

# Assessment of family and neighbors of an individual infected with *Wuchereria bancrofti* from a non-endemic area in the city of Maceió, Brazil

## ABSTRACT

The family and neighbors of a patient infected with *W. bancrofti* microfilariae were assessed aiming to evaluate the occurrence of cases of lymphatic filariasis in a non-endemic area in the city of Maceió, in the Brazilian state of Alagoas. The patient had previously lived in an endemic focus; however, he has been living in an area where the parasite has never been detected for the past ten years. Female ingurgitated *Culex quinquefasciatus* mosquitoes captured in the houses of the microfilaremic individual and of his neighbors in the non-endemic region were also examined by polymerase chain reaction (PCR) technique. The thick blood smear examination, blood membrane filtration, and rapid immunochromatography (antigen search) revealed no infected individuals in the family of the microfilaremic individual. All 334 neighbors undergoing the thick blood smear examination were negative for *W. bancrofti* microfilariae. In 478 ingurgitated *C. quinquefasciatus* mosquitoes examined by PCR, no *W. bancrofti* DNA was detected. The microfilaremic individual had a microfilaremia considered very low according to WHO standards (4 microfilariae/mL of blood). As the vectorial infection depends on microfilaremia, the patient's low parasite load did not determine the contamination of other individuals in the area. Our data have shown that the long-term residence of the microfilaremic individual in the non-endemic region was not sufficient to start a new transmission focus of lymphatic filariasis in Maceió.

**Keywords:** *Wuchereria bancrofti*, lymphatic filariasis, epidemiology, *Culex*.

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## INTRODUCTION

Lymphatic bancroftian filariasis, whose etiologic agent is the nematode *Wuchereria bancrofti* (Cobbold, 1877), is an exclusively human disease, endemic in several tropical regions. The economic losses and social stigma caused by the physical deformity of the patients in the chronic phase make bancroftosis the second world cause of work disability.<sup>1</sup>

Transmission occurs in 83 countries of tropical regions of Asia, Africa, Oceania, and Americas, with one billion people estimated to be at risk of transmission and 112 million people already infected by *W. bancrofti*.<sup>2</sup>

In the Americas, lymphatic filariasis is found in Guiana, Haiti, Dominican Republic, and Brazil.<sup>2</sup> Costa Rica, Suriname, and Trinidad Tobago, which until recently were endemic areas, are under surveillance and have not had active transmission for the past few years.<sup>2</sup>

In Brazil, bancroftosis is restricted to the city of Recife, Pernambuco State, some neigh-

boring cities, and the city of Maceió, Alagoas State. In the city of Belém, Pará State, an endemic area until recently, transmission has been controlled, which maintains the region under surveillance.<sup>3</sup>

In the city of Maceió, studies carried out in the 1990s showed that the distribution of *W. bancrofti* was restricted to three contiguous city sectors, Feitosa, Jacintinho, and Pitanguinha, at the edge of an open canal that receives part of the city's sanitary sewage, where basic sanitation is lacking.<sup>4</sup> Recent studies have shown that the transmission of lymphatic filariasis in Maceió tends to disappear, because the disease prevalence has been decreasing significantly since the implantation of the "Program to Eliminate Lymphatic Filariasis in Maceió" in 1999.<sup>5</sup>

In 2006, a hemoscopic survey was initiated aiming to reassess the distribution of parasitosis in Maceió. The sample comprised nocturnal students and public school employees living in

## Authors

Anderson B Leite<sup>1</sup>  
Ana RV de Lima<sup>1</sup>  
Renata B Leite<sup>1</sup>  
Rafael V Santos<sup>1</sup>  
Johnathan EL Gonçalves<sup>1</sup>  
Eliana MM Rocha<sup>1</sup>  
Gilberto Fontes<sup>1</sup>

<sup>1</sup>Institute of Biological Sciences and Health, Universidade Federal de Alagoas.

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## Correspondence to:

Prof. Dr. Gilberto Fontes  
Instituto de Ciências Biológicas e da Saúde,  
Universidade Federal de Alagoas/ICBS – UFAL  
Praça Afrânio Jorge,  
s/n – Prado  
Maceió – AL – Brazil  
CEP: 57010-020  
Phone: 55 82 32141704  
Fax: 55 82 32212501  
E-mail: gilberto.fontes@pq.cnpq.br

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the 50 neighborhoods of the city. Among the employees assessed, only one microfilaremic was detected, living outside the endemic area, but coming from Jacintinho, one of the endemic neighborhoods for lymphatic filariasis in the city.

This study aimed to assess the occurrence of cases of bancroftosis in non-endemic areas for this parasitosis in Maceió, Alagoas State, and its epidemiological significance.

## METHODOLOGY

**Studied area:** Part of the Benedito Bentes neighborhood, in the northern region of Maceió. The studied area, where the previously detected microfilaremic patient had been living for ten years, has geographic, environmental, and socio-economic similarities with the endemic area of lymphatic filariasis in the city, which favor the installation of a new focus of disease transmission. The area studied is 15 km away from the endemic focus of lymphatic filariasis in Maceió.

### Hemoscopic survey

**Population studied:** The family of the microfilaremic patient (four individuals) and 70 families (334 individuals) living within a 100-meter radius from his home (neighbors) were assessed.

**Human parasite diagnosis:** All individuals underwent examination by using blood thick smear technique (TS), collected after 10 PM, due to the nocturnal periodicity of microfilariae in peripheral blood of infected individuals in the region.<sup>6</sup> The microfilaremic individual was examined using the technique of blood filtration through polycarbonate membrane (MF),<sup>7</sup> to quantify his microfilaremia in 10 mL of blood, and rapid immunochromatographic tests (ICT “card test”), which detect the presence of *W. bancrofti* circulating antigens. To increase the sensitivity in the diagnosis of the family of the microfilaremic patient, in addition to TS, the MF technique (filtration of 20 mL of blood) and ICT “card test” were used.

### Entomological survey

**Mosquito collection:** Xenomonitoring,<sup>8</sup> consisting in the capture and examination by PCR of ingurgitated females of the *Culex quinquefasciatus* mosquito (Say, 1823), was performed. The mosquitoes were captured using a suction apparatus (Castro device), between 8 AM and 9 AM, at the house of the microfilaremic patient and at the neighboring houses, where some individuals underwent TS examination.

**Vectorial diagnosis:** The PCR system used the species-specific initiators NV1 and NV2, designed from cloning and characterization of the Sspl gene sequence of *W. bancrofti*.<sup>9</sup> The DNA was extracted according to the technique of Vasuki *et al.*,<sup>10</sup> and the reagents for DNA amplification were added to that material in a thermocycler programmed to 35 cycles of one minute at 92° C, one minute at 55° C, and one minute

at 72° C. Positive and negative controls were, respectively, as follows: DNA extracted from mosquitoes experimentally infected with *W. bancrofti* and DNA extracted from non-infected mosquitoes. The 188pb product was detected through 1.5% agarose gel electrophoresis.

**Ethical aspects:** This study was submitted to the Research Ethics Committee of Federal University of Alagoas (Register 014814/2006-76), and was approved. All individuals assessed provided written informed consent, which was signed by the examinee or his/her guardian.

## RESULTS

In the survey performed to describe the current situation of lymphatic filariasis in Maceió, which assessed 22,934 individuals in 2006 and 2007, one single microfilaremic individual was diagnosed. The patient was a 47-year-old male, living in the studied area for 10 years, but coming from Jacintinho, one of the endemic areas for lymphatic filariasis in the city where he had lived for more than 30 years. His microfilaremia, quantified by use of MF, was 4 microfilariae/mL of blood.

Assessment of the patient’s family (four women), by using MF and ICT, did not detect *W. bancrofti* infection. All 338 individuals examined by use of TS (four family members and 334 neighbors) were negative for *W. bancrofti* microfilariae.

At the patient’s house and at the neighboring houses, 478 ingurgitated specimens of *C. quinquefasciatus* were captured. Their examination by use of PCR showed no DNA of the parasite.

## DISCUSSION

Microfilaremic individuals constitute the source of infection with *W. bancrofti* of transmitting mosquitoes, being thus responsible for maintaining endemia. Most microfilaremic individuals are asymptomatic, and diagnosed only through active search, mainly through blood tests. In places with favorable conditions to the parasite transmission, the migration of microfilaremic individuals can account for the establishment of new foci of transmission. In Sri Lanka, infected migrants have originated foci of the parasitosis in areas where lymphatic filariasis did not exist.<sup>11</sup> A similar situation was observed in previously non-endemic areas of the metropolitan region of Recife, in the Brazilian state of Pernambuco, which currently have indigenous cases of bancroftosis.<sup>12</sup>

The focus is considered to be formed from the infection of household contacts with microfilaremic individuals, who have a greater chance of acquiring the parasitosis than the general population. Some studies have indicated that the prevalence of bancroftosis is higher in the patient’s family, whose members can have a chance seven-fold higher of being infected as compared to the general population of en-

demic areas.<sup>13</sup> In Maceió, while the prevalence of infected individuals within the family of microfilaremic individuals was 29.2%, among neighbors (20-meter radius) that prevalence was approximately five times lower (5.5%), coinciding with the prevalence observed in general population of the same endemic area.<sup>13</sup> The comparative analysis of family members of microfilaremic soldiers of the city army of Recife with family members of non-microfilaremic soldiers revealed a 4.2 times greater risk of infection among those living with microfilaremic individuals.<sup>14</sup> However, in the present study, the long-term exposure of the microfilaremic individual to the potential vector of *W. bancrofti* in a non-endemic area for lymphatic filariasis with environmental conditions similar to those existing in the endemic area was not sufficient to originate a new transmission focus in the city.

The possibility of acquiring the infection in the region where the patient has been living for the past 10 years cannot be completely eliminated. But both the lack of previous records of infected individuals in the area where the microfilaremic individual lives and the epidemiological analysis performed allow stating with a reasonable degree of certainty that the patient acquired the parasitosis during the approximately 30 years he lived in the endemic area of lymphatic filariasis in the city.

Microfilaremia of vertebrate hosts is one of the factors influencing the capacity of the vector to become infected and transmit the infection. The microfilaremic individual of the present study had a very low parasitemia according to the WHO criteria.

The infection rate of the mosquitoes is proportional to the density of microfilariae in the patient's peripheral blood. In Maceió, the infection rate of mosquitoes feeding on individuals with low microfilaremia (1-10 mf/mL), like the patient studied, was 0.07%, and the vector's efficiency was approximately 17%, that is, for six microfilariae ingested, only one would develop into infecting larva.<sup>15</sup> Those rates might not be sufficient to maintain the parasitosis transmission in the area. Studies have shown that the maintenance of endemia depends on the occurrence of high natural infection rates in mosquitoes. However, a consensus about the estimated number of infected mosquito bites required for the appearance of a new human case with patent microfilaremia has not been reached.<sup>16</sup> However, below a certain number of infecting bites, lymphatic filariasis is not endemic. Places whose microfilaria density is maintained under 5 microfilariae/60mm<sup>3</sup> are not considered at risk for transmission.<sup>17</sup>

Thus, the potential danger of importing bancroftosis and the establishment of sustainable foci of disease depend on the number of infected individuals and on the microfilaria density of patients, conditions that apparently did not exist at the places assessed in the present study.

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