

Acute visceral leishmaniasis in a domestic cat (*Felis silvestris catus*) from the state of Tocantins, Brazil

Leishmaniose visceral aguda em felino doméstico (*Felis silvestris catus*) do estado do Tocantins, Brasil

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Abstract

Visceral leishmaniasis (VL) is expanding in the Brazilian territory. Dogs are considered an important urban reservoir; however, studies have demonstrated the presence of infected cats in some Brazilian states. This report aimed to describe a case of *Leishmania (Leishmania) infantum* infection in a two-month-old domestic feline from a Brazilian region with a high incidence of human visceral leishmaniasis. The analyzed samples were the cat's blood, conjunctiva, spleen, liver, popliteal, submandibular and mesenteric lymph nodes, skin, lung and kidney. The diagnostic methods were: parasitological examination, polymerase chain reaction (PCR) and an immunofluorescence antibody test (IFAT). All tissues were positive. The title obtained using the IFAT was 1:160. The animal was negative for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV). This work addresses the first case of feline leishmaniasis in the state of Tocantins, and reveals data that may contribute to the knowledge of the disease, since it has been shown to be able to develop rapidly and fatally in kittens, with the ability to infect several tissues.

Key words: Cat. Infection. Visceral leishmaniasis.

Resumo

A leishmaniose visceral (LV) encontra-se em expansão no território brasileiro. O cão é considerado um importante reservatório urbano, no entanto, estudos tem demonstrado a presença de felinos infectados em alguns estados brasileiros. Este relato objetivou descrever um caso de infecção por *Leishmania (Leishmania) infantum* em um felino doméstico de dois meses proveniente de uma região brasileira

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com alta incidência de leishmaniose visceral humana. As amostras analisadas foram sangue, conjuntiva, baço, fígado, linfonodos poplíteo, submandibular e mesentérico, pele, pulmão e rim. Os métodos de diagnóstico utilizados foram: o exame parasitológico direto, a reação em cadeia pela polimerase (PCR) e a reação de imunofluorescência indireta (RIFI). Houve positividade de todos os tecidos e o animal apresentou elevada carga parasitária. O título obtido na RIFI foi de 1:160. O animal foi negativo para os vírus da imunodeficiência felina (FIV) e da leucemia felina (FeLV). Este trabalho aborda o primeiro caso de leishmaniose felina no estado do Tocantins e revela dados que podem contribuir para o conhecimento da doença, visto que esta mostrou-se capaz de se desenvolver de forma rápida e fatal em filhotes, podendo infectar vários tecidos.

Palavras-chave: Gato. Infecção. Leishmaniose visceral.

Introduction

Visceral leishmaniasis (VL) is caused by a protozoan of the species *Leishmania (Leishmania) infantum* Syn. *L. (L.) chagasi* (CHATZIS et al., 2014). Although dogs are considered an important urban reservoir of this infection, there are indications that domestic cats can present a relevant role in endemic areas (SILVA et al., 2010).

Studies in countries such as Israel and Greece show the presence of the infection in cats (CHATZIS et al., 2014; NASEREDDIN et al., 2008). In Brazil, infected domestic felines have already been observed in areas with a high incidence of canine and human VL (SILVA et al., 2010; SOBRINHO et al., 2012).

The state of Tocantins has shown a high incidence of VL, reaching 12.2/100,000 habitants in 2015. This index classifies it as the place with the highest incidence of this disease in the country (BRASIL, 2017).

Taking into account the distribution of visceral leishmaniasis in Brazil and the high infection rates observed in Tocantins State, the objective of this study was to describe the first case of *L. (L.) infantum* infection in a domestic feline from Araguaína city.

Case report

A domestic feline female, of no defined breed and about two-month-old, was treated at a veterinary clinic in the municipality of Araguaína-TO. The owner reported finding her abandoned on the streets of the city, showing signs of diarrhea.

The anamnesis showed normorexy and normodipsy. During the physical examination, the veterinarian observed a capillary filling time (CFT) of 2 seconds, the presence of hypocorac mucous membranes, a body temperature of 38.6 °C and the absence of changes during cardiopulmonary auscultation, abdominal palpation and palpation of lymph nodes. In addition, no skin and/or mucous lesions were observed.

Then, blood samples were collected for blood counts, which verified hyperchromic normocytic anemia (hemocytes: $4.98 \times 10^6/\text{mm}^3$; hematocrit: 18.2%; hemoglobin: 6.6 g/dl; MCV: 36.5 fl; MCH: 13.3 pg; CMCH: 36.3%; and lymphopenia: $284 \times 10^3/\text{mm}^3$) and thrombocytopenia (platelets: 68×10^3). During the microscopic examination of blood cells, anisocytosis, polychromasia and the presence of ovoid and acidophilic forms, containing a nucleus and kinetoplast, compatible with the amastigote forms of *Leishmania* spp., were observed within the leukocytes (Figure 1A). The number of leukocytes parasitized in 1,000 nucleated cells revealed a parasitemia of 1%.

Three days after the first consultation, during the recheck, the owner reported a worsening condition, characterized by hyporexy and apathy. In addition to the aforementioned clinical manifestations, the physical examination revealed a weak pulse, an increased CFT of 3 seconds and a body temperature of 39.3 °C. The patient was hospitalized and submitted to intravenous fluid therapy with 0.9% physiological solution, followed by food supplementation with Hiperkcal Nutricuper Cat

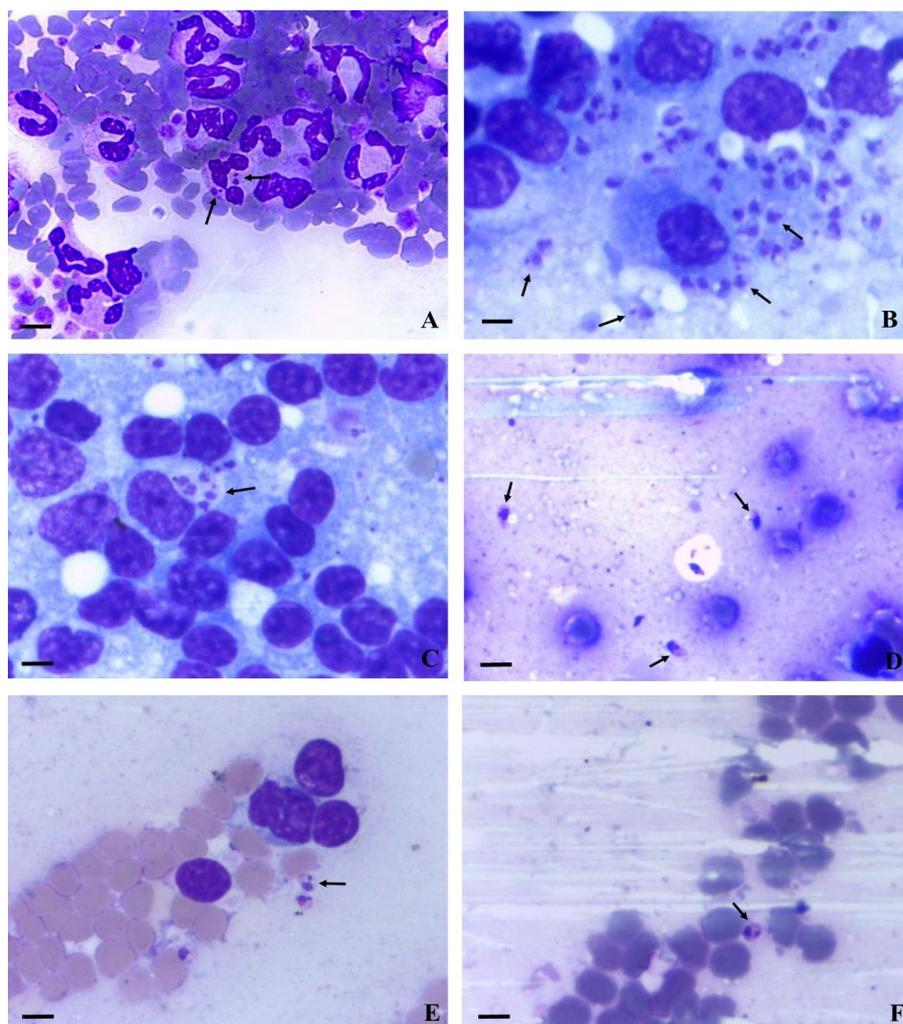
(Organnact®, Brazil), 1 g, orally, once per day, and Eritrós Cat (Organnact®, Brazil), 1 g, orally, once per day, in addition to an offer of pasty feed.

A second blood count was requested, followed by biochemical tests to evaluate hepatic and renal function. Material from skin scrapings and a puncture of the popliteal lymph node were collected in order to detect the probable *Leishmania* species present, through molecular analysis. At the time, the animal was treated with a 10% Imidacloprid collar and 4.5% Flumethrin 4.5%.

In the second hemogram, hematological abnormalities were observed: hypochromic

normocytic anemia (red blood cells: $3.82 \times 10^6/\text{mm}^3$; hematocrit: 16.6%; hemoglobin: 4.8 g/dl; MCV: 43.5 fl; MCH: 12.6 pg; and CMCH: 28.9%), leukocytosis (leukocytes: $24,600 \times 10^3/\text{mm}^3$) by neutrophilia with left deviation (neutrophils segmented: $18,696 \times 10^3/\text{mm}^3$; rod: $2.214 \times 10^3/\text{mm}^3$), monocytosis (monocytes: $1.476 \times 10^3/\text{mm}^3$), lymphocytosis (lymphocytes: $984 \times 10^3/\text{mm}^3$) and thrombocytopenia (platelets: $112 \times 10^3/\text{mm}^3$). Hematoscopy revealed anisocytosis, polychromasia, hypochromia, macroplots and the presence of amastigote forms of *Leishmania* spp. within the neutrophils and monocytes.

Figure 1. Visual demonstration of the amastigote forms of *Leishmania* spp. in tissue specimens of domestic felines from the municipality of Araguaína-TO. Note the protozoa (arrows) in the following types of samples: (A) blood; (B) popliteal lymph node; (C) submandibular lymph node; (D) liver; (E) spleen; (F) lung. (HE, 100X, scale = 6 μm).



The biochemical analytes were urea, creatinine, alanine aminotransferase (ALT/TGP), aspartate aminotransferase (AST/TGO) and total proteins and fractions (albumin and globulins). Hypocreatinemia (creatinine 0.4 mg/dl), hypoproteinemia (total protein: 4.1 g/dl), hypoalbuminemia (albumin: 1.3 g/dl) and increased serum ALT activity (124 IU/L) were found.

After 10 days, the animal demonstrated a worsening clinical picture and died. Then, she was sent to the Veterinary Medicine School and Animal Science of Tocantins Federal University for necropsy. During the procedure, tissue fragments from the liver, spleen, lung, kidney, conjunctiva, skin and popliteal, submandibular and mesenteric lymph nodes were collected.

Imprints of the popliteal and submandibular lymph nodes, liver, spleen and lung were obtained on microscopic slides. These were stained using the rapid Panoptic method and subjected to direct parasitological examination in an immersion objective, which revealed the presence of amastigote forms in all examined tissues (Figure 1B-F). Visually, the popliteal lymph node had the greatest number of amastigote forms. In every 1,000 nucleated cells, we observed 832 *Leishmania donovani* units (LDU index), an index that is considered high (REIS et al., 2006).

Samples obtained from both the necropsy and clinical examination were submitted to an extraction of total genomic DNA using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich®, United States), according to the manufacturer's recommendations. The subsequent molecular analysis was performed by PCR using primers MC1 (5'-GTTAGCCGATGGTGGTCTTG-3') and MC2 (5'-CACCCATTTTCCGATTTTG-3'), which amplified a 447 bp specific sequence for *L. (L.) infantum*, according to the methodology described by Cortes et al. (2004).

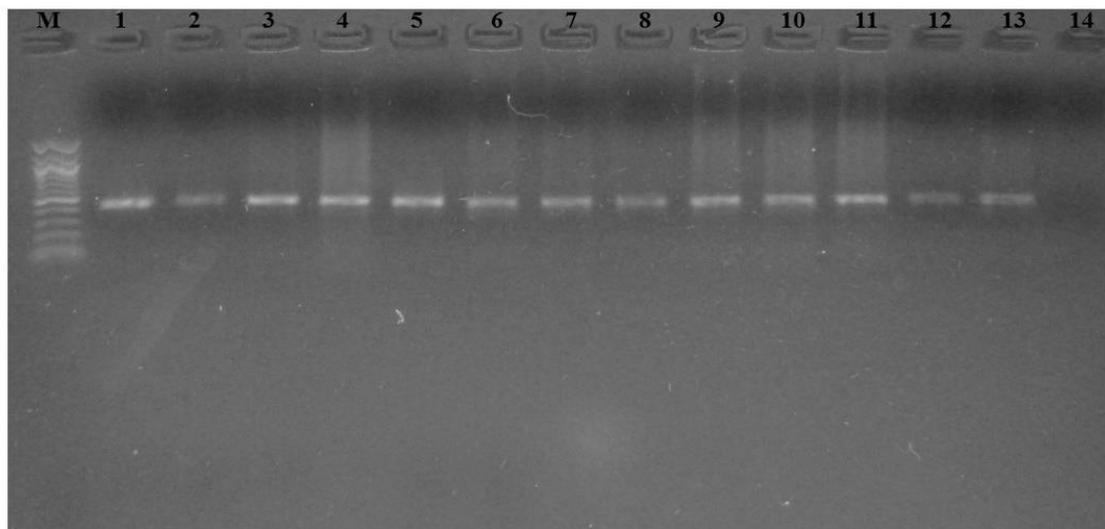
Amplification occurred in a Veriti 96 Well Thermal Cycler (Applied Biosystems, Thermo

Fisher Scientific®, United States) under the following conditions: an initial denaturation cycle of 95 °C for 5 min; 40 repeated cycles of 95 °C for 30 s, 61 °C for 30 s and 72 °C for 1 min; and a final extension cycle of 72 °C for 10 min. To read the reaction, 10 µL of the PCR products were homogenized in 2.5 µL of 2.5% bromophenol blue and applied on a 1.5% agarose gel (Agarose LE, Uniscience®, Brazil) containing 3.8 µL of ethidium bromide. Electrophoresis was conducted in an electrophoresis tub (Bio-Rad®, United States) under 140 V for 5 min, followed by a 45-min period at 110 V. Visualization was performed by means of an UV light transilluminator. Molecular analysis showed that all tested samples were positive (Figure 2).

Given the study of the presence of more *Leishmania* species, a cutaneous tissue sample was subjected to a second PCR using primers 150 (5'-GGG(G/T)AGGGGCGTTCT(C/G)CGAA-3') and 152 (5'-(G/C)(G/C)(G/C)(A/C)CTAT(A/T)TTACACCAACCCC-3'), which amplify 120 bp of the conserved region of the kinetoplast mini-circle (kDNA) of *Leishmania* spp. (PASSOS et al., 1999). The DNA of the strains *L. (L.) amazonensis* (IFA/BR/1967/PH8), *L. (V.) braziliensis* (MHOM/BR/2003/IMG) and *L. (L.) chagasi* (MHOM/BR/74/PP75) were used as positive controls.

Amplification occurred in a Mastercycler Personal (Eppendorf®, Germany) thermal cycler with an initial denaturation cycle of 94 °C for 5 min; 35 repeated cycles of 94 °C for 45 s, 59 °C for 45 s and 72 °C for 30 s; and a final extension cycle of 72 °C for 7 min. The result was visualized after electrophoresis in 6% polyacrylamide gel and 0.2% silver staining. The positive sample was submitted to restriction fragment length polymorphism (RFLP-PCR) analysis using the restriction endonuclease *Hae* III to observe the band pattern for the *Leishmania* species (VOLPINI et al., 2004). Three fragments (60 bp, 80 bp and 120 bp) were formed, demonstrating that only *L. (L.) Infantum* species were present in the tested sample.

Figure 2. Polymerase chain reaction for *Leishmania (Leishmania) infantum* in tissue specimens from a domestic feline from the municipality of Araguaína-TO. Note the following: (M) molecular weight marker of 100 bp; (1) positive control, strain P75; (2) blood; (3) conjunctive biopsy; (4) spleen biopsy; (5) liver biopsy; (6) lung biopsy; (7) kidney biopsy; (8) lymph node aspirate; (9) popliteal lymph node biopsy; (10) submandibular lymph node biopsy; (11) mesenteric lymph node biopsy; (12) deep skin scraping; (13) skin biopsy; (14) negative control. The positivity of all tested samples (wells 2 to 13) was evaluated by the distribution of the bands at a height of 447 bp.



Serum samples were submitted to an indirect immunofluorescence reaction (IFR) based on the methodology described by Pedras et al. (2008), considering a titer $\geq 1:40$ as a positive sample. The obtained titer was 1:160.

In order to obtain knowledge of a possible association between infections, the serum of the animal was also tested for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV). For this, a SNAP FIV/FeLV Combo Test (IDEXX®, United States) was used. Despite infection by *L. (L.) infantum*, the result for FIV/FeLV was negative.

In this report, the infection by the protozoan *L. (L.) infantum* was observed in a two-month-old female domestic feline, of an undefined breed. Tissues such as blood, conjunctiva, spleen, liver, popliteal, submandibular and mesenteric lymph nodes, skin, lung and kidney were all positive. Despite the presence of the parasite in all organ samples, the observed clinical signs were non-specific and differ from those reported in the literature; the literature outlines that ocular secretion

and nodular, ulcerative or crust lesions (in regions such as the nose, lips, ears and eyelids), paralysis of generalized dermatitis, hyperkeratosis, generalized ventral alopecia, nasal discharge, presence of the feline respiratory complex, uveitis, ear-tip lesions and gingivostomatitis are generally observed (NASEREDDIN et al., 2008; SHERRY et al., 2011).

Even with few clinical signs, the animal's condition rapidly evolved to death, which characterizes an acute evolution of the disease. Thus, the infection may be active in clinically normal animals or those with clinical signs, being cutaneous, ocular and/or systemic (CHATZIS et al., 2014).

Another relevant fact is the age of the feline. The possibility of infection in any age group has already been reported (SOBRINHO et al., 2012). Thus, the present description supports the fact that there is no minimum or maximum age for infection, and that the hypothesis of vertical transmission should be investigated.

With respect to the analysis performed via PCR, during the study of a case of feline leishmaniasis in Minas Gerais state, Silva et al. (2010) obtained results similar to those shown here. The authors observed positive samples from the marrow, skin, liver, spleen, popliteal lymph node and kidneys. However, it should be noted that the results of this report also include positive blood and skin scrapings obtained from the veterinary clinic office, which may be of great help in the establishment of a reliable and less invasive diagnosis using a specific PCR for *L. (L.) infantum*.

In the case of alterations evidenced by clinical pathology, normocytic anemia is frequently reported in cases of feline LV and hypoalbuminemi, and increased serum ALT activity may occur (PENNISI et al., 2015). Similarly, neutrophilic leukocytosis is observed in the clinical form of the infection (SILVA et al., 2010). Thus, these parameters may be of value for the clinical diagnosis of the disease.

Here, we described the presence of amastigote forms of *Leishmania* in 1% of the leukocytes visualized in the blood smear. Compared to this data, in a cat from Portugal, 4% of the neutrophils from the leukocyte smear contained the protozoan (PENNISI et al., 2015). Findings of this nature are relevant since they are not routine in the clinic environment.

Co-infection of *L. (L.) infantum* and FIV is frequently reported (SOBRINHO et al., 2012), but this was not observed in the studied animal, since the results for both FIV and FeLV were negative. In this way, it is possible that the death of the animal occurred solely because of multiple organ failure due to *Leishmania* infection.

This report addresses the first case of feline leishmaniasis in Tocantins state and provides an alert for veterinarians working in endemic regions or with risky transmission; it reveals that kittens can become infected and develop the disease, with rapid evolution and a high index of parasitism. When considering the use of PCR as a method

of molecular analysis, obtaining skin scrapings and blood samples is of value for a less invasive diagnosis in cats.

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