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BRIEF COMMUNICATION

IDENTIFICATION OF *Leishmania infantum* IN PUERTO IGUAZÚ, MISIONES, ARGENTINA

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SUMMARY

The emergence of zoonotic visceral leishmaniasis (ZVL) in Latin America is a growing public health problem. The urbanization of ZVL has been observed in different countries around the world, and there are a growing number of reports drawing attention to the emergence of this infection in new locations, as well as its increase in previously established areas of endemicity. In the city of Posadas, Misiones province, Northeastern Argentina, the transmission of ZVL associated with canines and *Lutzomyia longipalpis* was first reported in 2006. In the city of Puerto Iguazú, also in Misiones province, the first human case of ZVL was reported in February 2014. From 209 surveyed dogs, 15 (7.17%) were identified as positive by serological and/or parasitological methods. Amplification was observed in 14 samples and in all cases the species implicated was *Leishmania infantum*. To the authors' knowledge, this is the first molecular characterization of *L. infantum* from dogs in this area.

KEYWORDS: *Leishmania infantum;* Molecular characterization; New focus; Puerto Iguazú, Argentina.

Visceral leishmaniasis (VL) is one of the most important parasitic diseases in the world. The domestic dog (*Canis familiaris*) plays a fundamental role as a reservoir of zoonotic visceral leishmaniasis (ZVL), favoring the urban cycle of the disease in the presence of the phlebotomine vector. In Latin America, canine leishmaniasis is widespread and it is among the most important canine zoonotic vector-borne diseases. The estimated number of infected dogs is in the millions and a high prevalence of canine leishmaniasis (CanL) is associated with the transmission of infection to humans[4].

In 2006, the first autochthonous human case of VL in Argentina was reported in Posadas, Misiones province (Northeastern Argentina), and was associated with canines and *Lutzomyia longipalpis*[9]. The presence of *Leishmania infantum* was further described in *Lu. longipalpis* sandflies and dogs using molecular methods[1,5]. To date, 104 human cases of visceral leishmaniasis have been reported in Misiones province (0 in Puerto Iguazú). The department of Puerto Iguazú (northwest of Misiones) has been considered an endemic area for *Leishmania braziliensis,* and until February 2014 was considered free of *L. infantum*.

The city of Puerto Iguazú is located in the northwest region of Misiones province, Northeastern Argentina, 300 km from Posadas. The city is bordered by Brazil (Iguazú falls), to the north, and by Paraguay, to the east (Ciudad del Este). The objective of this study was to determine if *L. infantum* is the etiologic agent of canine visceral leishmaniasis in domestic dogs from the city of Puerto Iguazú. To date, CanL by *L. infantum* has not been reported in this area.

In May 2013, a parasitological and serological pilot survey was carried out on 209 domestic dogs randomly sampled from a total of 21,466 properties included in the City Land Registry. The research protocol was reviewed and approved by the bioethics committee of the Ministry of Health in Misiones, Argentina (Comité de Bioética, División de Zoonosis de la Subsecretaría de Atención Primaria y Salud Ambiental Salud del Ministerio de Salud de Misiones; Resolución Ministerial Nº: 2640/2008). Written informed consent was obtained from each dog owner before clinical examination, whole blood (1 mL) and popliteal node (with ethanol 70%) extraction was performed.

The detection of anti-*Leishmania* antibodies was carried out by two serological tests: i) Kalazar Detect® immunochromatographic test (ICT) was performed according to the manufacturer's instructions (InBios International, Seattle, WA, USA). Briefly, 20 µL of plasma plus three drops of chase buffer were placed on the pad of the dipstick, and; ii) *In house* immunofluorescent antibody test (IFAT) was performed following a standard method using 10 µL of 2 x 10^7 *L. infantum* promastigotes/mL in 1x PBS per well as antigen (reference strain MHOM/FR/78/LEM-75) and serial two-fold dilutions of plasma for the analysis[3]. The

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IFAT threshold title for positivity was 1/160. From 209 surveyed dogs, 13 (6.22%) were seropositive by IFI (13/209) and/or by ICT (12/209).

Lymph node samples were washed with 500 µL of PBS and DNA was extracted by conventional phenol-chloroform extraction and ethanol precipitation[7], and further eluted in 100 µL sterile distilled water. Purified DNA was stored at -80 °C until further use. Parasite DNA detection was done by means of PCR targeting the *Leishmania* intergenic transcript spacer (ITS-1)[10]. Amplicons were visualized in seven out of 209 dogs. A total of 15 (7.17%) dogs were positive by serological and/or parasitological methods.

Later, those 15 positive dogs were subjected to a nested-PCR with outer pair previously used[7] and inner pair SAC (5´-CATTTTCCGATGAT TACACC- 3´) and VAN2 (5´-GCGACACGTTATGTG AGCCG-3´) to amplify an internal region (280 to 330 bp) of the fragment as described by CRUZ *et al.* in 2013[6]. Direct sequencing of the PCR products was performed with forward and reverse primers; using the Big-Dye Terminator Cycle Sequencing Ready Reaction Kit V3.1 and the automated sequencer "3730 DNA analyzer" (Applied Biosystems, Foster City, CA). Sequences obtained were analyzed and edited using BioEdit v7.2.5.©1999-2013 software (Tom Hall, Ibis Biosciences, Carlsbad, CA). Amplification was observed in 14 of the 15 samples studied and in all cases the species implicated *was L. infantum*.

In February 2014, the first fatal case of VL in humans was reported in the city of Puerto Iguazú[8]. Although the infecting species in this patient is not known, 29 human-VL patients at species level have been identified in the following cities; Posadas, Oberá, San Ignacio, Candelaria and Apóstoles (Misiones Province), as well as in 52 dogs sampled in 2006[5] and 53 dogs in 2010, all from Posadas. *L. infantum* was the only *Leishmania* species identified infecting humans and dogs (unpublished data).

In conclusion, the changes in the urbanization of the vector, the existence of susceptible reservoir hosts (dogs), vectors (*Lu. Longipalpis*) and the proximity of the disease to Paraguay, Brazil[2] and other cities of Misiones, may have favored the establishment of ZVL by *L. infantum* in Puerto Iguazú. It is important to highlight the strategic position of the city of Puerto Iguazú as a contributing factor to the spread of the disease, because of its geographical location (a location at which the Argentine, Paraguayan and Brazilian borders meet) and the influx of tourists it receives throughout the year due to the nearby Iguazú National Park (Iguazú falls). CanL control policies should be established in order to prevent further human and canine cases.

RESUMEN

Identificación de *Leishmania infantum* en Puerto Iguazú, Misiones, Argentina

La emergencia de leishmaniosis visceral zoonótica (LVZ) en América Latina es problema de salud pública en aumento. La urbanización de la LVZ es un fenómeno observado en diferentes países alrededor del mundo y hay un número creciente tanto de denuncias respecto a la aparición de esta infección en nuevas ubicaciones, como su aumento en zonas endémicas previamente establecidas. En la ciudad de Posadas, provincia de Misiones, nordeste de Argentina, la transmisión de LVZ asociada a canes y *Lutzomyia longipalpis* fue descrita por primera vez en 2006. En la ciudad de Puerto Iguazú, provincia de Misiones, el primer caso humano de LVZ tuvo lugar en febrero de 2014. De 209 perros muestreados, 15 (7.17%) resultaron positivos mediante métodos serológicos y/o parasitológicos. Se observó amplificación en 14 muestras y en todos los casos la especie implicada fue *Leishmania infantum*. Según nuestro conocimiento, esta es la primera caracterización molecular de *L. infantum* en perros procedentes de este área.

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