

***Leishmania infantum* (syn. *chagasi*) INFECTION IN CATS FROM A BRAZILIAN  
SOUTHEASTERN ENDEMIC AREA FOR CANINE AND HUMAN  
LEISHMANIASIS**

Luciana C Baldini-Peruca<sup>1</sup>  
Simone Baldini Lucheis<sup>2</sup>  
Rozeani O Tome<sup>1</sup>  
Virgínia Bodelão Richini-Pereira<sup>3</sup>  
Ana Paula F. L. Correa<sup>1</sup>  
Marcella Z Troncarelli<sup>1</sup>  
Rodrigo Costa da Silva<sup>4</sup>  
Helio Langoni<sup>1\*</sup>

**ABSTRACT**

Visceral leishmaniasis (VL) is an important zoonosis caused by *Leishmania infantum* (syn. *chagasi*) in Americas and transmitted by *Lutzomyia longipalpis*, genus phlebotomines. The diagnosis of *Leishmania* infection in cats is very important to better comprehend the role of felines on LV's epidemiological chain. This study aimed to determine the occurrence of *L. infantum* (syn. *chagasi*) in 109 cats from Birigui, SP, Brazil, an endemic area for canine and human leishmaniasis, by using Indirect Fluorescent Antibody Test (IFAT), hemoculture and Polymerase Chain Reaction (PCR). Fifty two (47.7%; CI95% 38.6-57.0%) samples were positive by the hemoculture, three (2.8%; CI95% 1.0-7.8%) by IFAT and 17 (15.6%; CI95% 10.0-23.6%) by PCR. According to the results, we can conclude that cats from endemic areas for the infection can be frequently exposed to the agent and suggest the importance of these animals in the epidemiological cycle, demonstrating the need for further research and diagnostic alternatives to aid in the establishment of preventive steps to control this disease.

**Keywords:** cats, leishmaniasis, IFAT, hemoculture, PCR.

**INFECÇÃO POR *Leishmania infantum* (syn. *chagasi*) EM GATOS PROVENIENTES  
DE UMA ÁREA ENDÊMICA PARA LEISHMANIOSE CANINA E HUMANA, NA  
REGIÃO SUDESTE DO BRASIL**

**RESUMO**

Leishmaniose visceral (LV) é uma importante zoonose causada por *Leishmania infantum* (syn. *chagasi*) nas Américas e transmitida por flebotomíneos do gênero *Lutzomyia*. O diagnóstico da infecção por *Leishmania* em gatos é muito importante para a melhor compreensão do papel dos felinos na cadeia epidemiológica da LV. Este estudo objetivou determinar a ocorrência de *L. infantum* (syn. *chagasi*) em 109 gatos domésticos de Birigui-SP, Brasil, uma área endêmica para leishmaniose canina e humana, utilizando o Teste de Imunofluorescência Indireta (IFI), hemocultura e Reação em Cadeia pela Polimerase (PCR). Cinquenta e dois (47,7%; CI95% 38.6-57.0%) amostras foram positivas pela hemocultura; três (2.8%; CI95% 1.0-7.8%) pela IFI e 17 (15.6%; CI95% 10.0-23.6%) pela PCR. De acordo

<sup>1</sup> School of Veterinary Medicine and Animal Science, UNESP Univ Estadual Paulista, Department of Veterinary Hygiene and Public Health, Botucatu SP, Brazil.

<sup>2</sup> São Paulo Agency of Agribusiness Technology, Bauru, São Paulo, Brazil.

<sup>3</sup> Adolfo Lutz Institute, Regional Laboratory of Bauru, Bauru SP, Brazil.

<sup>4</sup> Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State MS, USA.

\* Corresponding author: hlangoni@fmvz.unesp.br

com os resultados apresentados, pode-se concluir que gatos provenientes de áreas endêmicas para leishmaniose podem estar frequentemente expostos ao agente e sugerem a importância desses animais no ciclo epidemiológico, demonstrando a necessidade de pesquisas e alternativas de diagnóstico para auxiliar no estabelecimento de medidas preventivas de controle da doença.

**Palavras-chave:** gatos, leishmaniose, RIFI, hemocultura, PCR.

## LA INFECCIÓN POR *Leishmania infantum* (syn. *chagasi*) EN LOS GATOS DE UNA ZONA ENDÉMICA PARA LEISHMANIASIS CANINA Y HUMANA EN EL SURESTE DE BRASIL

### RESUMEN

La leishmaniasis visceral (LV) es una zoonosis importante causada por *Leishmania infantum* (syn. *chagasi*) en las Américas, y transmitida por flebótomos del género *Lutzomyia*. El diagnóstico de la infección por *Leishmania* en los gatos es importante para una mejor comprensión del papel de los gatos en la cadena epidemiológica de LV. Este estudio tuvo como objetivo determinar la presencia de *L. infantum* (syn. *chagasi*) en 109 gatos de Birigui - SP, Brasil, una zona endémica de leishmaniasis canina y humana, mediante la prueba de inmunofluorescencia indirecta (IFI), hemocultivo y la Reacción en Cadena de la Polimerasa (PCR). Cincuenta y dos (47,7% ; IC95 % 38,6-57,0 %) muestras fueron positivas por el hemocultivo, tres (2,8 % ; IC 95 % 1,0-7,8 %) por el IFI y 17 (15,6 % ; IC95 % 10,0-23,6 %) por PCR. De acuerdo con los resultados presentados, puede concluir que los gatos de zonas endémicas para la infección pueden ser expuestos con frecuencia al agente y sugieren la importancia de estos animales en el ciclo epidemiológico, lo que demuestra la necesidad de investigación y alternativas diagnósticas para ayudar en el establecimiento de medidas preventivas para controlar esta enfermedad.

**Palabras clave:** gatos, leishmaniasis, IFI, hemocultivo, PCR.

### INTRODUCTION

Leishmaniasis have a large impact on the public health, being endemic in 98 countries or territories, with more than 350 million people at risk, affecting humans, as well as wild and domestic animals including dogs and cats (1). Among the domestic animals, dogs are involved in domestic transmission to humans, especially in cases of infection by *Leishmania chagasi* (2). However, the accelerated urbanization of the infection, other domestic species were found in the epidemiology in endemic foci, including the cat (3).

Several diagnostic methods have been used for the diagnosis of Visceral Leishmaniasis (VL) in dogs and humans (4). Hemoculture is a parasitological test that requires the presence of parasites in a sufficient amount to be visible in an optical microscope. Such parasitological exams enable the visibility of amastigotes of the parasite, even inside macrophages or in a free form (5). Serological exams such as the enzyme-linked immunosorbent assay (ELISA) and the indirect fluorescent antibody test (IFAT) are indirect diagnostic tests, detecting antibodies to *Leishmania* spp. Polymerase chain reaction (PCR) is a sensitive and highly specific method for detecting the DNA of the parasite (6,7).

According to the Information System for Reportable Diseases (8), in 2009 and 2010 there were 23 and 20 human VL cases reported and confirmed, respectively, in the city of

Araçatuba, 11 km from Birigui, an endemic area for canine and human visceral leishmaniasis. In Birigui, only one human VL case was reported and confirmed in 2009 and three in 2010.

It is important to know the role of the cat in the epidemiological chain of VL; therefore, we proposed to determine the prevalence of VL in cats from a Brazilian endemic area.

## MATERIAL AND METHODS

### Sampling

Blood samples were collected by cephalic venipuncture from 109 domesticated cats, (46 males and 63 females) randomly selected, without preference for age and breed. This material was assayed for all testes used in this study. The blood was divided into vacutainer flasks (BD Vacutainer®, USA), with and without EDTA. The blood samples with EDTA were separated in aliquots and processed for hemoculture and molecular methods. Those samples without EDTA were immediately centrifuged at 1600g for 15 minutes, and the serum samples were kept frozen at 20°C until the moment of serological test.

All cats from 14 residences were submitted to clinical exam, before the blood collection. They were examined for the detection of weight loss, lymphadenopathy, hepatomegaly and splenomegaly, and information about health management (worming, vaccination and castration). Epidemiological questions were also applied. These questions were related to the residential zone of the animal, physiological status, living environment, feeding type, nocturnal habits and contact with other animals from the same and other houses.

### Hemoculture

Three tubes with 5mL of sterile liver infusion tryptose medium (LIT) were used in a laminar flow chamber. The plasmatic portion was removed with a 1ml sterile syringe, and transferred slowly to the first tube. This procedure was repeated for the leukocytes portion, which was transferred to the second tube and, equally for the erythrocyte sediment, to the third tube. All tubes were identified with the respective number of animals. The cultures were kept in an incubator at 28-30°C, until four months after the inoculation, when they were submitted to the molecular technique.

Ten days after inoculation of the sample, the first reading was carried out, taking five microliters from each tube of inoculated culture, placing it between the coverslip and slide, and performing the reading of at least five slides per tube. The observation was made in an optical microscope using 400X magnification. Positive samples were defined at those presenting at least one flagellated structure similar to Trypanosomatidae. The cultures were observed every two weeks, for four months. The positive cultures were immediately processed for extraction of parasitic DNA, as were the negative cultures, after the end of four months of monitoring the readings (9).

### Serology

The research for *Leishmania* spp. IgG antibodies was performed by the indirect Fluorescent Antibody Test (IFAT), according to Camargo (10). using a cut-off titer as 40 (11). Additionally, a goat anti-cat IgG (h&l) antibody FITC conjugated, A20-120F (Erviagas, Brazil) was used. For the test, *L. major* antigen, kept by week passages at the Zoonoses Research Nucleus (NUPEZO), FMVZ - UNESP, was used (12). Positive and negative controls were also used. Positive reaction was considered when at least 50% fluorescent promastigotes per field was found, presenting fluorescence around the entire membrane.

### Polymerase Chain Reaction (PCR)

DNA was extracted from hemoculture samples using the commercial kit Blood Genomic Prep Mini Spin (GE Healthcare, USA) according to the instructions of the manufacturer, with some adaptations and the DNA concentration was measured by NanoVue™ (GE Healthcare, USA).

Each 0.2µL microtube was prepared with the PCR mixture composed by 1X PCR buffer (10mM Tris HCl pH 8.0, 50mM KCl), 1.5mM MgCl<sub>2</sub>, 0.2mM dNTP, 10µM of each primer, 0.5U Platinum *Taq* DNA polymerase, 10ng DNA template, in a total volume of 25µL. For specific detection of *L. chagasi*, the species-specific primers LC14 (5'-CGCACGTTATATCTACAGGTTGAG-3') and LC15 (5'-TGTTTGGGATTGAGGTAATAGTGA-3'), directed to minicircle kinetoplast DNA (kDNA) of *L. chagasi* was used (FMVZ-UNESP). The amplification protocol was: 94°C for 4min, 40 cycles at 94°C for 30s, 59°C for 30s, and 72°C for 30s, and a final extension at 70°C for 10min (13,14).

All amplifications were performed in a MasterCycler EP gradient (Eppendorf, USA). Negative controls were composed by DNA extraction ultrapure water and DNA extraction *T. cruzi* that was added to the mix-PCR. The sequences were analyzed by electrophoresis in 1.5% agarose with ethidium bromide (10mg.mL<sup>-1</sup>) gel stained (GIBCO, USA), and visualized in an image analyser, GelDoc-IT™ Imaging System (UVP, USA), by using VisonWorks®LS Software.

### Statistical analysis

All epidemiological data and results of the IFAT, hemoculture and PCR were inserted in an Excel spreadsheet. The associations between epidemiological variables and the PCR results were analyzed by chi-square ( $\chi^2$ ) or Fisher exact test, considering the significance level ( $\alpha$ ) of 7% (15). Statistics related to the performance of results obtained by IFAT, hemoculture and PCR were calculated by the use of PCR as the gold-standard, using the spreadsheet described by Mackinnon (16), considering  $\alpha$  of 5%. All tests were performed in EpiInfo™ v.3.5.1 software.

## RESULTS

Seventeen samples (15.6%; CI95% 10.0-23.6%) were positive by PCR. Of the 109 blood samples submitted to hemoculture, 52 (47.7%; CI95% 38.6-57.0%) were positive. The values for sensitivity and specificity were 88.2 and 59.8%, respectively, with a fair concordance ( $\kappa = 26.1\%$ ).

Only three serum samples (2.7%; CI95% 1.0-7.8%) presented specific antibodies for *Leishmania* spp. by the IFAT, with titer 40. The respective sensitivity and specificity values from IFAT, were 11.8 and 98.9%. The observed concordance was slight ( $\kappa=16.1\%$ ).

Analyzing the clinical signs, 8/109 (7.4%; CI95% 3.8-13.8%) presented emaciation, 1/109 (0.9%; CI95% 0.2-5.0%) hepato/splenomegaly and 100/109 (91.7%; CI95% 85.0-95.6%) did not present any clinical alteration. From eight cats with emaciation, three (37.5%; CI95% 13.7-70.1%) presented *L. chagasi* DNA, one (12.5%; CI95% 2.8-48.3%) was seropositive by IFAT and four (50%; CI95% 21.2-78.8%) were positive in hemoculture. Fourteen (14%) cats positive by PCR, two (2%) positive by IFAT and 57 (57%) positive by hemoculture were asymptomatic.

The results on vaccination, worming, house area location, sterilization, environment in which the cat lives, food type, nocturnal habits and contact with other animals from the same house did not present significant difference ( $P > 0.05$ ) compared to PCR results. In other way, the variable "contact with animals from other houses" presented significant difference, with OR = 2.9 ( $P = 0,06$ ).

## DISCUSSION

The absence of a significant difference between leishmaniasis and most of the epidemiological questions suggests little interference between susceptibility to the agent and the environment, lifestyle or cat diet type. Concerning to the contact with animals from other houses, it was observed that cats with this type of contact have 2.9 more chances (OR=2.9;  $P=0.06$ ) to be infected, with a PCR positive result, than the others. This data emphasizes the problem of public health when an owner or the neighborhood do not adopt sanitary measures for the destination of the organic materials, being accumulated, attracting the sand fly vector, *Lutzomyia longipalpis*, that increases the susceptibility of the animals and the humans from surrounding area.

Although hemoculture has emerged as an alternative for improving the positivity of indirect parasitological methods, Passos et al. (17) did not obtain success in culture in NNN from a lesion biopsy fragment and, interdigital region of a cat. Savani et al. (18) did not detect parasites in preparations of liver and spleen fragments inoculated in blood agar with brain-heart infusion. Nevertheless, Souza et al. (19) observed innumerable promastigote forms in NNN cultures aspirated from nodules located in the region of the nose, ears and interdigital area of a feline. The greatest problem is the contamination by bacteria and/or fungi and, due to this, it is a technique that requires aseptic conditions for the collection and handling of blood samples, in addition to such other limitations as prolonged time until the final result (four months) (20,21,22).

Fifty-two (47.7%; CI95% 38.6-57.0%) samples showed flagellated structures similar to leishmania. The flagellated forms observed in the 37 samples found negative by PCR and in the 51 negative by IFAT may be from other non-*Leishmania chagasi* Trypanosomatidae, due to the phylogenetic proximity among these parasites, namely belonging to the same family, since similar morphological presentation in culture media is also registered by Luciano et al. (23). Furthermore, the hemoculture possesses low sensitivity, and is capable of generating negative or false-negative results, particularly when the parasitemia is low, which hinders the visualization of parasites by optical microscopy. However, the present study obtained an elevated sensitivity value. The hemoculture sensitivity and specificity observed by Braga (24) were 69.4% and 71.4%, respectively.

From the 57 negative hemocultures, 55 presented negative results equivalent to those obtained by PCR, with the hemoculture technique presenting 59.8% specificity and 88.2% sensitivity. Only two cats were negative by hemoculture and positive by PCR, on account of the two samples not showing flagellated Trypanosomatidae, although *Leishmania* spp. antibodies were detected by IFAT (titer 40). Both results stem from the low parasite load at that moment of sampling.

The seropositivity in only 2.7% cats was superior not only to the data reported by Figueiredo et al. (13) who observed 0% positive results in serum samples of 43 cats from Barra Mansa, RJ, Brazil, by IFAT, but also the data observed by Poli et al. (25) in an Italian study with 0.9% positivity in 110 felines by IFAT. In Campo Grande, MS, Brazil, Braga<sup>3</sup> reported a seroprevalence of 30% by IFAT in 50 feline blood samples, with titers varying from 40 to 320, compared to Oliveira et al. (26) that found 45/89 (50.6%; CI 95% 40.3-60.7%) positive cats in the same State. Da Silva et al. (3) utilizing IFAT to examine eight serum samples from cats, obtained two (25.0%) samples positive for *Leishmania* spp., with titers ranging from 40 to 320. Maroli et al. (27) detected seropositivity of 10.7 and 13.0% by IFAT in blood samples from cats in two studies carried out in Fortaleza (Ceará state, Brazil). In Europe, specifically the southern region of Spain, in 2007, Martín-Sánchez et al. (28) found 28.3% seroprevalence by IFAT in 183 cats examined. Maia et al. (29) observed 17.3% seropositivity in 23 cats evaluated in Portugal. In Spain, Ayllon et al. (30) detected *Leishmania* spp. antibodies by

IFAT in ten of the 233 cat blood samples evaluated, with seroprevalence of 4.3%, a percentage higher than that observed in the present study.

Analyzing the three positive feline blood samples by IFAT, only two (66.6%) was positive by PCR. This is due to the probable occurrence of an infection or exposition by another *Leishmania* spp. or non-*Leishmania* spp. Cross reactions between *Trypanosoma cruzi* and *Leishmania* spp, detected by IFAT are reported in diverse studies (23,30,31,32).

One hundred and six samples were not reactive by IFAT for *Leishmania* spp., of which fifteen (14.2%; CI95% 8.8-22.1%) presented the DNA of the parasite *L. chagasi*. The sensitivity and specificity of IFAT were 11.8 and 98.9%, respectively, with PCR being considered the gold-standard. Braga (24) obtained a lower specificity value (70%) upon evaluating 50 cats in Campo Grande, MS, Brazil, as well as 80.6% sensitivity and 78.6% specificity by IFAT among 50 dogs from the same region. Costa et al. (33) evaluated 200 cats in Araçatuba, SP, Brazil, another endemic region for canine and human VL, and observed an 11.5% prevalence of the infection by the ELISA with sensitivity and specificity values of 25 and 89%, respectively, utilizing the direct parasitological exam as the gold-standard. These researchers concluded that the immune response to the *Leishmania* spp. infection in cats differs from that observed in dogs, whose sensitivity and specificity values in the serological methods are greater, which may explain the small number of cats that were infected and symptomatic (33).

The serology of infected cats is generally less specific than in dogs, since the production of antibodies for *Leishmania* spp. is lower, enabling the persistence of seronegativity. Furthermore, the appearance of antibodies leads to a variable period after the establishment of the infection. Nevertheless, they become distinctly elevated after the development of clinical signs or starting from the moment at which it is possible to isolate the parasite. It must also be taken into account that a significant portion of the population never develops high titers in response to the infection and neither becomes clinically sick nor transmits the parasite (34).

Although the serology validates the diagnosis of canine VL, the detection of circulating *Leishmania* spp. antibodies in cats is not equally observed in this species and, therefore, is not sufficient to determine *Leishmania* spp. infection in cats (35). Furthermore, the low specificity of IFAT observed in the present study suggests that this technique without association with the molecular test, can underestimate the true number of infected cats, thereby facilitating the transmission of the parasites. Serological surveys carried out by different techniques demonstrated that the prevalence of *Leishmania* spp. antibodies in cats assayed throughout the world ranged from zero to 68% (27). Feline studies that determine sensitivity and specificity values by IFAT are scarce. Thus, it becomes necessary to augment research studies that aim to associate the serological and molecular diagnostic methods in order to elucidate sensitivity and specificity of techniques for cats.

The DNA of *L. chagasi* was detected in 17/109 (15.6%; CI95% 10.0-23.6%) cat blood samples. This result is markedly lower than the findings of Maia et al. (28) who observed the presence of *Leishmania* spp. DNA in 7/23 (30.4%; CI95% 15.6-51.1%) cat blood samples. On the other hand, it is close to the report by Maia et al. (28) that detected *L. infantum* DNA in 28/138 (20.3%; CI95% 14.4-27.8%) cats in Lisbon, Portugal. Ayllon et al. (29) detected DNA of *L. infantum* in only 1/233 (0.4%; CI95% 0.1-2.4%) cats.

Out of the 92 cats negative by PCR only one (1.1%; CI95% 0.3-5.8%) was positive by IFAT and 37 (40.2%; CI95% 30.8-50.5%) by hemoculture. These results for culture and PCR may occur due the cross-reactions with other *Leishmania* spp. and non-*Leishmania* spp. Trypanosomatidae.

In 17 blood samples of the 109 cats evaluated, *L. chagasi* DNA was detected, of which 15 (88.2%; CI95% 65.3-96.4%) tested positive in hemoculture and two (11.8%; CI95% 3.6-34.7%) presented *Leishmania* spp. antibodies. Coelho et al. (6) reported the first case of feline

leishmaniasis in Andradina, SP, Brazil, another endemic area for canine and human VL, in which *L.chagasi* DNA was detected from aspiration cytology of the popliteal lymph node. Despite detecting DNA from the parasite by PCR, the IFAT was not reactive for *Leishmania* spp. The difficulty of to observe the parasite in an optical microscope, from hemoculture samples and the probable resistance of cats to the agent, justify the results observed in this study.

Costa et al. (33) found clinical alterations in 25% of the cats seropositive for *Leishmania* spp. Portús et al. (36) registered that the number of cats infected and symptomatic is small, corroborating with these and our data. There was a statistically significant difference only between the results from asymptomatic animals and those obtained by IFAT and PCR.

## CONCLUSIONS

The results indicate the occurrence of *Leishmania chagasi* in cats from Birigui/SP, an endemic area for canine and human visceral leishmaniasis, where most of the positive cases were associated with the contact with animals from other houses, which increases the risk for public health.

The association of the three diagnostic methods proved to be crucial for improving the diagnostic accuracy of leishmaniasis in the studied cats.

It is suggested that more studies are necessary to determine the true role of cat in the epidemiological cycle of visceral leishmaniasis.

## ACKNOWLEDGEMENTS

The authors thank the National Council for Scientific and Technological Development (CNPq) for providing the Masters scholarship, and the technical team of the Center for the Control of Zoonoses (CCZ) from Birigui/SP, Brazil, for the help with the studied animals.

## REFERENCES

1. World Health Organization. Control of the Leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. Geneva: WHO; 2010. p.22-6. (Report; n°. 949).
2. Simões-Mattos L, Teixeira MJ, Costa DC, Prata Jr JR, Bevilaqua CM, Sidrim JJ, et al. Evaluation of terbinafine treatment in *Leishmania chagasi*-infected hamsters (*Mesocricetus auratus*). Vet Parasitol. 2002;103:207-16.
3. Da Silva AVM, Cândido CDS, Pereira DP, Brazil RP, Carreira JCA. The first record of american visceral leishmaniasis in domestic cats from Rio de Janeiro, Brazil. Acta Trop. 2008;105:92-4.
4. Gontijo CMF, Melo MN. Leishmaniose visceral no Brasil: quadro atual, desafios e perspectivas. Rev Bras Epidemiol. 2004;7:338-49.
5. Simões-Mattos L, Mattos MRF, Teixeira MJ, Oliveira-Lima JW, Bevilaqua CML, Prata Jr RC, et al. The susceptibility of domestic cats (*Felis catus*) to experimental infection with *Leishmania braziliensis*. Vet Parasitol. 2005;127:199-208.

6. Coelho WMD, Lima VMF, Soutel RVG, Amarante AFT, Gennari SM, Meireles MV, et al. Ocorrência de anticorpos contra *Leishmania* spp. em gatos domésticos do município de Andradina-SP-Brasil. *Vet Zootec.* 2008;15:75.
7. Ikonopoulou J, Kokotas S, Gazouli M, Zavras A, Stoitsiou M, Gorgoulis VG. Molecular diagnosis of leishmaniosis in dogs. Comparative application of traditional diagnostic methods and the proposed assay on clinical samples. *Vet Parasitol.* 2003;113:99-103.
8. Ministério da Saúde (BR). Sistema de Informação de Agravos de Notificação. Leishmaniose visceral: casos notificados confirmados no Sinan [Internet]. Brasília: Ministério da Saúde; 2004 [cited 2016 Mar 5]. Available from: <http://dtr2004.saude.gov.br/sinanweb/tabnet/tabnet?sinanet/leishvi/bases/leishvbrnet.def>.
9. Luz ZMP. Changes in the hemoculture methodology improve the test positivity. *Mem Inst Oswaldo Cruz.* 1999;94:295-8.
10. Camargo ME. Introdução às técnicas de imunofluorescência. *Rev Bras Patol Clin.* 1974;10:143-71.
11. Ministério da Saúde (BR). Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de vigilância e controle da leishmaniose visceral. Brasília: Ministério da Saúde; 2006. (Série A. Normas e manuais técnicos).
12. Rosypal AC, Cortés-Vecino JA, Gennari SM, Dubey JP, Tidwell RR, Lindsay DS. Serological survey of *Leishmania infantum* and *Trypanosoma cruzi* in dogs from urban areas of Brazil and Colombia. *Vet Parasitol.* 2007;149:172-7.
13. Figueiredo FB, Bonna IC, Nascimento LD, Costa T, Baptista C, Pacheco TM, et al. Serological evaluation for detection of anti-*Leishmania* antibodies in dogs and cats in the district of Santa Rita de Cássia, municipality of Barra Mansa, State of Rio de Janeiro. *Rev Soc Bras Med Trop.* 2009;42:141-5.
14. Lachaud L, Marchegui-Hammami S, Chabbert E, Dereure J, Dedet JP, Bastien P. Comparison of six PCR methods using peripheral blood for detection of canine visceral leishmaniasis. *J Clin Microbiol.* 2002;40:210-5.
15. Triola MF. Introdução à estatística. 9a ed. Rio de Janeiro: LTC; 2005.
16. Mackinnon DP. Introduction to statistical mediation analysis. New York: Routledge Academic; 2000.
17. Passos VMA, Lasmar EB, Gontijo CMF, Fernandes O, Degraive W. Natural infection of a domestic cat (*Felis domesticus*) with *Leishmania* (*Viannia*) in the metropolitan region of Belo Horizonte, State of Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz.* 1996;91:19-20.
18. Savani ES, Camargo MCO, Carvalho MR, Zampieri RA, Santos MG, D'Auria SR, et al. The first record in the Americas of an autochthonous case of *Leishmania* (*Leishmania*) *infantum chagasi* in a domestic cat (*Felis catus*) from Cotia County, São Paulo State, Brazil. *Vet Parasitol.* 2004;120:229-33.



19. Souza AI, Barros EMS, Ishikawa E, Ilha IMN, Marin GRB, Sundar S, et al. Laboratory diagnosis of visceral leishmaniasis. *Clin Diag Lab Immunol.* 2002;9:951-8.
20. Chiari E. Chagas disease diagnosis using polymerase chain reaction, hemoculture and serologic methods. *Mem Inst Oswaldo Cruz.* 1999;94:299-300.
21. De Carli GA. *Parasitologia clínica: seleção de métodos e técnicas de laboratório para o diagnóstico de parasitoses humanas.* Rio de Janeiro: Atheneu; 2001.
22. Junqueira ACV, Chiari E, Wincker P. Comparison of the polymerase chain reaction with two classical parasitological methods for the diagnosis of Chagas disease in an endemic region of North-eastern Brazil. *Trans R Soc Trop Med Hyg.* 1996;90:129-32.
23. Luciano RM, Lucheis SB, Troncarelli MZ, Luciano DM, Langoni H. Avaliação da reatividade cruzada entre antígenos de *Leishmania* spp. e *Trypanosoma cruzi* na resposta sorológica de cães pela técnica de Imunofluorescência Indireta (RIFI). *Braz J Vet Res Anim Sci.* 2009;46:181-7.
24. Braga ARC. Hemocultura, reação de imunofluorescência indireta (RIFI) e reação em cadeia pela polimerase para *Leishmania* spp. em cães e gatos provenientes de área endêmica e não endêmica para leishmaniose [dissertação]. Botucatu: Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista; 2009.
25. Poli A, Abramo F, Barsotti P, Leva S, Gramiccia M, Ludovisi A, et al. Feline leishmaniasis due to *Leishmania infantum* in Italy. *Vet Parasitol.* 2002;106:181-91.
26. Oliveira ALL, Paniago AMM, Dorval MCE, Oshiro ET, Leal CR, Sanches M. Emergent outbreak of visceral leishmaniasis in Mato Grosso do Sul State. *Rev Soc Bras Med Trop.* 2002;39:446-50.
27. Maroli M, Penissi MG, Muccio T, Khoury C, Gradoni L, Gramiccia M. Infection of sandflies by a cat naturally infected with *Leishmania infantum*. *Vet Parasitol.* 2007;145:357-60.
28. Martín-Sánchez J, Acedo C, Muñoz-Pérez M, Pesson B, Marchal O, Morillas-Márquez F. Infection by *Leishmania infantum* in cats: epidemiological study in Spain. *Vet Parasitol.* 2007;145:267-73.
29. Maia C, Nunes M, Campino L. Importance of cats in zoonotic leishmaniasis in Portugal. *Vector Borne Zoonotic Dis.* 2008;8:555-9.
30. Ayllon T, Tesouro MA, Amusatogui I, Villaescusa A, Rodrigues-Franco F, Sainz A. Serologic and molecular evaluation of *Leishmania infantum* in cats from Central Spain. *Ann NY Acad Sci.* 2008;1149:361-4.
31. Beard CB, Pye G, Steurer FJ, Rodriguez R, Campman R, Peterson AT, et al. Chagas disease in a domestic transmission cycle, southern Texas, USA. *Emerg Infect Dis.* 2003;9:103-5.

32. Silveira TGV, Teodoro U, Lonardoni MVC, Toledo MJD, Bertolini DA, Arraes SMAA, et al. Investigação sorológica em cães de área endêmica de leishmaniose tegumentar no Estado do Paraná, Sul do Brasil. *Cad Saude Publica*. 1996;12:89-93.
33. Costa TAC, Rossi CN, Laurenti MD, Gomes AAD, Vides JP, Sobrinho LSV, et al. Ocorrência de leishmaniose em gatos de áreas endêmicas para leishmaniose visceral. *Braz J Vet Res Anim Sci*. 2010;47:213-7.
34. Dye C, Vidor E, Dereure J. Serological diagnosis of leishmaniasis: on detecting infection as well as disease. *Epidemiol Infect*. 1993;103:647-56.
35. Maia C, Gomes J, Cristóvão J, Nunes M, Martins A, Rebêlo E, et al. Feline *Leishmania* infection in a canine leishmaniasis endemic region, Portugal. *Vet Parasitol*. 2010;174:336-40.
36. Portús M, Gállego M, Riera C, Aisa M, Fisa R, Castillejo S. Wild and domestic mammals in the life cycle of *Leishmania infantum* in Southwest Europe. A literature review and studies performed in Catalonia (Spain). *Rev Iber Parasitol*. 2002;62:72.

**Recebido em: 04/07/2016**

**Aceito em: 08/02/2017**