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Research paper

Infection of *Lutzomyia longipalpis* in cats infected with *Leishmania infantum*

Ivete Lopes de Mendonça[a,*], Joilson Ferreira Batista[b], Kayo Sandro Pimentel do Prado Lopes[c], Francisco das Chagas Ribeiro Magalhães Neto[b], Diana Sousa Alcântara[b], Yslla Fernanda Fitz Balo Merigueti[d], Carlos Henrique Nery Costa[e]

[a] Federal University of Piauí, Department of Clinical and Veterinary Surgery, Teresina, PI, Brazil
[b] Federal University of Piauí, Graduate Program in Animal Science, Teresina, PI, Brazil
[c] Federal University of Piauí, Animal Health Laboratory, Teresina, PI, Brazil
[d] University of Western Paulista (UNOESTE), Veterinary Teaching-Hospital, Veterinary Parasitology Laboratory, Presidente Prudente, São Paulo, Brazil
[e] Federal University of Piauí, Department of Community Medicine, Teresina, PI, Brazil

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ABSTRACT

The clinical manifestations most frequently observed in cats with leishmaniasis caused by *Leishmania infantum* are cutaneous alterations, which suggest a high parasitic load in the skin and the possibility of infecting a vector. This study evaluated the infectiousness of to phlebotomine sand flies cats infected with *L. infantum*. A total of 12 cats with infection by *L. infantum* from the city of Teresina, Piauí, Brazil, were included in the study. Cats were diagnosed by direct visualization of the parasite. Laboratory-bred insects, free from infection by *Leishmania* spp. were offered a blood meal for 60 min on cats infected with *L. infantum*. On the fifth and sixth day after the blood meal, flies were dissected to assess promastigote forms of the parasite in the digestive system. Eight cats (67 %) were able to infect the vectors. The frequency of infected insects per cat ranged 0.0–94.4%. The mean frequency of insects feeding on cats was 95.2 %. Large numbers of the parasite were observed per insect, but were not quantified. The result confirm that cats are able to infect *L. longipalpis*, indicating that cats are part of the epidemiological chain of VL, acting as reservoir of the disease.

1. Introduction

Visceral leishmaniasis (VL) is caused by a protozoa of the genus *Leishmania*, with the species *L. infantum* most commonly infecting dogs and cats in the Old World, and in Central and South America (Pennisi and Persichetti, 2018). The infection and disease are increasingly reported in cats, mainly in areas endemic for human and canine leishmaniasis. In Brazil, there are reported cases in the city of Cotia, state of São Paulo (Savani et al., 2004), Araçatuba, São Paulo (Vides et al., 2011), Andradina, São Paulo (Coelho et al., 2011), Belo Horizonte, Minas Gerais (Silva et al., 2010), and Teresina, Piauí (Mendonça et al., 2017a,b).

Leishmaniasis in cats is not well understood and there may be important differences in the development of the disease, in comparison to dogs. In addition, research in transmission, immunopathogenesis, development, management, prevention, and therapy of the disease in cats is still in the early stages (Pennisi and Persichetti, 2018). Pennisi and Persichetti (2018) have reported that published cases of feline leishmaniasis probably represent only a minimal proportion of all diagnosed cases, and those that are diagnosed may represent only a fraction of all existing cases.

Regarding transmission of infection to cats, there is no specific information (Pennisi et al., 2015). However, with the large amount of information available regarding the vectorial transmission of *L. infantum*, there is no doubt that the infection of these animals is through the bite of infected phlebotomine sand flies, and this means that, in areas where the transmission of *Leishmania* spp. occurs in dogs, cats are likely to be in contact with the parasite and may be infected (Pennisi et al., 2015). However, transmission of the infection from cats to other animals and humans, needs to be demonstrated.

There are only two studies that have reported transmission of *L. infantum* infection from cat to a vector, in different regions. The first case was in Messina, Italy, where the infection of *Phlebotomus perniciosus* was identified by xenodiagnosis in a cat infected with *L. infantum* (Maroli et al., 2007). The second case occurred in Belo Horizonte, Minas Gerais, Brazil, where Silva et al. (2010) reported the intensity of infection by *L. infantum* in *L. longipalpis* midguts determined by xenodiagnosis in a naturally infected cat.

* Corresponding author. Present address: Department of Clinical and Veterinary Surgery, Federal University of Piauí, Teresina, PI, 65.049-550, Brazil.
E-mail address: etemendonca54@gmail.com (I.L.d. Mendonça).

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Despite reports showing the possibility of vector infection, there is a lack of information about the frequency of insects that feed when in contact with cats, and the characteristics of the animals that determine vector infection. In addition, the low efficiency of leishmaniasis control measures used in dogs, the increase in the number of feline leishmaniasis cases reported in previous years, the natural susceptibility of cats to infection by *L. infantum*, the frequent cutaneous involvement in feline leishmaniasis, indicate the need to evaluate from an epidemiological and control perspective the real importance of cats in the *Leishmania* domestic cycle (Maia and Campino, 2011). Therefore, the objective of this study was to evaluate the infectiousness to phlebotomine sand flies, feeding on cats infected by *L. infantum*, and to verify which clinical changes and characteristics of cats interfere with vector infection.

2. Materials and methods

2.1. Study site and ethics

The study was conducted in the Animal Health Laboratory (LASAN), of the Federal University of Piauí (UFPI), Teresina, Piauí, Brazil. Teresina is located at latitude 5°5′21″ S and longitude 42°48′6″ W. The study was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Piauí, under protocol number 102/2015.

2.2. Experimental design

The study included 12 cats from residences located in the city of Teresina, Piauí, obtained from a parallel study of the prevalence of feline leishmaniasis, in which 307 cats were examined and 20 were diagnosed with *L. infantum* and 12 of the infected animals the owners authorized the realization of xenodiagnosis. Animal ages ranged from 1 years and 2 months to 14 years, included males and females of various breeds, and were all positive for VL, diagnosed by visualization of the parasite in bone marrow, popliteal lymph node or skin. None of the 12 cats had been treated with anti-*Leishmania* drugs.

Polymerase chain reaction (PCR) was used to confirm *L. infantum* infection, using specific primers to amplify 300–350 bp fragments of the *L. infantum ITS1* gene, followed by restriction fragment length polymorphism (RFLP) analysis, using the *HaeIII* enzyme (Mendonça et al., 2017a). After confirming *L. infantum* infection, all animals underwent xenodiagnosis to identify possible infection of the vector.

Blood samples were used to detect feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), using the Alere Ivf Ac/FeLV Ag quick test (Bionote Inc. 2-9 Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea 440440 in accordance with the manufacturer's recommendations.

2.3. Evaluation of infection of the L. longipalpis

For xenodiagnosis, cats were sedated with 15 mg/kg of ketamine hydrochloride, 0.3 mg/kg diazepam and 0.03 mg/kg of acepromazine, administered intramuscularly. *L. longipalpis* were obtained from the sand fly colony of LASAN. A mean of 45 (range 14–83) first generation, 5 day-old females, without any food source during the 8 h prior to the test, were used for each application. The insects were placed in opaque plastic containers, approximately 5 cm in diameter and 6 cm in height, with one side open and covered with organza fabric. The containers were positioned on the outer ear skin of each animal for 60 min. (Fig. 1). This provided the sole source of blood meal for the insects.

Subsequently, the insects were placed in a bio-oxygen demand (BOD) incubator at 26 °C, with a cotton cloth soaked in a 50 % sugar solution. On the 5th and 6th day after the blood meal, the insects were dissected on a sterile slide, for microscopical assessment of promastigotes using a 40x objective (Mendonça et al., 2017b). Parasite assessment was performed on the digestive system (Fig. 2A) and proboscis of the insect, and the *L. longipalpis* species was confirmed by morphological characteristics of the spermathecae (Fig. 2B).

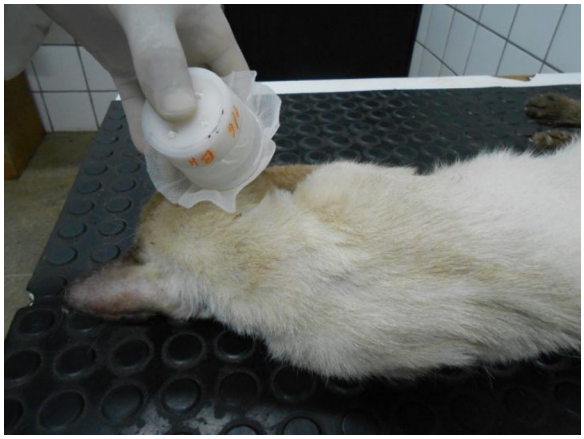

Fig. 1. Xenodiagnosis performed on the back of the ear, in a cat infected with *Leishmania infantum*.

2.4. Data analysis

A possible relationship between the number of clinical signs and the frequency of infected insects was assessed with the Spearman correlation test, using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) with an error rate of 5%.

3. Results

Of the 12 cats with leishmaniasis, eight (67 %) were shown to have infected the vector. Age of the animals ranged from 1 y and 2 mos to 14 y (mean 5.8 y), 8 were male, 11 were without defined breed, and one was Siamese. All had clinical signs suggestive of VL. The more frequent changes were enlarged lymph nodes, alopecia, and skin lesions (Fig. 3).

The 12 animals were submitted to xenodiagnosis and eight (67 %) infected the vector. For the 12 cats assessed, a total of 542 insects were used, of which 158 became infected (29.2 %). The frequency of feeding insects per animal ranged from 87 % to 100 % (mean 95.2 %) and the infection frequency of insects ranged from 0% to 94 % (mean 35.3 %) (Table 1).

Assessment of the 12 cats submitted to xenodiagnosis revealed that all animals with *L. infantum* in bone marrow and skin infected the vector (Table 2). Therefore, this group of cats constitutes a risk for disease transmission to other susceptible animals and humans.

Regarding the clinical signs related to xenodiagnosis, 85.7 % of the animals that presented more than three clinical changes, infected the vector (Table 2). There was a significant positive correlation between the number of clinical signs and the frequency of infected insects (Spearman correlation $p = 0.03$). Infection with FIV had no influence on the infection of the vector (Table 2). All 12 cats were negative for FeLV.

Furthermore, insects had a high load of *L. infantum*. The rupture of the digestive system caused the release of large amounts of parasite, although it was not possible to quantify these with optical microscopy (Fig. 4, Video 1 and Video 2).

In the molecular identification of *Leishmania* sp. isolated from the cats submitted to xenodiagnosis, it was observed that the fragments (187 bp, 72 bp and 55 bp) generated after the PCR and RFLP, using the parasite DNA isolated from all 12 cats were compatible with *L. infantum*, etiologic agent of VL in dogs and humans in Brazil.

4. Discussion

This study demonstrated that 67 % of cats infected by *L. infantum*

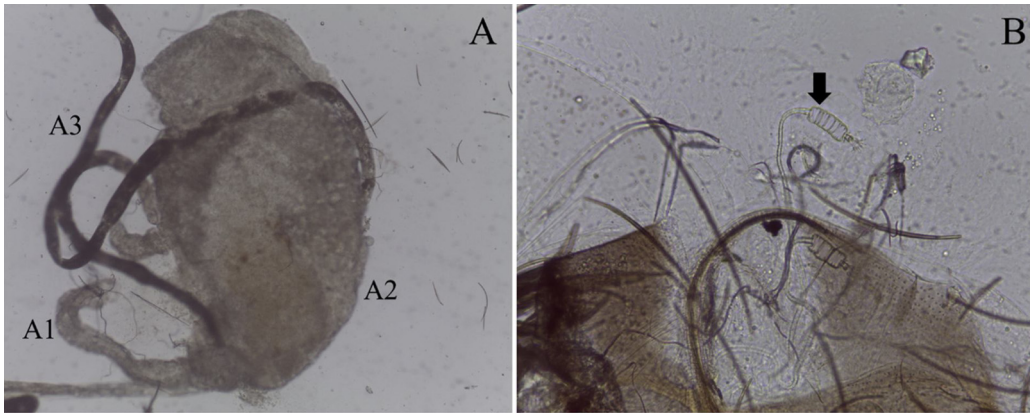

Fig. 2. Anatomical segments of insect vector submitted for xenodiagnosis in cats infected with *Leishmania infantum*. Legend: A - Digestive system. A1 - Anterior intestine. A2 - Stomach and A3 - Hindgut. B - Spermatheca presenting morphological characteristics compatible with the species *Lutzomyia longipalpis* (arrow).

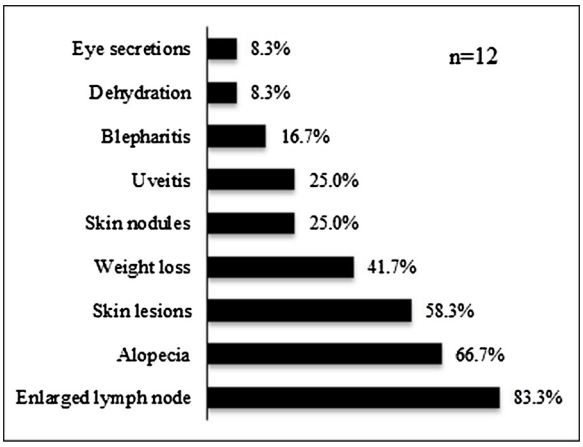

Fig. 3. Percentage of clinical signs observed in 12 cats with leishmaniasis.

Table 1
Xenodiagnosis result performed on the 12 cats infected by *Leishmania infantum* using the *Lutzomyia longipalpis* vector.

	Total	Mean per animal	Minimum	Maximum
Insects used (n)	542	45.2	38	64
Insects feeding n (%)	516 (95.2)	43 (95.2)	32 (87)	64 (100)
Infected insects n (%)	158 (29.2)	13.2 (35.3)	0 (0)	31 (94)

were able to infect a vector. Up until completion of this study, only two other studies had demonstrated the possibility of phlebotomine sand fly infection with *L. infantum*. These studies were carried out in Messina in Italy (Maroli et al., 2007), and Belo Horizonte, Minas Gerais, Brazil (Silva et al., 2010), although in both studies only one animal was assessed.

Due to the number of animals used in this study, it was also possible to analyze the characteristics of cats that were capable of infecting the vector. One of the findings is that in all animals in which *L. infantum* was found in the skin, the vector was infected. Animals without *L. infantum* in the skin also had negative xenodiagnosis results. Furthermore, 85.7 % of the animals that had more than three clinical signs, and 40 % of those who had fewer than three clinical signs suggestive of VL, were able to infect the vector. Moreover, a high percentage of infected insects per animal (21.7 %) was observed. These findings are highly relevant and show that cats are able to participate actively in the transmission of *L. infantum*, in endemic areas.

The identification of *L. infantum* in the skin, and the observation of clinical changes in the animals were efficient alternatives for

Table 2
Clinical characteristics and infection related to the outcome of xenodiagnosis in cats infected by *Leishmania infantum*.

Characteristics		Xenodiagnosis		
		Examined (n = 12)	Positive (n = 8)	Frequency (%)
Age	Young (< 3 y)	3	3	100
	Adult (3–8 y)	5	3	60.0
	Senile (> 8 y)	3	1	33.3
Sex	Male	8	6	75.0
	Female	4	2	50.0
Breed	WDB	11	7	63.6
	Siamese	1	1	100
Number of clinical signs	≤3	5	2	40.0
	> 3	7	6	85.7
L. infantum in bone marrow	Yes	8	8	100
	No	4	0	0
L. infantum in lymph node	Yes	12	8	66.7
	No	0	0	0
L. infantum in skin	Yes	8	8	100
	No	4	0	0
FIV	Positive	3	2	66.7
	Negative	9	6	66.7

WDB - Without defined breed; FIV - Feline Immunodeficiency Virus.

identifying cats capable of infecting a vector. However, studies using dogs and humans as reservoirs have presented alternatives for detecting infection hosts for sand flies (Mendonça et al., 2017a,b; Silva et al., 2016), that can be used to verify the infection performance in cats. One of these is the detection of parasitemia, using quantitative polymerase chain reaction (qPCR). When used in humans, this method demonstrated that patients with the parasite in their blood had a high probability of infecting a vector (Silva et al., 2016). Serological testing has also been employed. In dogs from areas with high transmission rates, serological tests fail to discriminate dogs with visceral leishmaniasis that transmit *L. infantum* to the vector *L. longipalpis* (Mendonça et al., 2017b). However, results may prove different in cats.

Two xenodiagnostic studies in dogs, also performed in Teresina, reported a proportion of infected insects per animal of only 1.0 % (Soares et al., 2011), and 5.8 % (Mendonça et al., 2017b), both using a similar methodology to that of the present study. Comparing the xenodiagnostic findings in dogs, with those found in cats in the present study (21.7 %), it is evident that cats have a much higher capacity to infect a sand flies. In the studies conducted in Teresina, the percentage of dogs that were able to infect the vector, 29 % (Soares et al., 2011) and 46.5 % (Mendonça et al., 2017b), was also lower than that observed in cats in the present study (67 %). These results confirm that cats are important in the maintenance of the high VL rates in the city of

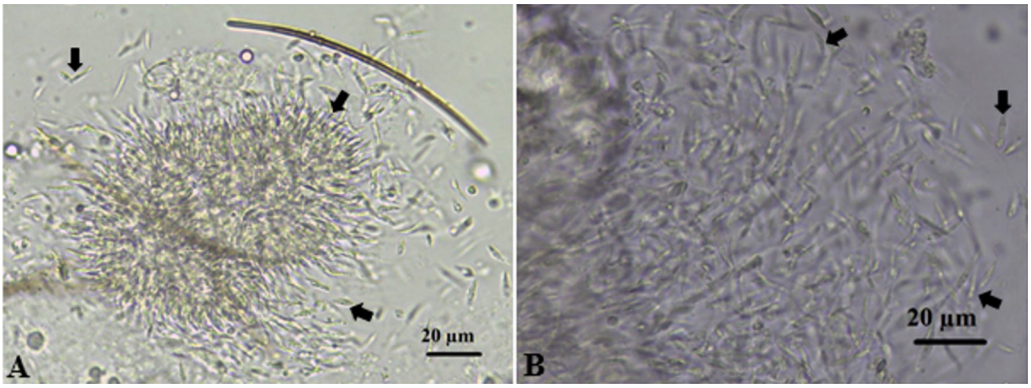

Fig. 4. Promastigote *Leishmania infantum* in the digestive system of *Lutzomyia longipalpis*, 6 days after xenodiagnosis in a cat infected with *L. infantum*. A- *L. infantum* release due to the rupture of the anterior intestine of the insect. B - *L. infantum* freed from the stomach of the vector after rupture of the stomach.

Teresina.

The identification of cats infected with *Leishmania* spp., especially *L. infantum*, and the possibility of infection of phlebotomine sand flies with this parasite, as identified by xenodiagnosis in naturally infected cats in Teresina, state of Piauí, Brazil (an area of high transmission of VL), should encourage the authorities to seek new measures to control the disease. The euthanasia of seropositive dogs alone (the main measure adopted by the Brazilian Ministry of Health for the control of human and canine VL) may not be enough to control the disease in endemic areas, and the importance of cats as reservoirs should be taken into account.

In conclusion, we demonstrated that domestic cats infected with *L. infantum* are able to infect the biological vector *L. longipalpis* and are part of the transmission cycle of VL. The disease is zoonotic, and the species of parasite found in cats is responsible for causing leishmaniasis in dogs and humans, posing a serious threat to public health.

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Declaration of Competing Interest

No competing interests to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vetpar.2020.109058.

References

Coelho, W.M., Richini-Pereira, V.B., Langoni, H., Bresciani, K.D., 2011. Molecular detection of *Leishmania* sp. in cats (*Felis catus*) from Andradina Municipality, São Paulo State, Brazil. Vet. Parasitol. 176, 281–282.

Maia, C., Campino, L., 2011. Can domestic cats be considered reservoir hosts of zoonotic leishmaniasis? Trends Parasitol. 27, 341–344.

Maroli, M., Pennisi, M.G., Di Muccio, T., Khoury, C., Gradoni, L., Gramiccia, M., 2007. Infection of sandflies by a cat naturally infected with *Leishmania infantum*. Vet. Parasitol. 145, 357–360.

Mendonça, I.L., Batista, J.F., Ribeiro, I.M.M., Rocha, F.S.B., Silva, S.O., Melo, M.N., 2017a. *Leishmania infantum* in domestic cats from the municipality of Teresina, state of Piauí, Brazil. Parasitol. Open 3, E1.

Mendonça, I.L., Batista, J.F., Werneck, G.L., Soares, M.R.A., Costa, D.L., Costa, C.H.N., 2017b. Serological tests fail to discriminate dogs with visceral leishmaniasis that transmit *Leishmania infantum* to the vector *Lutzomyia longipalpis*. Rev. Soc. Bras. Med. Trop. 50, 483–488.

Pennisi, M.G., Persichetti, M.F., 2018. Feline leishmaniosis: is the cat a small dog? Vet. Parasitol. 251, 131–137.

Pennisi, M.G., Cardoso, L., Baneth, G., Bourdeau, P., Koutinas, A., Miró, G., Oliva, G., Solano-Gallego, L., 2015. LeishVet update and recommendations on feline leishmaniosis. Parasit. Vectors 8, 1–18.

Savani, E.S., de Oliveira Camargo, M.C., de Carvalho, M.R., Zampieri, R.A., dos Santos, M.G., D'Auria, S.R., Shaw, J.J., Floeter-Winter, L.M., 2004. The first record in the Americas of an autochthonous case of *Leishmania* (*Leishmania*) *infantum chagasi* in a domestic cat (*Felix catus*) from Cotia County, São Paulo State, Brazil. Vet. Parasitol. 120, 229–233.

Silva, S.M., Rabelo, P.F., Gontijo, N.F., Ribeiro, R.R., Melo, M.N., Ribeiro, V.M., Michalick, M.S., 2010. First report of infection of *Lutzomyia longipalpis* by *Leishmania* (*Leishmania*) *infantum* from a naturally infected cat of Brazil. Vet. Parasitol. 174, 150–154.

Silva, J.C., Zacarias, D.A., Silva, V.C., Rolão, N., Costa, D.L., Costa, C.H., 2016. Comparison of optical microscopy and quantitative polymerase chain reaction for estimating parasitaemia in patients with kala-azar and modelling infectiousness to the vector *Lutzomyia longipalpis*. Mem. Inst. Oswaldo Cruz 111, 517–522.

Soares, M.R., de Mendonça, I.L., do Bonfim, J.M., Rodrigues, J.A., Werneck, G.L., Costa, C.H., 2011. Canine visceral leishmaniasis in Teresina, Brazil: relationship between clinical features and infectivity for sand flies. Acta Trop. 117, 6–9.

Vides, J.P., Schwardt, T.F., Sobrinho, L.S., Marinho, M., Laurenti, M.D., Biondo, A.W., Leutenegger, C., Marcondes, M., 2011. *Leishmania chagasi* infection in cats with dermatologic lesions from an endemic area of visceral leishmaniosis in Brazil. Vet. Parasitol. 178, 22–28.