Short Communication

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 world1. 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 control2.

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, Manganhotiba1, Maricá4, and Volta Redonda2, and in different urban areas of the capital1,5,6,7. Canine VL cases historically precede spatial and temporal human cases; therefore, the geographical expansion of canine cases in the State of Rio de Janeiro has put epidemiological surveillance authorities on alert8.

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 547,226km². 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 Piraí, Resende, and Porto Real, and forms a conurbation with the Cities of Volta Redonda and Pinhal9.

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/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 Health4. 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 Control2.

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...
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. 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 1980 in this state, 1977, in the State of Rio de Janeiro, the first human case of VL in Brazil, and the results of this study corroborate this. In 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, 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.

<table>
<thead>
<tr>
<th>Clinical specimen</th>
<th>Percentage (number of isolates/number of dogs) (CI95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node fragment</td>
<td>73.8% (48/65; 84.5-63.0)</td>
</tr>
<tr>
<td>Spleen fragment</td>
<td>69.5% (98/141; 77.0-61.9)</td>
</tr>
<tr>
<td>Skin fragment</td>
<td>58.5% (38/65; 70.4-46.5)</td>
</tr>
<tr>
<td>Bone marrow aspirates</td>
<td>56.7% (80/141; 60.8-52.5)</td>
</tr>
<tr>
<td>Lymph node aspirates</td>
<td>52.3% (33/63; 64.6-40.0)</td>
</tr>
</tbody>
</table>

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.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES