



Cytological diagnosis of *Leishmania Spp.* in a cutaneous nodule of a cat: case report

<https://doi.org/10.56238/homeIIsevenhealth-111>

Anna Karollyne Martins Rodrigues

Student of the Postgraduate Program in Animal Health and Public Health, Universidade Federal do Tocantins, UFT, Araguaína, TO, Brazil
annakrmartins@gmail.com

Gustavo Costa Freitas

Student of the Postgraduate Program in Animal Health and Public Health, Universidade Federal do Tocantins, UFT, Araguaína, TO, Brazil

Andressa Francisca Silva Nogueira

Professor Universidade Federal do Tocantins, UFT, Araguaína, TO, Brazil

Ruth Martins Santos

Student of the Postgraduate Program in Animal Health and Public Health, Universidade Federal do Tocantins, UFT, Araguaína, TO, Brazil

Helcileia Dias Santos

Professor Universidade Federal do Tocantins, UFT, Araguaína, TO, Brazil

1 INTRODUCTION

Leishmaniasis is a chronic, systemic protozoonosis that, when untreated, can progress to death in more than 90% of cases. The transmission of the disease occurs as long as there is parasitism in the skin or peripheral blood of the reservoir, through the bite of vectors infected by *Leishmania* spp. In Brazil, two species, so far, are considered vectors of the disease, *Lutzomyia longipalpis* and *Lutzomyia cruzi*. (BRASIL, 2016).

Leishmania is a protozoan belonging to the family Trypanosomatidae, divided into two subgenera: *Leishmania* and *Viannia*. The species *Leishmania (Leishmania) infantum* (synonymous with *L. chagasi*) is responsible for visceral leishmaniasis, while the species *Leishmania braziliensis*, *Leishmania amazonensis*, *Leishmania guyanensis*, *Leishmania lainsoni*, *Leishmania naiffi*, *Leishmania lindenberg* and *Leishmania shawi* are responsible for tegumentary leishmaniasis. All of the aforementioned species have been identified in states in the North and Northeast regions of Brazil (LAINSON 2010; BRASIL 2007).

It is an obligate intracellular parasite of the cells of the mononuclear phagocytic system, with two main forms: one flagellated or promastigote, found in the digestive tract of the vector insect, and another aflagellated or amastigote, observed in the tissues of vertebrate hosts (BRASIL, 2007).

The protozoan is transmitted to cats by sandflies. To date, non-vectorial transmission has not been described in cats, but blood transfusion may be a mode of infection in cats similar to what happens in humans and dogs (PENNISI 2015).



According to Batista (2020), the ability of cats to transmit *Leishmania* spp. to the dog was proven through a study, and cats are then considered important reservoirs for Leishmaniasis.

Felines generally have a low frequency of infection for *Leishmania* spp., which is believed to be due to failures in antibody detection or the fact that felines have natural resistance to leishmaniasis, probably related to genetic factors (DOS SANTOS 2018; COSTA 2010).

The disease may present in three clinical forms: mucocutaneous, cutaneous or visceral. Anorexia, lymphadenomegaly, dermatitis, uveitis, diffuse alopecia, emaciation, hyperthermia, onychogryphosis are classic clinical signs of visceral leishmaniasis in dogs. The domestic cat can be infected by several species of *Leishmania*, and may or may not be symptomatic and present nonspecific clinical signs, which commonly include nodular or ulcerated lesions on the muzzle, lips, ears and eyelids and diffuse alopecia (Pirajá et al., 2013).

CBC, biochemical profile and urinalysis are required in any suspected case to identify hyperglobulinemia, non-regenerative anemia, renal disease or other laboratory changes less frequently associated with leishmaniasis. Testing for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) is recommended in case of risk of exposure and for other concomitant diseases that alter feline immunocompetence (LeishVet 2018).

Among the forms of diagnosis for leishmaniasis there are parasitological, immunological and molecular tests. Due to the high cost, molecular tests such as Polymerase Chain Reaction (PCR) are predominant only in research. Immunological tests are the most used, such as the Indirect Immunofluorescence Reaction (RIFI) and the Immunoenzymatic Assay (ELISA) (Ruiz, 2019).

For Vides et al. (2011) and Bresciani et al. (2010) serological tests are not as effective when compared to the results obtained in dogs. Martín-Sánchez et al. (2007) observed that PCR positive animals had lower antibody titres than non-reactive animals in immunological tests.

Symptoms precede antibody production and it is assumed that the feline serologically negative by ELISA and RIFI and positive in PCR for *Leishmania* has a possibly low humoral response (Martín-Sánchez et al. 2007; Bresciani 2010).

LeishVet (2018) recommends that for diagnostic confirmation of the disease, quantitative serologic testing or western blot (immunodetection) should be performed on sera from cats with clinical signs or laboratory changes compatible with leishmaniasis.

Although cytological analyses of tissue aspirates have a high degree of specificity in diagnosis, they are rarely requested for the diagnosis of leishmaniasis by clinicians, who prefer to request serological and molecular tests first. According to Pirajá et al, (2013) and Oliveira et al, (2011), cytologies of material such as lymph node, bone marrow and suspicious cutaneous and mucocutaneous nodules have great help in closing the diagnosis.



Cytology is a low-cost test, easy to perform and fast, however, it requires knowledge on the part of the evaluator. The visualization of amastigote forms in the analyzed material allows a definitive diagnosis for leishmaniasis.

There are no published controlled studies on the therapy of feline leishmaniasis. In the absence of evidence to the contrary, empirical treatment with the same drugs recommended for dogs, allopurinol, 10 mg/kg every 12 h, or 20 mg/kg, SID, PO, for at least 6 months, is generally considered effective and apparently safe.

2 OBJECTIVE

The aim of this paper is to report the diagnosis of feline leishmaniasis by cytologic analysis of a cutaneous nodule.

3 CASE REPORT

On October 6, 2020, a 3-year-old male feline was seen at the Mundo dos Bichos Veterinary Clinic, in Araguaína do Tocantins -TO, presenting nodules in both ears, measuring approximately 1cm in diameter, in the pina region (Figure 1). According to the tutor, the nodules appeared approximately 6 months ago, without any other complaint regarding the animal's clinical condition. The feline lived on the farm, rural area of the municipality of Araguaína -TO and currently resides in the urban area of the same municipality.

Figure 1: Nodular lesions in a cat parasitized by *Leishmania* spp. A: lateral view of the right ear; B: frontal view of both ears; C: approximate view of the lesion.

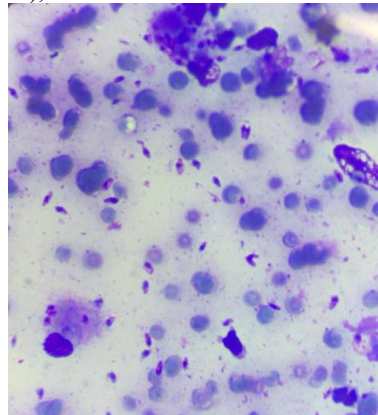


The suspicion was neoplasia, and cytology of the nodules was requested as a complementary examination. The technique used for collection was fine needle aspiration puncture (FNAP). After collection, slides were made by the squash method of the cytological material on glass slides for microscopy. The slides were stained with New Prov® rapid panoptic dye and analyzed by optical microscopy with a 100x objective and immersion oil.



RBCs, inflammatory cells, intermediate epithelial cells and small oval-shaped structures containing a nucleus and a kinetoplast were observed, compatible with amastigote forms of *Leishmania* spp. (Figure 2). Such structures were present within epithelial cells and defense cells, as well as free in the extracellular space. The cytology result was compatible with *Leishmania* spp. associated with inflammatory process.

Figure 2: Photomicrograph of cytological material from a cutaneous nodule in the ear pinna region of a cat. Presence of amastigote forms of *Leishmania* spp. (arrows), red blood cells and some cell debris.



Treatment for leishmaniasis was instituted with allopurinol tablet, 10mg/kg, every 12 hours, indefinitely and use of repellent collar, with change every 8 months.

To date, there has been no regression of the nodules in the animal, however, it is stable.

4 DISCUSSION

In Brazil, the northern region is endemic for leishmaniasis, confirmed by the high occurrence of affected dogs throughout the state of Tocantins and surrounding states (BRASIL 2016). The high prevalence of this zoonosis in the state may have contributed to the diagnosis of the disease in the cat of the reported case since the diagnosis in felines is uncommon.

According to Pennisi (2015), clinical feline leishmaniasis remains rare, even in areas where the disease is frequent in dogs. Cats are therefore thought to be more resistant than dogs to infection. According to Dos Santos (2018) and Costa (2010), unlike dogs, which have a humoral immune response, cats have a cellular response to leishmaniasis, which ensures greater resistance to infection, justifying the low number of symptomatic cats.

The domestic cat can be infected by several species of *Leishmania* spp. and may or may not be symptomatic and present nonspecific clinical signs. When the animal is symptomatic, it may present a single clinical manifestation or nonspecific clinical signs, which commonly include nodular or ulcerated lesions on the muzzle, lips, ears and eyelids, alopecia, without suffering from the disease (POLI et al., 2002; PIRAJÁ et al., 2013; NOÉ et al., 2015; METZDORF et al., 2017). Corroborating the present study, the animal had only nodules in the region of the auricular pinna, in both ears.



In addition, in felines the tegumentary form is the most common, with nonspecific cutaneous symptoms such as ulcerated or crusted lesions in the nasal plane, lips, ear pinna, eyelids (CONTIJO 2011).

However, in a case reported by Sousa (2019), in the same city as the case in question, a 2-month-old feline, diagnosed with *Leishmania (L.) infantum*, presented nonspecific clinical signs, such as diarrhea, desiccation, anemia, rapidly evolving to the animal's death. It should be considered, especially based on the literature, that felines can carry the disease and remain clinically stable and sometimes present some nonspecific symptoms.

According to Dos Santos (2018), cytologies of material such as lymph node, bone marrow, cutaneous and mucocutaneous nodules are very helpful in closing the diagnosis. Aspirates from lesions in positive animals may show the amastigote form of the parasite extracellularly or within macrophages. As observed in the present study, where the visualization of amastigote forms of *Leishmania* spp. in the cytological examination was essential for the diagnosis.

The advantages of cytology are clear and rewarding, and its use as a rapid and accurate diagnostic method is important in the routine (DOS SANTOS 2018).

Recently, albeit experimentally, it is possible to use flow cytometry for the differentiation of visceral leishmaniasis from tegumentary leishmaniasis using specific immunoglobulins (IgGs) (CONTIJO 2011).

The Polymerase Chain Reaction (PCR) is the most efficient test capable of identifying the species involved, with 100% specificity and a positive diagnosis rate almost 60% higher than those achieved with serology (CONTIJO 2011). PCR is a high-cost test and this was one of the reasons why it was not performed in the work described. The PCR test would be extremely important for the complete resolution of the case and better understanding of this disease in cats.

The treatment instituted for the patient in the case described followed that recommended in the literature, which consists of the same drugs used for dogs, allopurinol, 10 mg/kg, every 12 h, or 20 mg/kg, SID, PO, for at least 6 months (PENNISI 2015). Treatment is generally considered effective and apparently safe for cats.

5 CONCLUSION

Feline leishmaniasis is a disease that still requires many studies to better elucidate the epidemiology of the disease. Taking into account the symptomatology of the disease in cats, which in most cases is nonspecific, or these animals may present a single nonspecific skin lesion, it is necessary to suggest leishmaniasis as a differential diagnosis, and it is important to perform complementary tests. FNAB of cutaneous nodule proved to be a fast, easy and efficient method in the identification of amastigote forms for the diagnosis of feline leishmaniasis.



REFERENCES

- BATISTA, Joilson Ferreira et al. Transmission of *Leishmania infantum* from cats to dogs. *Revista Brasileira de Parasitologia Veterinária*, v. 29, n. 4, 2020.
- BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Manual de vigilância, prevenção e controle de zoonoses: normas técnicas e operacionais [recurso eletrônico] / Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância das Doenças Transmissíveis. – Brasília: Ministério da Saúde, 2016. 121 p.
- BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de Vigilância da Leishmaniose Tegumentar Americana / Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. – 2. ed. – Brasília: Editora do Ministério da Saúde, 2007. 182 p. – (Série A. Normas e Manuais Técnicos).
- BRAZ, Paulo Henrique et al. Citopatologia: Uma forma de diagnóstico em casos de tumores de pele. *Brazilian Journal of Animal and Environmental Research*, v. 3, n. 2, p. 334-344, 2020.
- COSTA, Thiago André Carreo et al. Ocorrência de leishmaniose em gatos de área endêmica para leishmaniose visceral. *Brazilian Journal of Veterinary Research and Animal Science*, p. 213-217, 2010.
- DOS SANTOS, Camila Maria et al. Análise citológica para diagnóstico de leishmaniose em um gato oligossintomático em área endêmica, Campo Grande, MS, Brasil. *Brazilian Journal of Animal and Environmental Research*, v. 1, n. 1, p. 59-71, 2018.
- RUIZ, Valeska Regina Reque. *Estudos em Patologia Veterinária*. Ponta Grossa, PR: Atena Editora, 2019.
- GONTIJO, Bernardo B. et al. Esporotricose e Leishmaniose Tegumentar em cães e gatos: semelhanças e diferenças. *Pubvet*, v. 5, p. Art. 1245-1250, 2011.
- LAINSON, Ralph et al. *Espécies neotropicais de Leishmania: uma breve revisão histórica sobre sua descoberta, ecologia e taxonomia*. 2010.
- LEISH VET - LEISHMANIOSE CANINA E FELINA. Informação concisa para o médico veterinário 4ª edição setembro 2018. <http://www.leishvet.org/wp-content/uploads/2018/09/PO-Guidelines.pdf>
- PENNISI, Maria-Grazia et al. LeishVet update and recommendations on feline leishmaniosis. *Parasites & vectors*, v. 8, n. 1, p. 1-18, 2015.
- PIRAJÁ, Gabriela Villa et al. Leishmaniose felina: revisão de literatura. *Veterinária e Zootecnia*, p. 203-216, 2013.
- ROCHA, N.S. 2008. Exame citológico no diagnóstico de lesões da pele e subcutâneo. *Clinica Veterinária*. n.76, p.76- 80.
- SOUSA, Sebastiana Adriana Pereira et al. Acute visceral leishmaniasis in a domestic cat (*Felis silvestris catus*) from the state of Tocantins, Brazil. *Semina: Ciências Agrárias*, v. 40, n. 4, p. 1723-1730, 2019.