Transactions of the Royal Society of Tropical Medicine and Hygiene 105 (2011) 579–585

Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Wild, synanthropic and domestic hosts of *Leishmania* in an endemic area of cutaneous leishmaniasis in Minas Gerais State, Brazil

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ARTICLE INFO

Article history:
Received 14 December 2010
Received in revised form 8 July 2011
Accepted 8 July 2011
Available online 3 September 2011

Keywords:
Leishmania
Host reservoirs
Dogs
Mixed infection
PCR
Brazil

ABSTRACT

Domestic, synanthropic and wild hosts of *Leishmania* spp. parasites were studied in an area endemic for American tegumentary leishmaniasis (ATL), specifically in northern Minas Gerais State, Brazil. Domestic dogs and small forest mammals are reservoir hosts for *L.* (*Leishmania*) *infantum*. However, the role that these animals play in the transmission cycle of the *Leishmania* spp. that cause cutaneous leishmaniasis is not well known. This study evaluated 72 rodents, 25 marsupials and 98 domestic dogs found in two villages of the Xakriabá Indigenous Territory, an area of intense ATL transmission. A total of 23 dogs (23.47%) were shown to be positive according to at least one test; 8 dogs (8.16%) tested positive in a single serological test and 15 dogs (15.31%) tested positive by IFAT and ELISA. Eleven dogs were euthanised to allow for molecular diagnosis, of which nine (81.8%) tested positive by PCR for *Leishmania* in at least one tissue. Seven animals were infected only with *L.* (*L.*) *infantum*, whilst two displayed a mixed infection of *L.* (*L.*) *infantum* and *L.* (*V.*) *braziliensis*. Isoenzymatic characterisation identified *L.* (*L.*) *infantum* parasites isolated from the bone marrow of two dogs. Of the 97 small mammals captured, 24 tested positive for *Leishmania* by PCR. The results showed that *L.* (*V.*) *braziliensis*, *L.* (*L.*) *infantum* and *L.* (*V.*) *guyanensis* are circulating among wild and synanthropic mammals present in the Xakriabá Reserve, highlighting the epidemiological diversity of ATL in this region.

1. Introduction

Leishmaniasis is a parasitic disease caused by protozoa of the *Leishmania* genus, which are transmitted by phlebotomine sand flies to different vertebrate hosts. The primary reservoir hosts of *Leishmania* are wild mammals, mainly rodents and canines. However, with the increased domiciliation of the transmission cycle of leishmaniasis, domestic and synanthropic animals have assumed an important role in maintaining the parasite in transmission areas.[1] In the Americas, more than 40 species of mammals can be carriers of *Leishmania* spp. parasites.[2] However, only some of these animals function as reservoirs, acting as sources of infection for phlebotomine sand fly vectors and

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doi:10.1016/j.trstmh.2011.07.005

thereby contributing to the establishment of *Leishmania* in the area.

Identification of the vertebrate hosts for the *Leishmania* species that cause cutaneous leishmaniasis (CL) is an object of intense study. Few animals have been definitively identified as reservoirs. In fact, the natural reservoirs of *L.* (*Viannia*) *braziliensis*, the most widely spread aetiological agent for American tegumentary leishmaniasis (ATL) in Brazil, are not well known. Because the transmission cycle of *L.* (*V.*) *braziliensis* is primarily forest-based and is frequently associated with human penetration into forests or regions of vegetation, there is a strong suspicion that wild mammals could be the natural reservoirs for this species.[3,4] Reports of small rodents and marsupials infected with *L.* (*V.*) *braziliensis* as well as identification of parasite DNA in the tissues of these mammals suggest that they may help to maintain this species in the natural environment.[2,5]

Another relevant factor is the recent modification of the epidemiological cycle of ATL. With the disease now established in rural and urbanised areas, the transmission cycle in these places appears to be maintained through the participation of synanthropic or even domestic reservoirs. Epidemiological and experimental evidence indicates dogs as the principal reservoir hosts for *L.* (*Leishmania*) *infantum* in urban settings.[1] However, it is unclear whether these animals serve as domestic reservoirs for *L.* (*V.*) *braziliensis* and other dermotropic species.[6–12] There are many reports of dogs found naturally infected with *L.* (*V.*) *braziliensis*,[10,13–18] but their capacity for phlebotomine infection appears to be low.[19] Considering that domestic dogs are not the principal reservoir hosts of the aetiological agents of CL,[3,20–22] it remains to be determined which mammals play this role in rural and urban areas.

This study investigated infection by *Leishmania* spp. in domestic, wild and synanthropic animals in an area where ATL is endemic, specifically in the Xakriabá Indigenous Reserve in northern Minas Gerais State, Brazil.

2. Materials and methods

2.1. Area of study

The Xakriabá Indigenous Reserve is located in the municipality of São João das Missões (latitude 14°53′01″ south and longitude 44°05′26″ west), in northern Minas Gerais, Brazil. The reserve covers 78% of the entire municipality and has a population of 8380 inhabitants.[23] The region predominantly displays Brazilian savannah (cerrado) vegetation mixed with areas of caatinga (scrubland).

Two villages within the Xakriabá Territory with the greatest number of leishmaniasis cases were selected for this study.

2.2. Wild and domestic animal capture and sample collection

Traps were distributed across transects such that the entire area was covered, and four trails were defined between the houses for capturing small mammals (rodents and marsupials) in selected villages. Thirty traps were placed along each trail, two at each collection point. The collection points were nearly 20 m apart from each other. Twenty-one houses were also chosen and two traps were placed around each one. Thus, a total of 42 traps around houses and 120 traps along the trails were used in each 2 months during the 1 year of capture (May 2008 to June 2009). The collection points were established so as to sample different habitats related to patterns of human activity. The field method used was that of Lacher et al.,[24] Paglia et al.[25] and Fonseca et al.[26]

To perform molecular tests for *Leishmania* detection, all collected mammals were euthanised and samples of blood, bone marrow, tail skin, ear skin, spleen and liver were collected from all of the small mammals captured. Species identification was performed by analysing the morphological characteristics of each animal and comparing them with specimens deposited in the Mastozoology Collection of the Federal University of Minas Gerais (UFMG) (Belo Horizonte, Brazil).

All domestic dogs living in the selected villages were included in the study. The animals were examined and peripheral blood samples were collected for serological diagnosis. Animals displaying one or more ulcers in their hide had a fragment of a lesion collected for molecular diagnosis. Dogs testing positive for the disease were collected and euthanised by the local team responsible for zoonosis control.

After sedating and anesthetising seropositive animals, the bone marrow was punctured in the region of the tibia crossbow. The aspirated mesenteric or popliteal lymph node was collected and inoculated into a culture medium to isolate promastigotes of *Leishmania*. The following tissue fragments were also collected for direct parasitological examination and for molecular diagnosis: ear skin; spleen; liver; and the mesenteric lymph node.

2.3. Serological diagnosis of canine leishmaniasis

IFAT and ELISA tests were performed using antigen made from the promastigotes of *L.* (*L.*) *infantum* (MHOM/BR/1967/BH46 strain) and following the protocols of the *Leishmania* Serology Laboratory of the UFMG.

2.4. Detection and identification of Leishmania in clinical samples from small mammals and domestic dogs

2.4.1. Parasite isolation in the culture medium

Five-hundred microlitres of aspirated bone marrow from seropositive dogs was added to tubes containing blood agar-enriched LIT culture medium and was maintained at 25 ± 1 °C. Each culture was examined weekly and was considered positive if promastigote forms of *Leishmania* were observed. Isolated samples were cryopreserved for subsequent characterisation by multilocus enzyme electrophoresis (MLEE) and molecular methods. Identification of the parasite by MLEE was performed by typing processes at the *Leishmania* Collection of the Oswaldo Cruz Institute (Brazil).

2.4.2. Molecular diagnosis

Presence of the parasite was also investigated by amplifying DNA isolated directly from the tissue samples