

# Outbreak of canine visceral leishmaniasis in Barra Mansa, State of Rio de Janeiro

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## ABSTRACT

**Introduction:** In Brazil, visceral leishmaniasis (VL) has spread to various regions. This study reports canine cases of VL in Barra Mansa, where human VL cases were recently reported. **Methods:** Using the human index case, a canine survey was performed by dual-path platform immunochromatography and enzyme-linked immunosorbent assay. Seropositive animals were euthanized. Cultures were collected to detect *Leishmania* parasites. **Results:** Serological tests detected 141 canine VL cases, and *Leishmania chagasi* were isolated from 82.2% animals. **Conclusions:** *Leishmania chagasi* is in circulation in Barra Mansa. This study broadens information on the parasite's distribution in the State of Rio de Janeiro.

**Keywords:** Barra Mansa. Canine survey. Canine visceral leishmaniasis.

Visceral leishmaniasis (VL) is of serious public health importance, and affects approximately 1-2 million people annually around the world<sup>1</sup>. Autochthonous transmission has been recorded in nearly all Brazilian states. The domestic dog is an important reservoir of infection, therefore constituting a primary target for disease control<sup>2</sup>.

Human cases are rare in the State of Rio de Janeiro, although canine cases have often been recorded over the years in municipalities such as Angra dos Reis, Mangaratiba<sup>3</sup>, Maricá<sup>4</sup>, and Volta Redonda<sup>5</sup>, and in different urban areas of the capital<sup>3,6</sup>. Canine VL cases historically precede spatial and temporal human cases<sup>7</sup>; therefore, the geographical expansion of canine cases in the State of Rio de Janeiro has put epidemiological surveillance authorities on alert<sup>8</sup>.

Barra Mansa is a municipality in the Southern region of the state where nine human cases of VL were recently reported<sup>9</sup>. Confirmation of these cases triggered a series of actions in Barra Mansa, in particular, the initiation of a canine survey directed at tracking the occurrence and extent of VL among domestic dogs. In this paper, our focus was to report the initial results of the canine survey in Barra Mansa during the years 2011-2013 and to identify *Leishmania* species in seroreactive animals.

Barra Mansa is a Brazilian municipality in the southern State of Rio de Janeiro. It is located at latitude 22°32'39" S, longitude 44°10'17" W, altitude 381m, and has an area of 547,226km<sup>2</sup>. The average annual temperature is 28°C with rainy summers and a relative humidity of 77%. The population is approximately 177,000 inhabitants. Barra Mansa borders the municipalities of Valença, Quatis, Rio Claro, Pirai, Bananal, Barra do Pirai, Resende, and Porto Real, and forms a conurbation with the Cities of Volta Redonda and Pinheiral<sup>10</sup>.

The canine survey included domestic dogs living within a radius of approximately 100 meters of recorded humans VL cases. The animals were evaluated by a dual-path platform immunochromatographic test [DPP rapid test; Bio Manguinhos/Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil], and DPP-positive animals were confirmed by an enzyme-linked immunosorbent assay [*Ensaio imunoenzimático para diagnóstico da leishmaniose visceral canina* (EIE-LVC); Bio Manguinhos/FIOCRUZ/Rio de Janeiro, Brazil] at the Central Laboratory Noel Nutels [Laboratório Central (LACEN), Rio de Janeiro, Brazil], as recommended by the Ministry of Health<sup>8</sup>. All animals confirmed as seropositive by enzyme-linked immunosorbent assay (ELISA) were collected and sent for euthanasia at the Laboratório de Pesquisa Clínica em Dermatozoonoses/FIOCRUZ, Rio de Janeiro, Brazil. Euthanasia followed the technical norms defined by Resolution no 714/02 from the Federal Council of Veterinary Medicine and by the screening-culling procedure adopted by the Brazilian Ministry of Health in its Program for Visceral Leishmaniasis Control<sup>2</sup>.

Before euthanasia, the animals were sedated with ketamine (10mg/kg) mixed with acepromazine (0.2mg/kg) and clinically classified as *asymptomatic* (i.e., dogs without clinical signs

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**Received** 22 February 2014  
**Accepted** 29 May 2014

of VL upon physical examination), *oligosymptomatic* (i.e., dogs with 1-3 clinical signs), and *symptomatic* (i.e., dogs that had >3 clinical signs of VL such as emaciation, skin lesions, lethargy, lymph node enlargement, or signs of hepatomegaly or splenomegaly). Euthanasia was performed with an overdose of sodium thiopental 5%. Tissue samples (from liver, spleen, and intact skin fragments) and bone marrow and lymph node aspirates were collected and processed by culture for the isolation of parasites using the protocol described by Madeira et al.<sup>3</sup>. Tissue samples were first placed in sterile saline solution with antibiotics and an antifungal, and later transferred to a biphasic culture medium (Novy-MacNeal-Nicolle medium + Schneider medium supplemented with 10% fetal bovine serum) and incubated at 27°C ± 1°C, whereas the material obtained by puncture was immediately seeded in test tubes containing the same culture medium. Cultures were examined weekly for 30 days. When positive, they were reserved for etiological characterization by isoenzyme electrophoresis (i.e., multilocus enzyme electrophoresis) which employed five enzymatic systems: malic enzyme (ME, EC1.1.1.40), nucleosidase (NH, EC3.2.2.1), glucose-6-phosphate dehydrogenase (G6PDH, EC1.1.1.49), glucose phosphate isomerase (GPI, EC5.3.1.9), and 6-phosphogluconate dehydrogenase (6PGDH, EC1.1.1.43). *Leishmania braziliensis* (MHOM/BR/75/M2903) and *Leishmania chagasi* (MHOM/BR/74/PP75) were the reference samples in all experiments.

During the study period, 3,103 dogs were evaluated. Of these, 141 (4.5%) dogs were seropositive and hence euthanized. Promastigote forms were isolated from different sites in 116 (82.2%) euthanized animals (Table 1). Twenty-five euthanized dogs were culture-negative. Among these, 15 dogs were asymptomatic, nine dogs were oligosymptomatic, and one dog was symptomatic.

At least one sample of the isolated organisms from each animal was processed by multilocus enzyme electrophoresis. *Leishmania chagasi* was confirmed in 100% (n = 116) of dogs.

The expansion of VL has been reported in various regions of Brazil, and the results of this study corroborate this. In 1977, in the State of Rio de Janeiro, the first human case of VL occurred in the municipality of Rio de Janeiro. From this case, the protocol of the canine survey was officially established in

1980 in this state<sup>6</sup>. At that time and in subsequent years, this survey was performed only in certain areas of the City of Rio de Janeiro. However, it has recently been extended to other regions of the state such as Barra Mansa because of the discovery of human and/or canine cases in these regions. The displacement of infected animals and the high adaptability of the vector have been the main reasons for the dispersal of VL in Brazil, and the State of Rio de Janeiro is no exception to this rule. Barra Mansa is bordered by the municipalities of Resende and Volta Redonda where VL cases have also recently been described<sup>5</sup>.

In this respect, a point that should be emphasized is the need for epidemiological surveillance in regions with favorable conditions for the establishment of VL. In Barra Mansa, the presence of *L. chagasi* has been confirmed, which places the region on alert.

Canine seropositivity may vary, depending on the epidemiological characteristics of the region. In Belo Horizonte, is approximately 7% which is an area of intense transmission<sup>11</sup>. In Barra Mansa, we reported a prevalence of 4.5%. It is interesting that by 2009 canine leishmaniasis seropositivity had already been reported at levels that reached 10% in Barra Mansa<sup>12</sup>. At that time, *L. chagasi* was not detected in the dogs studied; however, one dog was infected with *Leishmania braziliensis*. This suggests that canine visceral leishmaniasis (CVL) was perhaps already spreading silently in Barra Mansa, and led to an explosion in the number of cases such as those reported in this paper.

The limitation of serological testing is a major argument against euthanasia of seropositive dogs<sup>11</sup>. In our study, *L. chagasi* was confirmed by culture in 82% of the 141 euthanized seropositive dogs. This speaks in favor of the routine - DPP followed by enzyme-linked immunosorbent assay that is currently employed for the diagnosis of CVL in Brazil<sup>8</sup>. In 25 seropositive dogs, parasitological confirmation was not possible. This may be explained by the clinical condition of the animal at the time of euthanasia because 15 (60%) dogs were classified as asymptomatic animals. Studies have shown that the chance of parasite isolation is less in asymptomatic dogs than in symptomatic animals<sup>13</sup>.

Another point that should be emphasized is the need for laboratory confirmation, primarily in areas with no record of VL. Human cases have been reported in Barra Mansa; however, this is the first time that *L. chagasi* has been shown in VL cases in this region.

The culture is the standard reference tool for the diagnosis of leishmaniasis; however, this approach is not used in epidemiologic studies<sup>14,15</sup>.

The isolation of *L. chagasi* from the assessed dogs reinforces the involvement of this animal in the transmission cycle of VL in Barra Mansa. Despite reports of *L. braziliensis* in this region, this study did not detect it in any of the evaluated dogs. In the present study we evaluated a variety of clinical specimen, and the highest rates of positivity were in lymph node fragments, whereas interestingly the lowest rates were in lymph node aspirates. We believe this result may be because of variations in the volume of material collected by puncture. In recent years,

**TABLE 1 - Results of parasitological cultures using different clinical specimens from euthanized seropositive dogs.**

Clinical specimen	Percentage (number of isolates/number of dogs) (CI:95%)
Lymph node fragment	73.8% (48/65; 84.5-63.0)
Spleen fragment	69.5% (98/141; 77.0-61.9)
Skin fragment	58.5% (38/65; 70.4-46.5)
Bone marrow aspirates	56.7% (80/141; 60.8-52.5)
Lymph node aspirates	52.3% (33/63; 64.6-40.0)

CI95%: 95% confidence interval.

leishmaniasis has been classified, along with other arthropod-borne diseases, as an emerging and re-emerging disease with a spread that can be associated with numerous factors. Barra Mansa, which was previously an area without recorded cases, now has an explosion of VL canine cases. The sequence of events demonstrated in this study suggests that CVL was possibly already initiated and spreading silently. The canine survey continues to be conducted in Barra Mansa, along with health education activities to professionals from different sectors that are aimed at containing the spread of VL in this region.

## ACKNOWLEDGMENTS

The authors would like to thank Evandro Chagas National Institute of Infectology (*Instituto Nacional de Infectologia Evandro Chagas*)/*Fundação Oswaldo Cruz* (FIOCRUZ) for the infrastructure to realize the research.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## FINANCIAL SUPPORT

This study was supported by the municipal prefecture of Barra Mansa and the Evandro Chagas National Institute of Infectology (*Instituto Nacional de Infectologia Evandro Chagas*)/*Fundação Oswaldo Cruz* (FIOCRUZ). Maria de Fátima Madeira and Fabiano Borges Figueiredo hold a grant from *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for their productivity in the research.

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