Ciência Rural, Santa Maria, v.54:08, e20230127, 2024
http://doi.org/10.1590/0103-8478cr20230127
ISSNe 1678-4596
PARASITOLOGY

Detection of antibodies against *Leishmania* species using enzyme-linked immunosorbent assay in cats from the western border of Brazil

Gabriela Döwich Pradella[1*] Taiane Acunha Escobar[2] Thália Pacheco dos Santos[2] Jennifer Stello Minuzzi[2] Lívia Kmetzsch Rosa e Silva[3] Isac Junior Roman[4] Fernanda Silveira Flôres Vogel[4] Claudia Acosta Duarte[2] Irina Lübeck[2]

[1]Programa de Pós-graduação em Ciência Animal, Universidade Federal do Pampa (UNIPAMPA), 97501-970, Uruguaiana, RS, Brasil. E-mail: gabrieladowich.aluno@unipampa.edu.br. *Corresponding author.
[2]Universidade Federal do Pampa (UNIPAMPA), Uruguaiana, RS, Brasil.
[3]Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil.
[4]Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.

ABSTRACT: Feline leishmaniosis is infrequent worldwide, and cats have been suggested as secondary reservoirs for the parasite. However, specific diagnostic techniques for feline samples are scarce. In this study, we standardized an in-house indirect enzyme-linked immunosorbent assay (ELISA) using crude *Leishmania infantum* antigen to detect antibodies in feline samples from an endemic canine visceral leishmaniosis (CVL) area in the western border of Brazil. The results were compared with those of an indirect immunofluorescence assay (IFA). We tested semi-domiciled felines residing in Uruguaiana and Barra do Quaraí, Rio Grande do Sul. Among the 41 samples, 25 (61%) were positive using ELISA and 24 (58%) were positive using IFA (1:40). Our findings demonstrated a high seropositivity of feline samples from the endemic CVL area in the western border of Brazil, and we proposed the use of an in-house ELISA with crude antigen for population screening. This is the first serological survey on felines in a region where CVL is well established.
Key words: feline, leishmaniasis, serodiagnosis, immunodiagnostic, *Leishmania infantum*.

Detecção de anticorpos anti *Leishmania* spp. utilizando ensaio de imunoabsorção enzimática em gatos na fronteira Oeste do Brasil

RESUMO: A leishmaniose felina é relatada esporadicamente em todo o mundo e, a espécie foi sugerida como um reservatório secundário para o parasita. Apesar disso, há carência de técnicas diagnósticas específicas para amostras de felinos. O presente estudo teve como objetivo padronizar um Ensaio de Imunoabsorção Enzimática (ELISA) indireto interno, utilizando o antígeno cru de *L. infantum* para detectar anticorpos em amostras felinas em uma área endêmica de CVL na fronteira oeste do Brasil e, comparar os resultados com o ensaio de imunofluorescência indireta (IFA). Foram testados felinos residentes em Uruguaiana e Barra do Quaraí – RS, semi-domiciliados. Entre as 41 amostras testadas, 25 (61%) foram positivas no ELISA e 24 (58%) no IFA (1:40). O presente estudo elucidou uma alta soropositividade de amostras felinas em uma área endêmica de leishmaniose visceral canina na fronteira Oeste do Brasil e demonstrou a possibilidade de aplicar um ELISA com antígeno bruto para diagnóstico de triagem populacional. Além disso, este é o primeiro inquérito sorológico em felinos na região onde a leishmaniose visceral canina está bem estabelecida.
Palavras-chave: felino, leishmaniose, sorodiagnóstico, imunodiagnóstico, *Leishmania infantum*.

INTRODUCTION

Species of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) are the etiological agents of leishmaniasis, a neglected tropical disease. Over 20 species of *Leishmania* have been identified to infect humans and other mammals (WHO, 2020). While dogs are the primary urban reservoir for *Leishmania* species (spp), and their role in zoonotic transmission is well established, other mammals infected with *Leishmania* spp. have been reported in Brazil (DANTAS-TORRES, 2007; LIMA et al., 2013).

The epidemiological role of felines in the disease cycle has been reported worldwide (PENNISI et al., 2013, 2015; PENNISI & PERSICHETTI, 2018). The first case of feline leishmaniosis (FeL) was documented in Algeria in 1912. Since then, FeL has been reported in several countries. Cats are susceptible to *Leishmania* infection and often reside in close proximity to both dogs and humans. A few studies have proposed the potential role of cats as secondary reservoirs, contributing to the persistence of the disease in areas endemic to canine visceral leishmaniosis (CVL) (ASFARAM et al, 2019). *L.*

Received 03.06.23 Approved 10.31.23 Returned by the author 01.19.24
CR-2023-0127.R3
Editor: Rudi Weiblen

infantum is the main species involved in FeL in Brazil (NASCIMENTO et al., 2022). Although, CVL has been reported in the border region between Argentina and Brazil since 2008 (SOUZA et al., 2009), no cases of FeL have been reported in the state of Rio Grande do Sul.

Diagnostic techniques for FeL are not well defined and primarily rely on methods used for CVL samples. There are no commercial tests specifically designed for FeL diagnosis, and the accuracy of serological tests (commercial tests for CVL or in house serological methods for cats) has not been evaluated. Most epidemiological studies involving the serological evaluation of feline samples employ immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Additionally, parasitological and molecular techniques are used for confirmation (OLIVEIRA et al., 2015; IATTA et al., 2020; FOROUGHI-PARVAR et al., 2021; NASCIMENTO et al., 2022).

In this study, we standardized an indirect ELISA using crude *L. infantum* antigen to detect antibodies in feline samples from an endemic CVL area in western border Brazil.

MATERIALS AND METHODS

The present study was conducted in urban areas of Uruguaiana (29° 44′ 58″ S and 57° 5′ 18″ W) and Barra do Quaraí (30° 11’ 59’’ S and 57° 31’ 12’’ W) municipalities, located on the western border of Brazil, adjacent to Argentina and Uruguay. The collection of samples from individually-owned cats was carried out from September 2018 to February 2020. The study included semi-domiciled, mixed breed cats (*Felis silvestris catus*) of both sexes, with an average age of 4 years.

A total of 41 biological samples were collected from the cats via venipuncture of the external jugular or cephalic veins while under physical restraint and stored in tubes with ethylenediaminetetraacetic acid (EDTA) or without anticoagulant. The major clinical signs present were increase of lymph nodes (Table 1). Along with this, some animals presented complex stomatitis gingivitis and one of them weight loss. The blood was centrifuged at 400xG for 20 minutes, and the serum was stored in 2-mL microtubes and frozen at -20 ºC for subsequent use.

For the positive control, we selected a cat living in an endemic CVL area in Uruguaiana. The cat cohabited with a dog showing clinical signs of CVL and tested positive in polymerase chain reaction (PCR) and serology tests. The positive control cat also tested positive in a dual-path platform chromatographic immunoassay rapid test (DPP-Bio Manguinhos®) and quantitative PCR (qPCR) for leishmaniasis. For the negative control, an adult cat without contact with CVL-infected dogs was selected. The blood sample from this cat tested negative for both DPP® and qPCR. Blank controls for primary and secondary antibodies were included to verify the absence of any non-specific reactions.

The *L. infantum* strain (International code MHOM/BR/2002/LPC-RPV), selected as the antigen owing to its prevalence in the region (ESCOBAR et al., 2020; PRADELLA et al., 2020), was obtained from the *Leishmania* Collection of the Oswaldo Cruz Institute (CLIOC -FIOCRUZ/RJ). The culture was maintained and cultivated according to the manual with modifications (BRAZIL, 2018).

The antigen preparation followed the protocol described by SOARES (2012) with slight modifications. The plates were fixed with crude *L. infantum* antigen at a concentration of 10 µg/mL. Subsequently, blocking was performed by adding 300 µL/well of 1% nonfat dried milk (Molico®), and the plates were incubated at 37 °C for 1 hour, as previously standardized for canine samples (PRADELLA et al., 2023).

The first antibody dilutions were 1:20, 1:40, 1:80, and 1:100. The second antibody dilution was tested at concentrations of 1:10,000 and 1:40,000 (FIGUEIREDO et al., 2009; SZARGIKI et al., 2009). The optimal combination was determined based on the signal-to-noise ratio, calculated by dividing the mean absorbance of the positive control by that of the negative control. The cutoff value was determined as the mean plus three times the standard deviation of the optical density (OD) from the negative control, following the method described by RAJASEKARIAH (2001).

The complete in-house indirect ELISA protocol has been previously described (PRADELLA et al., 2023). Briefly, the first antibody was diluted in phosphate-saline dilution buffer (PBS) supplemented with 0.05% polysorbate 20 (PBS Tween) and 0.25% casein, at a concentration of 1:80, as previously defined. The second antibody used in this assay was species-specific (Goat pAb to cat IgG [HRP] ab112801) and was diluted in PBS Tween at a concentration of 1:10,000. The substrate solution, tetramethylbenzidine (TMB), was incubated for 15 minutes in the dark. Following incubation, 25 µL/well of the stop solution (2M sulfuric acid) was added, and the absorbance

Table 1 - General information for each animal included in the study group and the relation with the presence of increased lymph nodes and ELISA results.

Animal identification	Sex	Age	Municipality	Increased lymph nodes	ELISA
F10	Female	4	Uruguaiana	No	Negative
F11	Female	8	Uruguaiana	No	Positive
F12	Female	7	Uruguaiana	No	Positive
F13	Female	5	Uruguaiana	Yes	Positive
F14	Female	5	Uruguaiana	Yes	Negative
F15	Male	3	Uruguaiana	No	Positive
F17	Male	8	Uruguaiana	No	Negative
F18	Male	6	Uruguaiana	No	Negative
F19	Male	5	Uruguaiana	Yes	Positive
F20	Male	8	Uruguaiana	Yes	Positive
F21	Female	7	Uruguaiana	No	Negative
F22	Female	7	Uruguaiana	No	Negative
F23	Male	8	Uruguaiana	No	Positive
F25	Female	8	Uruguaiana	Yes	Positive
F29	Male	2	Uruguaiana	No	Negative
F30	Female	8	Uruguaiana	Yes	Positive
F31	Male	3	Uruguaiana	Yes	Positive
F38	Male	4	Uruguaiana	No	Negative
F39	Male	7	Uruguaiana	No	Negative
F43	Female	7	Uruguaiana	No	Positive
F44	Female	3	Uruguaiana	Yes	Negative
F46	Female	2	Uruguaiana	Yes	Positive
F47	Male	2	Uruguaiana	Yes	Negative
F48	Female	2	Uruguaiana	Yes	Negative
F49	Male	2	Uruguaiana	Yes	Positive
F53	Male	4	Uruguaiana	No	Positive
F61	Male	2	Uruguaiana	No	Positive
F62	Female	2	Uruguaiana	Yes	Positive
F63	Male		Uruguaiana	Yes	Positive
F64	Male	0,8	Uruguaiana	No	Positive
F65	Female	1	Uruguaiana	No	Negative
F67	Female	8	Uruguaiana	Yes	Positive
F69	Female	2	Uruguaiana	Yes	Positive
F73	Male	8	Uruguaiana	No	Negative
F74	Male	6	Uruguaiana	No	Positive
F77	Male		Uruguaiana	Yes	Negative
F82	Male	0,41	Barra do Quaraí	No	Positive
F83	Male	0,41	Barra do Quaraí	No	Negative
F84	Female	2	Barra do Quaraí	No	Positive
F86	Male	2	Barra do Quaraí	Yes	Positive
F89	Male	0,5	Uruguaiana	No	Positive

was measured at 450 nm using an ELISA reader (Multiskan FC- Thermo Fisher Scientific®), according to the TMB manufacturer's instructions.

Additionally, the samples were tested using indirect IFA, in which multispot slides were coated with promastigote forms of *L. infantum*. Serum samples were diluted in PBS at 1:40, 1:80, 1:160, and 1:320, using positive and negative sera as test controls. Commercial fluorescein-labeled anti-Cat IgG© (Goat Anti-Cat IgG FITC®, F4262, Sigma-Aldrich, San Luis, Missouri, USA) was used as the secondary antibody. Slides were observed at 400X magnification under a fluorescent microscope (Optiphase INV403F).

RESULTS AND DISCUSSION

In this study, we first identified a feline infected with *Leishmania* in an endemic area of Uruguaiana, Rio Grande do Sul, using qPCR tests and the DPP-Bio Manguinhos® method. Considering that serological methods, such as IFA and ELISA, are widely used for the diagnosis of *Leishmania* infections, we developed an in-house indirect ELISA standardization methodology for feline samples. No studies on FeL have been reported in the state of Rio Grande do Sul (PENNISI & PERSICHETTI, 2018; ASFARAM et al., 2019; NASCIMENTO et al., 2022).

Using the in-house indirect ELISA standardization, we achieved a signal-to-noise ratio of 4.64 when the serum was diluted 1:80 and the second antibody was diluted 1:10,000. The positive and negative control exhibited an OD of 2.058 and 0.443, respectively. The cutoff value was set at 0.419 (values 5% higher were considered positive and 5% lower negative). Out of the 41 cat samples, 25 (61%) tested positive via ELISA (Figure 1) for the detection of antibodies to *Leishmania* spp. (Table 2). Among the total of 41 cats tested using the IFA technique, 36 (88%), 26 (63%), 15 (37%) and 7 (17%) were positive using the dilutions 1:40, 1:80, 1:160 and 1:320, respectively. TREVISAN et al. (2015) reported that the widespread use of ELISA for detecting anti-*Leishmania* antibodies in Brazil indicates both infection and exposure to the parasite.

The recommended dilution for IFA in cat samples is 1:80, which has been applied under field conditions after test validation (PENNISI et al., 2015; IATTA et al., 2020). However, in our study, we also assessed the 1:40 dilution, which has been used in studies carried out in different states of Brazil and has evidenced higher sensitivity compared with the 1:80 dilution (NASCIMENTO, 2021). It has also reported a seroprevalence of 10.9% for FeL (VIDES et al., 2011). Similar to our study, VIDES et al. (2011) performed indirect ELISA using crude antigen of *L. infantum* and the calculations included signal-to-noise ratio and cutoff values. The calculated cutoff value was 0.2765 for ELISA, and the seroprevalence of FeL was 14/55 (25.4%) which was lower than that observed in our research.

Anti-*Leishmania* antibody detection using ELISA has been widely used in feline serological evaluations. The validation of the test is crucial to determine its optimal conditions, as demonstrated in various studies comparing different sample dilutions and secondary antibodies (FIGUEIREDO et al., 2009; VIDES et al., 2011; SOBRINHO et al., 2012;). One limitation of the study was lack of *Leishmania* characterization in the positive samples and it is important to clarify that seropositive cats indicate exposition and not infection with *Leishmania*. In the same way, the accuracy (sensitivity and specificity) of the serological tests used were not evaluated.

This research represents a significant contribution as a pioneering study conducted in a triple border region between Brazil, Uruguay, and Argentina, where reports on *Leishmania* infection are limited. The motivation to conduct it based on previous research on feline infections in the region and the absence of a specific diagnostic

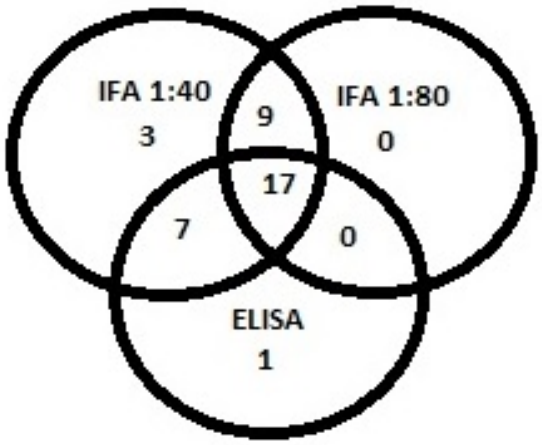

Figure 1 - Correlation between positive samples using different techniques: enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA) with a 1:40 dilution, and IFA with a 1:80 dilution.

Table 2 - Optical density (OD) for positive (OD >0.439) and negative (OD <0.398) samples tested using ELISA for the detection of antibodies against *Leishmania* spp.

Positive Sample	ELISA OD 450 nm	Negative Sample	ELISA OD 450 nm
F11	0.666	F10	0.371
F12	0.581	F14	0.217
F13	0.761	F17	0.409
F15	0.990	F18	0.366
F19	0.714	F21	0.225
F20	1.673	F22	0.370
F23	0.528	F29	0.328
F25	0.968	F38	0.359
F30	0.874	F39	0.360
F31	0.529	F44	0.391
F43	0.439	F47	0.377
F46	0.480	F48	0.308
F49	1.105	F65	0.173
F53	0.633	F73	0.188
F61	0.445	F77	0.311
F62	0.565	F83	0.164
F63	0.443		
F64	0.533		
F67	0.764		
F69	0.456		
F74	0.611		
F82	0.462		
F84	0.812		
F86	0.604		
F89	0.915		

methodology for felines. The challenge was to identify infected felines in order to develop this technique, which required an active search in the main endemic areas for CVL.

CONCLUSION

This study reports the first documented case of FeL in an endemic area of CVL, providing the first serological test for leishmaniasis in feline samples from the western border region of Rio Grande do Sul, Brazil. In-house ELISA allowed for the serological detection of *Leishmania* antibodies in feline samples. Out of the 41 cat samples tested, 61% (25/41) were positive via ELISA for the detection of antibodies against *Leishmania* spp., and among them, 58% (24/41) were also positive via IFA. The identification of positive cat samples in an endemic area for CVL is highly relevant and raises concerns for human health. Based on our initial findings, further analyses are necessary to identify the prevalence of *Leishmania* infections in feline species and determine the circulating feline *Leishmania* species in the western border region of Rio Grande do Sul. Additionally, understanding the role of FeL in the leishmaniasis cycle is crucial for effective control and prevention strategies.

ACKNOWLEDGMENTS

This work was supported by FAPERGS/PPSUS (Research Program for SUS: Shared Health Management; PPSUS 2017) and financed in part from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brazil, CAPES- finance code 001). The *Leishmania* strains were kindly provided by Coleção de Leishmania do Instituto Oswaldo Cruz (CLIOC FIOCRUZ), Rio de Janeiro, thus enabling development of this study. FAPERGS/PPSUS (Research Program for SUS: Shared Health Management; PPSUS 2017) number 17/2551-001 405-7; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasil) by PhD scholarship to G. D. Pradella, Finance code 001.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interests

AUTHORS' CONTRIBUTIONS

Gabriela Döwich Pradella: all steps; Taiane Acunha Escobar: animal collection and data curation; Thália Pacheco dos Santos and Jennifer Stello Minuzzi: animal collection; Lívia Kmetzsch Rosa e Silva: real time PCR- positive controls; Isac Junior Roman and Fernanda Silveira Flôres Vogel: IFA; Claudia Acosta Duarte and Irina Lübeck: all steps, project coordinators.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

Ethics Committee on Animal Experimentation from Universidade Federal do Pampa with number 014/2020.

REFERENCES

ASFARAM, S. et al. Is the cat an important reservoir host for visceral leishmaniasis? A systematic review with meta-analysis. **Journal of Venomous Animals and Toxins Including Tropical Diseases**, v.25, n.June, p.1–10, 2019. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6583674/pdf/1678-9199-jvatitd-25-e20190012.pdf>. Accessed: Dec. 01, 2023. doi: 10.1590/1678-9199-JVATITD-2019-0012 .

DANTAS-TORRES, F. The role of dogs as reservoirs of *Leishmania* parasites, with emphasis on *Leishmania* (*Leishmania*) *infantum* and *Leishmania* (*Viannia*) *braziliensis*. **Veterinary Parasitology**, v.149, n.3–4, p.139–146, 2007. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0304401707003597>. Accessed: Dec. 01 2023. doi: 10.1016/j.vetpar.2007.07.007.

ESCOBAR, T. A. et al. Molecular detection of *Leishmania* spp. in Brazilian cross-border south region mammalian hosts. **Transboundary and Emerging Diseases**, v.67, n.2, p.476–480, 2020. Available from: <https://onlinelibrary.wiley.com/doi/epdf/10.1111/tbed.13361>. Accessed: Jun. 22, 2023, doi: 10.1111/tbed.13361.

FIGUEIREDO, F. B. 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. **Revista da Sociedade Brasileira de Medicina Tropical**, v.42, n.2, p.141–145, 2009. Available from: <https://www.scielo.br/j/rsbmt/a/QwHw9XvNMk3KjqGn54Vcfhy/?format=pdf>. Accessed: Jun. 22, 2023. doi: 10.1590/S0037-86822009000200009.

FOROUGHI-PARVAR, F. et al. FML-ELISA a novel diagnostic method for detection of feline leishmaniasis in two endemic areas of Iran. **Journal of Parasitic Diseases**, v.45, n.1, p.279–284, 2021. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7921240/pdf/12639_2020_Article_1316.pdf>. Accessed: Aug. 18 2023. doi: 10.1007/s12639-020-01316-3.

IATTA, R. et al. Validation of a new immunofluorescence antibody test for the detection of *Leishmania infantum* infection in cats. **Parasitology Research**, v.119, n.4, p.1381–1386, 2020. Available from: <https://pubmed.ncbi.nlm.nih.gov/32107620/>. Accessed: Jun. 01, 2023. doi: 10.1007/s00436-020-06627-1.

LIMA, B. S. et al. Small mammals as hosts of *Leishmania* spp. in a highly endemic area for zoonotic leishmaniasis in north-eastern Brazil. **Trans R Soc Trop. Med Hyg**, v.107, p.592–597, 2013. Available from: <https://pubmed.ncbi.nlm.nih.gov/23868744/>. Accessed: Jul. 15, 2023, doi: 10.1093/trstmh/trt062.

BRAZIL [FIOCRUZ]. **Training LPL/LRNTL/CLIOC**, 2018.

NASCIMENTO, L. F. J. et al. Epidemiological and diagnostic aspects of feline leishmaniasis with emphasis on Brazil: a narrative review. **Parasitology Research**, v.121, n.1, p.21–34, 2022. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8580739/pdf/436_2021_Article_7372.pdf>. Accessed Sept. 05, 2023, doi: 10.1007/s00436-021-07372-9.

OLIVEIRA, G. C. et al. Antibodies to *Leishmania* spp. in domestic felines. **Revista Brasileira de Parasitologia Veterinária**, v.24, n.4, p.464–470, 2015. Available from: <https://www.scielo.br/j/rbpv/a/yTHpmvpzGkbFxMLVRW4hgwx/?format=pdf&lang=en>. Accessed: Jun. 22, 2023. doi: 10.1590/S1984-29612015071.

PENNISI, M. G. et al. Leishmaniosis in cats: ABCD guidelines on prevention and management. **J Feline Med Surg**, v.15, p.638-642, 2013. Available from: <https://orbi.uliege.be/bitstream/2268/188001/1/410%20pub_thiry.pdf>. Accessed: May, 16, 2023, doi: 10.1177/1098612X13489229.

PENNISI, M. G. et al. LeishVet update and recommendations on feline leishmaniosis. **Parasites and Vectors**, v.8, n.1, p.1–18, 2015. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4462189/pdf/13071_2015_Article_909.pdf>. Accessed: Dec. 02, 2022. doi: 10.1186/s13071-015-0909-z.

PENNISI, M. G.; PERSICHETTI, M. F. Feline leishmaniosis: is the cat a small dog? **Vet Parasitol**, v.251, p.131–137, 2018. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7130840/pdf/main.pdf>. Accessed: Aug. 13, 2022. doi: 10.1016/j.vetpar.2018.01.012.

PRADELLA, G. D. et al. Identification of *Leishmania* spp. in horses and a dog from rural areas of Uruguaiana, Rio Grande do Sul, Brazil. **Semina: Ciências Agrárias**, v.41, n.6, p.2687–2694, 2020. Available from: <https://ojs.uel.br/revistas/uel/index.php/semagrarias/article/view/38423/28070>. Accessed: Sept. 20, 2022. doi: 10.5433/1679-0359.2020v41n6p2687.

PRADELLA, G. D. et al. In-house serological ELISA as a leishmaniosis diagnostic test: development and applications in canines from the western border of Brazil. **Ciência Rural**, v.53, n.4. 2023. Available from: <https://www.scielo.br/j/cr/a/xRJFHggRWzHgtGpBs5hV3cS/>. Accessed: May, 10, 2022. doi: 10.1590/0103-8478cr20210907.

RAJASEKARIAH, G. H. R. et al. Optimization of an ELISA for the serodiagnosis of visceral leishmaniasis using in vitro derived promastigote antigens. **Journal of Immunological Methods**, v.252, n.1–2, p.105–119, 2001. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0022175901003416?via%3Dihub>. Accessed: Jan. 13, 2022. doi: 10.1016/S0022-1759(01)00341-6.

SOARES, I. R. **Avaliação clínica e laboratorial de equinos sororreagentes para *Leishmania* sp. no município de Belo Horizonte, Minas Gerais, Brasil**. 2012. 133f. Dissertation (Master's in Animal Science)- Postgraduate Coure in Animal Science. Federal University of Minas Gerais, 2012. Available from: <https://repositorio.ufmg.br/bitstream/1843/BUOS-95ZGQZ/1/isabel_roussouli_res_soares_disserta__o_2012.pdf>. Accessed: Feb. 24, 2022.

SOBRINHO, L. S. V. et al. Coinfection of *Leishmania chagasi* with *Toxoplasma gondii*, Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) in cats from an endemic area of zoonotic visceral leishmaniasis. **Veterinary Parasitology**, v.187, n.1–2, p.302–306, 2012. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0304401712000131?via%3Dihub>. Accessed: Feb. 10, 2022. doi: 10.1016/j.vetpar.2012.01.010.

SOUZA, G. D. et al. The first report of the main vector of visceral leishmaniasis in America, *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae: Phlebotominae), in the state of Rio Grande do Sul, Brazil. **Memórias do Instituto Oswaldo Cruz**, v.104, n.8, p.1181–1182, 2009. Available from: <https://www.scielo.br/j/mioc/a/ry8DNNK7nr9RBDws5Z7PH4Q/?format=pdf&lang=en>. Accessed: Jun. 05, 2022. doi: 10.1590/S0074-02762009000800017.

SZARGIKI, R. et al. Comparison of serological and parasitological methods for cutaneous leishmaniasis diagnosis in the state of Paraná, Brazil. **Brazilian Journal of Infectious Diseases**, v.13, n.1, p.47–52, 2009. Available from: <https://pubmed.ncbi.nlm.nih.gov/19578630/>. Accessed: May, 01, 2022. doi: 10.1590/s1413-86702009000100011.

TREVISAN, D. A. C. et al. Diagnostic methods to cutaneous leishmaniasis detection in domestic dogs and cats. **An Bras Dermatol**, v.90, p.868–872, 2015. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4689076/pdf/abd-90-06-0868.pdf>. Accessed: Mar. 13, 2022. doi: 10.1590/abd1806-4841.20153716.

VIDES, J. P. et al. *Leishmania chagasi* infection in cats with dermatologic lesions from an endemic area of visceral leishmaniosis in Brazil. **Veterinary Parasitology**, v.178, n.1–2, p.22–28, 2011. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0304401711000112?via%3Dihub>. Accessed: Jun. 22, 2022. doi: 10.1016/j.vetpar.2010.12.042.

WHO -Global leishmaniasis surveillance, 2017–2018, and first report on 5 additional indicators. **Geneva**: World Health Organization. Weekly Epidemiological Record. v.25, p.265–280, 2020. Available from: <https:// www.who.int/publications/i/item/who-wer95 25>. Accessed: Jun. 12, 2022.