigens. Briefly, capillary blood samples were obtained from finger pricks in a calibrated microhematocrit tube (100 µL) and transferred to the ICT card, according to the manufacturer's instructions. The results of the tests were read by a trained technician after 10 minutes, and positives recorded.

Ethics approval

This study was approved by the Research Ethics Committee of the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus (CAAE No. 06959813.0.0000.0005). All data obtained and herein reported were treated anonymously by the investigators.

RESULTS

A total of 3 000 ICT-based tests were performed in schoolchildren attending a sample of public elementary schools. Table 1 shows the stratified school-age children sample by age and gender. The average age was 7.9 ± 1.1 years, and the gender distribution was 1 757 (58.6%) females and 1 243 (41.4%) males. No test was found positive for the presence of *W. bancrofti* circulating antigens. All 709 of the adolescents and adults (ages 11–85 years) tested had a negative result for circulating filarial antigen (Table 2).

TABLE 1. Distribution, by age and sex, of school students examined for *Wuchereria bancrofti* antigenemia, Manaus, Amazonas State, 2016

Age	Male		Female		Total		ICT* results
	n	%	n	%	n	%	
6	328	41.5	462	58.5	790	26.3	Negative
7	308	41.5	435	58.6	743	24.8	Negative
8	278	41.6	392	58.6	669	22.3	Negative
9	186	41.3	264	58.5	451	15.0	Negative
10	143	41.2	204	58.8	347	11.6	Negative
Total	1 243	41.4	1 757	58.6	3 000	100.0	Negative

* Immunochromatographic Card Test
Source: Prepared by authors from the study data.

TABLE 2. Distribution, by age group and sex, of adolescents and adults examined for *Wuchereria bancrofti* antigenemia, Manaus, Amazonas State, 2016

Age	Male		Female		Total		ICT* results
	n	%	n	%	n	%	
11–20	47	40.9	68	59.1	115	16.2	Negative
21–30	126	51.2	120	48.8	246	34.7	Negative
31–40	108	61.4	68	38.6	176	24.8	Negative
41–50	50	64.9	27	35.1	77	10.9	Negative
≥51	56	58.9	39	41.1	95	13.4	Negative
Total	387	54.6	322	45.4	709	100.0	Negative

* Immunochromatographic Card Test
Source: Prepared by authors from the study data.

DISCUSSION

Despite the efforts to control LF around the world, the disease remains endemic in 49 countries, including Brazil (1), where the microfilariae of *W. bancrofti* were described for the first time by Otto Wucherer in 1866 (17). However, the earliest systematic epidemiological studies on the distribution of Bancroftian filariasis in Brazil were conducted decades later (11, 18). In a study conducted between 1951 and 1958, a total of 811 361 individuals were examined (using thick blood film), and 120 399 specimens of mosquito vectors were dissected (18). The simultaneous discovery of microfilaremic individuals and infected mosquitoes provided evidence of local LF transmission in 11 cities of the country, including Manaus (Amazonas State) (18). In fact, a previous study in Manaus in 1949 had already demonstrated a prevalence of 2.0% (48/2 405) of *W. bancrofti* microfilariae carriers in the neighborhoods of São Raimundo, Santo Antônio, Vila da Prata, Cachoeirinha, São Francisco, and Presidente Vargas (14). In 1956, Rachou and Lacerda (15) examined 10 889 persons in Manaus and identified that 0.2% were microfilariae-positive. Since then, beyond the selective treatment of infected patients, no specific measures focusing on the prevention of filarial infections were implemented, and no epidemiological surveys to investigate LF had been carried out. The Ministry of Health no longer considered Manaus to be LF endemic (19).

The present study revealed that LF had indeed been successfully eliminated in Manaus, despite the persistence of risk factors. The examined individuals live in areas where the environmental conditions and the lack of urban infrastructure and sanitation favor vector proliferation and *W. bancrofti* transmission. Furthermore, some of the surveyed schools are located in the very same neighborhoods where LF had been found to be endemic. These factors could very well facilitate LF reintroduction, should Manaus eventually receive infected people coming from LF-endemic areas.

Reintroduction of the infection through migration was described in Sri Lanka (20) and in the metropolitan areas of Recife in Brazil, where LF had already been eliminated (21). Surveillance measures must therefore be sustained, focusing specially on immigrants from LF-endemic areas and countries. A study conducted by Silva et al. in 2014 (22) on a population of Haitian refugees in Manaus showed very low prevalence rates of LF. Only one immigrant among the 244 evaluated (0.4%) was found to be positive. The possibility of reintroduction of LF should not be ruled out, as there is a large density of the main vector *C. quinquefasciatus* in the region, as well as adequate conditions for the transmission of the parasite (23).

Three other cities (Humaitá, Guajará-Mirim, and Porto Velho) in the Western Brazilian Amazon region were studied by Korte et al. in 2013. There was a history of allochthonous cases of *W. bancrofti* infections dating back to the 1950s in all three cities (24). No humans or mosquitoes were found to be infected with the parasite in the 2013 study, indicating that the area was no longer positive for LF transmission (24). It appears that the small number of people infected and the mild nature of the microfilarial infection reported in the past were not sufficient to sustain transmission (24).

ICT card tests are recommended by WHO as the diagnostic tool of choice to be used in LF elimination programs to define

areas where mass drug administration is required and surveillance is to be maintained (8). The test is easy to perform in field settings and has a high level of sensitivity for the detection of filarial antigen (25).

According to WHO (8, 9), children between 6 and 10 years old should be the target of epidemiological surveys, because it is in this age group where a positive test will indicate a recent infection. The sample of 3 000 children as defined by WHO (8) is not useful to measure LF prevalence, but it is a fast way to determine whether transmission is still occurring in the area. The rationale for examining young children is therefore to assess recent infections. Negative findings provide a good indicator that transmission has ceased in the area (26). According to WHO, antigenemia levels of >0.1% indicate ongoing transmission. In this situation, a population survey to determine LF prevalence and morbidity would be required.

A sample of adolescents and adults was used in this study to assess the status of LF in people born, raised, and living for many years in a former LF-endemic area. This was important, as no LF survey had been carried out in the city since the 1950s. The Brazilian LF Program needs to look at all historical LF foci in order to prepare a dossier for submission to WHO to obtain the validation of elimination of LF as a public health problem in Brazil.

This is the first study that relied on the WHO criteria to evaluate the current LF situation in Manaus. The results presented here are quite promising, indicating that the historical LF focus in Manaus has indeed been eliminated. The Ministry of Health of Brazil considers that LF transmission has been interrupted in almost all the foci in the country, including Belém, Maceió (27, 28), Salvador, Castro Alves, Florianópolis, Ponta Grossa, and Barra de Laguna (11). It has since 2011 been working intensively to eliminate the remaining active foci in the Recife metropolitan region (28).

Certification of LF elimination as per the WHO standardized procedures and those of the Strategic and Technical Advisory Group for Neglected Tropical Diseases will require careful preparation of the dossier (29, 30). This study constitutes an important element of the dossier.

Author contributions. GF and MM conceived the original idea, planned the experiments, analyzed the data, interpreted the results and wrote the manuscript. MM and RCSG collected and analyzed the data. All authors reviewed and approved the final version.

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Conflicts of interest. The authors declare no conflicts of interest.

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Interrupción de la transmisión de la filariasis linfática en Manaus, anteriormente un foco de *Wuchereria bancrofti* en la Amazonia occidental de Brasil

RESUMEN

Objetivo. Confirmar la ausencia de casos autóctonos de *Wuchereria bancrofti* en Manaus, anteriormente un foco de filariasis linfática en la Amazonia occidental de Brasil.

Métodos. En el 2016 se llevó a cabo una encuesta en el terreno con pruebas rápidas inmunocromatográficas (tiras inmunocromatográficas) para detectar antígenos filáricos circulantes en sangre. La muestra constó de un grupo de 3 000 escolares de 6 a 10 años matriculados en escuelas de diferentes zonas urbanas de Manaus (incluida la zona que anteriormente era el foco de filariasis linfática en la ciudad) y de un grupo de 709 adolescentes y adultos, de edades comprendidas entre 11 y 85 años, nacidos y criados en diferentes áreas de Manaus.

Resultados. Todas las personas dieron negativo en la prueba de antígeno de *Wuchereria bancrofti*.

Conclusiones. Aunque hubo un tiempo en que Manaus se consideraba zona endémica, parece que este foco de transmisión de la filariasis linfática ya no está activo. Los resultados de este estudio podrían brindar apoyo a la certificación de la Organización Mundial de la Salud respecto de los esfuerzos realizados en Brasil para eliminar la transmisión de la filariasis linfática.

Palabras clave *Wuchereria bancrofti*; filariasis linfática; Brasil.

Interrupção da transmissão da filariose linfática em Manaus, anteriormente um foco de *Wuchereria bancrofti* na parte leste da Amazônia brasileira

RESUMO

Objetivo. Confirmar a ausência de casos autóctones de *Wuchereria bancrofti* em Manaus, anteriormente um foco da filariose linfática na parte leste da Amazônia brasileira.

Métodos. Uma pesquisa de campo foi realizada em 2016 com o uso de teste rápido por imunocromatografia (cartão ICT) para detecção de antígenos de microfilárias circulantes no sangue. A amostra estudada consistiu de um grupo de 3 000 crianças escolares entre 6 e 10 anos de idade matriculados em escolas de diferentes áreas da zona urbana de Manaus (englobando a área anteriormente com o foco de filariose linfática) e um grupo de 709 adolescentes e adultos entre 11 e 85 anos de idade nascidos e crescidos em diferentes áreas de Manaus.

Resultados. Todos os indivíduos pesquisados tiveram teste negativo para o antígeno da *W. bancrofti*.

Conclusões. Apesar de Manaus ter sido anteriormente uma área endêmica, parece que não existe mais foco ativo de transmissão da filariose linfática na cidade. Os resultados deste estudo podem servir para embasar a certificação pela Organização Mundial da Saúde da eliminação da transmissão da filariose linfática no Brasil.

Palavras-chave *Wuchereria bancrofti*; filariose linfática; Brasil.
