

## Case Report

# First autochthonous case of canine visceral leishmaniasis in Rondônia, Brazil, a region with no history of visceral leishmaniasis

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### Abstract

This report describes the first autochthonous case of canine visceral leishmaniasis in Rondônia, northern Brazil. A canine resident of the municipality of Cacoal, with clinical signs and symptoms of visceral leishmaniasis, was treated by a veterinarian. Samples were analyzed by a reference laboratory. Dual-path platform (DPP) assay, indirect immunofluorescence technique (IIT), enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), isolation in a culture medium, and direct parasitological analysis were performed. DPP assay, IIT, and ELISA revealed positive results for *Leishmania*; PCR identified the species as *Leishmania infantum*. Based on the clinical presentation and test results, canine visceral leishmaniasis was diagnosed.

**Keywords:** Canine visceral leishmaniasis. *Leishmania infantum*. Autochthonous case.

### INTRODUCTION

Canine visceral leishmaniasis (CVL) is a worldwide public health concern, and areas at risk for the incidence of this disease are expanding<sup>1</sup>. Approximately 90% of cases of CVL in Latin America occur in Brazil<sup>1</sup>. Most Brazilian regions have recorded cases of either human or canine infection with *Leishmania infantum*. No cases have been recorded in the Rondônia State prior to this report. Rondonópolis City (approximately 200km from Cacoal), is an emerging urban focus of intense transmission of visceral leishmaniasis (VL); its reported prevalence of CVL is 19.2%<sup>2</sup>.

Canines are important reservoirs of the parasite<sup>3</sup>. The occurrence of CVL is directly related to the occurrence of visceral leishmaniasis (VL) in humans. Clinical manifestations of CVL are variable. The disease evolves slowly and has an insidious onset; it is considered to be a serious systemic disease. Its clinical manifestations are related to the type of immune response of the infected animal, and they range from an asymptomatic infection to a very severe disease. The most common signs and symptoms are weight loss; alopecia; skin

ulceration with formation of crusts, usually on the muzzle, ears and extremities; conjunctivitis; irregular fever; apathy; and onychogryphosis<sup>4</sup>.

This report describes the first autochthonous case of CVL recorded in the municipality of Cacoal, which is located 500km from Porto Velho, the capital of Rondônia. Cacoal has approximately 85,000 inhabitants and is home to a large university center. The county shares its northern border with the Mato Grosso State, a region where the incidence of CVL is increasing rapidly<sup>5</sup>.

### CASE REPORT

The infection was diagnosed in a 3-year-old male American pit bull terrier from an urban region of Cacoal. The dog's owner was also its breeder. The sick animal was in the third generation in its lineage, and none of the animals in its lineage had traveled to other counties or states. The dog was often taken to a horse farm in rural Cacoal, where it had contact with other canines, including a male border collie, an Australian cattle dog, and a Blue Heeler cattle dog from the Minas Gerais State.

About a year before the diagnosis, the dog started to show the first signs of infection: these included weight loss; skin lesions, mainly on the muzzle and ears; opaquing of the fur; and mucopurulent nasal discharge. A consulted veterinarian identified the following additional signs: small shallow ulcers on the skin, especially in the joint regions; hyperkeratosis in the

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nasal region; onychogryphosis; and bilateral keratoconjunctivitis with severe blepharitis (**Figures 1A to 1D**).

A blood sample was collected from the animal by a veterinarian and sent to a laboratory accredited by the Minas Gerais Canine Visceral Leishmaniasis Diagnostic Laboratory Network; accreditation was performed as recommended by the Ministry of Health [*Ministério da Saúde* (MS)]. Three tests were performed: Dual-Path Platform (DPP®) rapid immunoassay, indirect immunofluorescence test (RIFI), and enzyme-linked immunosorbent assay (ELISA). The results of DPP assay, ELISA, and RIFI tests for *Leishmania* were positive; the highest titer was 1/160 in the RIFI test.

Following the diagnosis of CVL, an additional blood sample was collected in order to repeat the serological tests, and a popliteal lymph node biopsy was performed in order to isolate the parasite. Blood samples were also collected from the canines that had been in direct contact with the infected canine.

The samples of the collected materials were sent to the Oswaldo Cruz Foundation [Fundação Oswaldo Cruz (FIOCRUZ)], Rondônia, FIOCRUZ Rondônia and Laboratório Central de Rondônia.

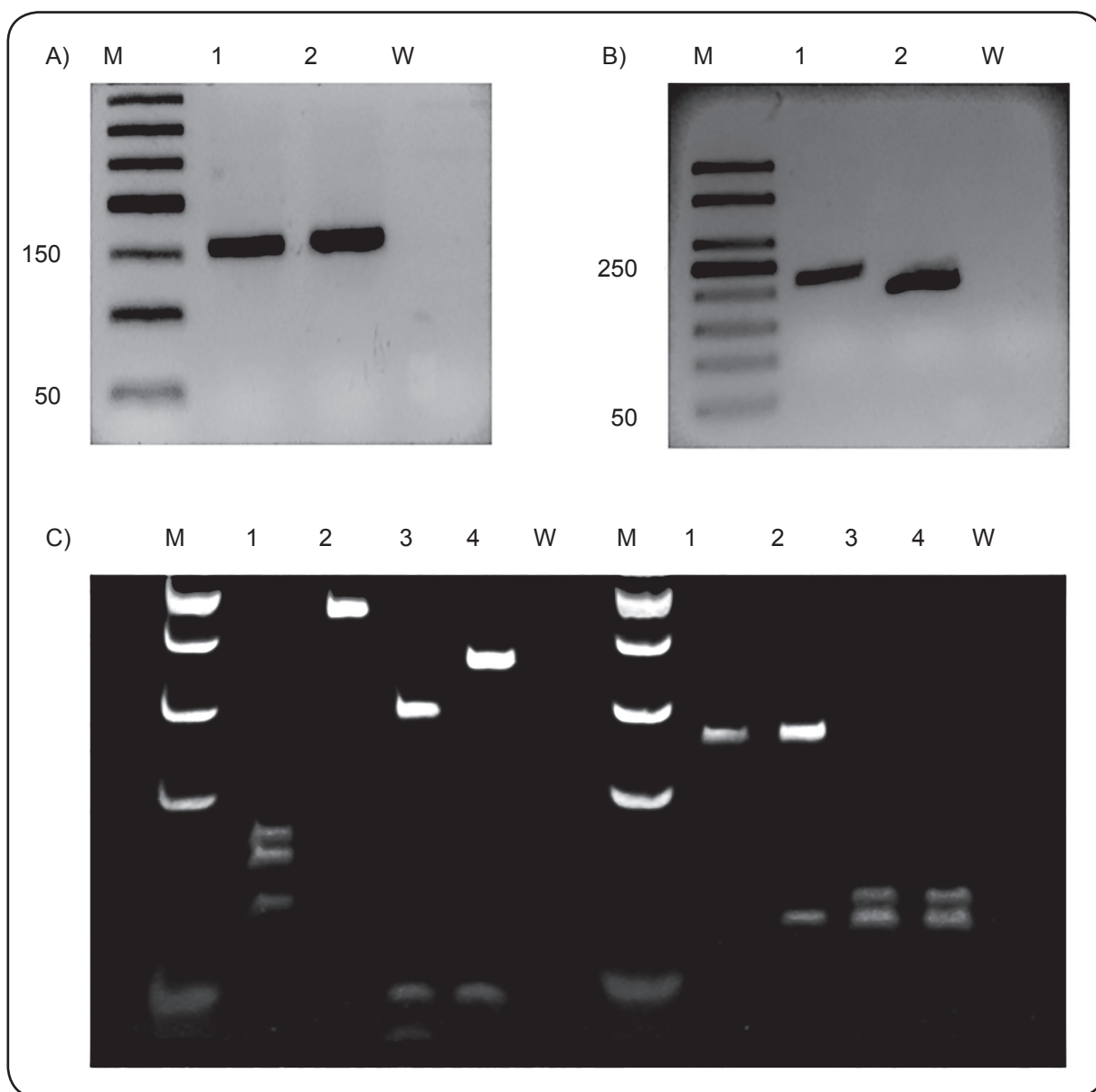
At FIOCRUZ Rondônia, a sample of the canine's lymph node was subjected to polymerase chain reaction (PCR) directed at two targets: the kinetoplast DNA (kDNA)<sup>6</sup> and the gene for heat shock protein 70 (hsp70). Restriction fragment size polymorphism (RFLP) was performed for the parasite species identification<sup>7</sup>. Imprints were made on slides stained using panoptic methods, and isolation was attempted using a biphasic culture medium (Novy-MacNeal-Nicolle + Schneider) supplemented with 20% fetal bovine serum and 2% filtered human urine.

All serologic tests, including DPP assay and ELISA, were carried out at the Clinical Research Laboratory on Dermatozoonoses of the Evandro Chagas Domestic Animal Infectious Diseases National Institute [*Instituto Nacional de Infectologia Evandro Chagas* (INI)] at FIOCRUZ in Rio de Janeiro, as recommended by the Ministry of Health. Direct examination of the slides of the popliteal lymph node aspirate for parasites was also performed at this laboratory.

The results of PCR were positive for the two molecular targets tested (**Figure 2**).



**FIGURES 1A to 1D:** A dog with VL having hyperkeratosis on the nasal region (Figures 1A and 1B), alopecia on the tips of the ears, conjunctivitis, and bilateral blepharitis (Figures 1C and 1D). **VL:** visceral leishmaniasis.



**FIGURE 2:** Agarose gels stained with 2% GelRed® (A and B). Fragments amplified by PCR for kDNA (A) and hsp70 (B). Fragments of 120 and 234bp are shown. **Line M:** ladder of 50bp; **Line 1:** positive sample; **Line 2:** positive control; **Line W:** writhe control. **C:** 10% polyacrylamide gel stained with silver nitrate. Fragments are shown in comparison to the *L. infantum* control after enzymatic digestion with *HaeIII* and *BstUI*. **Line M:** ladder of 50bp; **Line 1:** positive sample identified as *Leishmania infantum*; **Lines 2 – 4:** positive *Leishmania* controls from *L. amazonensis*, *L. braziliensis* and *L. guyanensis*, in that order; **Line W:** writhe control. **PCR:** polymerase chain reaction; **kDNA:** kinetoplast DNA; **bp:** base pairs; **L.:** *Leishmania*.

The results of all laboratory tests are given in **Table 1**. The results of DPP assay conducted at two FIOCRUZ locations and ELISA performed at INI were positive. Amastigotes were observed on direct examination of the tissues for parasites. Isolation in the biphasic culture medium was unsuccessful due to contamination, which prevented growth of the parasite. Blood samples collected from the other canines were also tested using DPP assay and ELISA, and one of the dogs tested positive in the DPP assay.

## DISCUSSION

This is the first report of an autochthonous case of CVL in the Rondônia State, and the case identification was based

on the clinical, laboratory, and epidemiological findings. Canine infection serves as an important indicator in public health surveillance because canines are the primary reservoirs of the infection that can be transmitted to humans<sup>8</sup>. The epidemiological history of this canine and the fact that it never left the municipality of Cacoal suggest that the infection was transmitted by a vector; this hypothesis is corroborated by the proven presence of *Lutzomyia longipalpis* in the central region of the Rondônia State<sup>9,10</sup>. *Lutzomyia longipalpis* is the primary vector of CVL in Brazil. Although the density of *L. longipalpis* has been reported to be low in Rondônia, the presence of *L. longipalpis* in canines can abet the introduction and spread of the disease throughout the state. This risk is heightened because



**TABLE 1:** Results of tests for the canines in the municipality of Cacoal, Rondônia.

Canines	Direct examination for parasites	Isolation in culture	DPP®	ELISA	RIFI	PCR
Pit bull	Negative*	Negative*	Positive*/**	Positive**	Positive***	Positive*
Blue heeler	NP	NP	Positive*	Negative**	NP	NP
Border collie	NP	NP	Positive*/**	Negative**	NP	NP

**DPP:** Dual-Path Platform; **ELISA:** enzyme-linked immunosorbent assay; **RIFI:** indirect immunofluorescence test; **PCR:** polymerase chain reaction; **NP:** test not performed; **FIOCRUZ:** Fundação Oswaldo Cruz; **INI:** Instituto Nacional de Infectologia Evandro Chagas. \*Test performed at FIOCRUZ Rondônia. \*\*Test performed at INI. \*\*\*Test performed in a private laboratory.

*L. longipalpis* has been found in municipalities of the Mato Grosso State that border the Rondônia State<sup>11</sup>.

Another possible hypothesis is that transmission occurred between canines without the presence of a vector. The infected canine had had direct contact with a canine from an endemic area; however, ELISA test in the latter canine was negative. Regardless of which hypothesis is correct, it is important to point out that CVL is endemic in Mato Grosso, which shares a border with the municipality of Cacoal<sup>12</sup>. A canine acting as a reservoir in Mato Grosso could have easily carried the disease into Rondônia, and the identification of the current case in this report suggests that other municipalities bordering Mato Grosso may harbor autochthonous cases of the disease that have not been recorded. The likely modes of entry for the disease are large transit vehicles; people and animals traveling along highway BR 364 and other roads that connect the two states; and the expansion of livestock and agricultural activity by several producers who have farms in both Rondônia and Mato Grosso.

This autochthonous case alerts us to the need for VL surveillance programs in Rondônia that take into consideration the border area with Mato Grosso and the proven presence of *L. longipalpis* within Rondônia itself. Therefore, it is imperative that we implement epidemiological and entomological surveillance measures, conduct an active search for asymptomatic human cases, and train professionals to use early identification methods.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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## REFERENCES

- Romero GAS, Boelaert M. Control of visceral leishmaniasis in latin america systematic review. PLoS Negl Trop Dis. 2010;19(1):e584.
- Carvalho AG, Luz JGG, Rodrigues LD, Dias JV, Fontes CJF. High seroprevalence and peripheral spatial distribution of visceral leishmaniasis among domestic dogs in an emerging urban focus in Central Brazil: a cross-sectional study. Pathogens and Global Health. 2018;112(1):29-36.
- Dantas-Torres F. The role of dogs as reservoirs of *Leishmania* parasites, with emphasis on *Leishmania (Leishmania) infantum* and *Leishmania (Viannia) braziliensis*. Vet Parasitol. 2007;149(3-4):139-46.
- Ministério da Saúde (MS). Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de Vigilância e Controle da Leishmaniose Visceral. Brasília: MS; 2007
- Mestre GLC, Fontes CJF. A expansão da epidemia da leishmaniose visceral no Estado de Mato Grosso, 1998-2005. Rev Soc Bras Med Trop. 2007;40(1):42-8.
- Degrave W, Fernandes O, Campbell D, Bozza M, Lopes U. Use of molecular probes and PCR for detection and typing of *Leishmania* a mini-review. Mem Inst Oswaldo Cruz. 1994;89(3):463-9.
- Graça GC, da Volpini AC, Romero GAS, Oliveira Neto MP de, Hueb M, Porrozzi R, et al. Development and validation of PCR-based assays for diagnosis of American cutaneous leishmaniasis and identification of the parasite species. Mem Inst Oswaldo Cruz. 2012;107(5):664-74.
- Lainson R, Rangel EF. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. Mem Inst Oswaldo Cruz. 2005;100(8):811-27.
- Gil LHS, Basano SA, Souza AA, Silva MGS, Barata I, Ishikawa EA, et al. Recent Observations on the Sand Fly (Diptera: Psychodidae) Fauna of the state of Rondônia, Western Amazônia, Brazil: the Importance of *Psychodopygus davisi* as a vector of zoonotic cutaneous leishmaniasis. Mem Inst Oswaldo Cruz. 2003; 98:751-5.
- Teles CBG, Basano SA, Zagonel-Oliveira M, Campos JJ, Oliveira AFJ, Freitas RA, et al. Epidemiological aspects of American cutaneous leishmaniasis and phlebotomine sandfly population, in the municipality of Monte Negro, State of Rondônia, Brazil. Rev Soc Bras Med Trop. 2013;46(1):60-6.
- Missawa NA, Lima GBM. Distribuição espacial de *Lutzomyia longipalpis* (Lutz & Neiva, 1912) e *Lutzomyia cruzi* (Mangabeira, 1938) no estado de Mato Grosso. Rev Soc Bras Med Trop. 2006;39(4):337-40.
- Cunha RS, Andreotti A, Cominetti MC, Silva EA. Detection of *Leishmania infantum* in *Lutzomyia longipalpis* captured in Campo Grande, MS. Braz J Vet Parasitol. 2014;23(2):269-73.