



Diagnosis of leishmaniasis by cytology in earpoint in canine – case report

Diagnóstico de leishmaniose por citologia em ponta de orelha em canino – relato de caso

Thais Melo da Silveira¹, Marcos Vinícius Ramos Afonso^{2*}, Elza Alice de Quadros²

¹Graduate Student in Veterinary Medicine – UNICERP

²Professor at the University Center in Cerrado do Patrocínio

*Corresponding author: markvinycius@hotmail.com

DOI: 10.4025/revcivet.v12i1.62793

RESUMO

A *Leishmaniose* é uma zoonose de grande importância para a saúde pública, existindo diversos métodos de diagnósticos, entretanto, cada qual apresenta características próprias. Dentre os métodos destaca-se a citologia por apresentar facilidade na execução, diagnóstico preciso e com baixo custo operacional. Objetivou-se descrever um caso clínico e o diagnóstico de canino com leishmaniose tegumentar através do exame citológico em ponta de orelha. Foi atendido um animal com histórico de lesões de pele, ocular e epistaxe. Durante a anamnese foi relatado que o cão apresentava lesões de pele que não cicatrizavam, estava esbarrando em objetos, anorexia, apatia e adipsia há três dias. Durante o exame físico foi identificado alopecia e hipotricose em região de face, linfadenomegalia, sem alteração em frequência cardíaca e respiratória. Foi solicitado a realização de raspado de pele e citologia de ponta de orelha. O material coletado foi avaliado em microscópio óptico, sendo possível a identificação de hemácias, associado e células de defesa e macrófagos com formas amastigotas do protozoário *Leishmania* e presença do mesmo no líquido extracelular confirmando assim o diagnóstico de leishmaniose. Já no raspado de pele foi detectado a presença do fungo *Microsporum canis*, dermatófilo comum na pele dos cães, causador das dermatofitoses que também podem acometer humanos. A citologia em ponta de orelha é um método de diagnostico que se mostrou eficaz detectando diretamente as formas infectantes do protozoário, sendo este de custo acessível e de simples execução.

Palavras-chave: Cães, Calazar, Dermatofitose. Leishmania. Pele.



ABSTRACT

Leishmaniasis is a zoonosis of significant importance for public health, and there are several diagnostic methods; however, each one has its own characteristics. Among the methods, cytology stands out for its ease of execution, accurate diagnosis and low operational cost. The objective was to describe a clinical case and the diagnosis of a canine with cutaneous leishmaniosis through cytological examination of the ear tip. An animal with a history of skin and ocular lesions and epistaxis was treated. During the anamnesis it was reported that the dog had skin lesions that did not heal, was bumping into objects, anorexia, apathy and adipsia for three days. During the physical examination, alopecia and hypotrichosis in the face, lymphadenopathy, without changes in heart and respiratory rate were identified. A skin scraping and ear tip cytology were requested. The collected material was evaluated under an optical microscope, making it possible to identify red blood cells, associated defense cells and macrophages with amastigote forms of the *Leishmania* protozoan and their presence in the extracellular fluid, thus confirming the diagnosis of leishmaniasis. The presence of the fungus *Microsporum canis*, a common dermatophile on the skin of dogs, which causes dermatophytosis that can also affect humans, was detected in skin scrapings. Ear tip cytology is a diagnostic method that has proven to be effective in directly detecting the infective forms of the protozoan, which is affordable and simple to perform.

Keywords: Calazar. Dermatophytosis. Dogs. *Leishmania*. Skin.

INTRODUCTION

Leishmaniasis is an infectious disease caused by a protozoan of the *Leishmania* kind, and it can be manifested in two kinds, namely cutaneous and visceral leishmaniasis. *Leishmaniasis* is a disease of cosmopolitan distribution, with foremost importance in public health, due to its zoonotic character. In Brazil, *Leishmaniasis* is present in all regions, being one of the most relevant diseases which is neglected in some places (BRASIL, 2014).

Cutaneous *Leishmaniasis* is the most found kind of impairment, and animals can be infected through blood meal of phlebotomine, of the *Lutzomyia* kind, becoming host and container of the protozoan (GREENE, 2015). Among the main symptoms that are manifested by the animal, there can be cited anorexia, cachexia, onychogryphosis, diffuse or in ends skin lesions, difficulty of scarring, alopecia, hypotrichosis, hyperkeratosis, seborrhea, among others (BRUM et al., 2007; WHO, 2014).

Clinical manifestations in the animal are linked to its immune system, being that the contaminated organism becomes more susceptible to secondary infections (SAMPAIO et al., 2002).



Dogs, when infected, can become immunosuppressed, and they become more susceptible to secondary infections, such as fungal and bacterial dermatitis (GOMES et al., 2012).

There are diverse diagnostic methods, but they show high costs, variations in specificity, sensitivity and runtime (MEGID et al., 2018). Cytology is a diagnostic exam that can be used when the animal shows lesions or cutaneous changes. In the cytological exam, it is possible to observe changes in animals' cells, as well as the presence of parasite (SAMPAIO et al., 2009).

The cytological method can be held through smears, biopsy or through the technique of *imprint*, which consists in the disposition of the biological material of crusts, or blade flaking. Then, it is possible to see the protozoan in its amastigote type, in the microscope (NOGUEIRA et al., 2009).

Cytology, in the diagnosis of leishmaniasis, stands out because it is a less invasive, of low cost, absence of sophisticated equipment and fast execution. When the etiologic agent is identified, the diagnosis is accurate, and it shows high specificity (DOTTA et al., 2009).

This way, the cytology of lesions in animals, in case of suspicion of *Leishmaniasis*, can favor the early diagnosis, enabling higher accuracy and assertiveness in the therapeutic treatment, favoring the remission of clinical signals, better quality and the animal's well-being. This present study aims to report on the case of a dog that had been diagnosed with *Leishmaniasis* through cytology of ear point.

DEVELOPMENT

A female dog, without a defined breed, 1-year-old, body weight of 5,3kg, coming from the municipality of Patrocínio –MG, was attended in the Center of Animal Health at UNICERP. The main complaint pointed out by the tutor was that the animal, for three days already, had been presenting eye irritation, intense itching with wounds in the head and epistaxis. The tutor also said that he had used saline solution to clean the animal's wounds.

During the anamnesis, it was reported that the patient had been in contact with another dog that had wounds in the body. The patient was apathetic, with picky appetite, adipsia, normuria, impaired breathing and without behavioral changes, but, with impaired sight, because he usually bumped into objects. It was also reported that the animal had already been exposed to the presence of rodents, was sneezing a lot, and there were eye and nasal secretions. The tutor mentioned that he did not believe in the possibility of poisoning, that the itching was more intense in the face, the animal was not castrated, did not have a history of vaccination and neither of previous diseases. The animal did not have access to the street, was always in the house and in its external areas, but there was a lot of soil and bush in the backyard, but the animal did not have access to it.



During the physical exam, the presence of lesions in the head and ears were noticed, with characteristics of crusts, and the skin had cutaneous odor and flaking all over the body, onychogryphosis and the presence of ectoparasites. The animal's sight was impaired, with a lot of ocular secretion, congested episcleral vessels, being that the tutor had cleaned the animal's eyes with saline solution.

It was possible to identify lymphadenomegaly in sub mandibular, pre-scapular, inguinal and pale mucous membranes, tachycardia (200 heartbeats per minute), normal respiratory frequency (20 respiratory movements per minute), dehydration level of 5%, temperature of 39°C and score of body condition – 3 (in a scale from 1 to 9).

In the beginning, they suspected of Ehrlichiosis and Leishmaniasis. Complementary exams were requested, quick test for ehrlichiosis, skin scarring, complete blood count, ALT and serum dosage of creatinine.

The test for leishmaniasis was cytology in ear tip; it was also held the skin scarring for the evaluation of the presence of mites or opportunistic fungal. During the consultation, biological material was collected in the animal's area of ear tip for the holding of cytology through the scalpel's blade and *Imprint* of the lesion, and the material was conditioned and disposed in a microscope blade (Figure 1-A), which was stained with the Kit for Fast Panoptic Dying Laborclin (ARRAES *et al.*, 2008). After the drying of the blade, the direct observation in microscope was held.

Skin scarring was also done (Figure 1-B), which consisted in deepening the scalpel's blade on the skin, this way collecting the material; this material was dyed in violet and acetate's adhesive tape (LACAZ *et al.*, 1998).

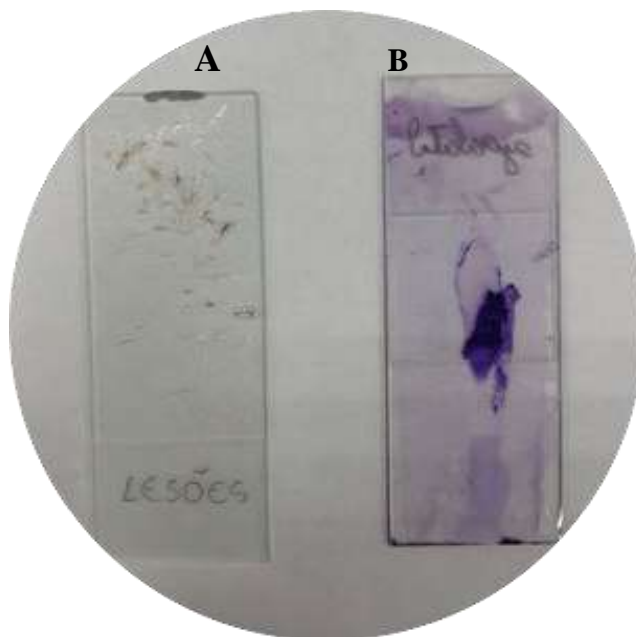


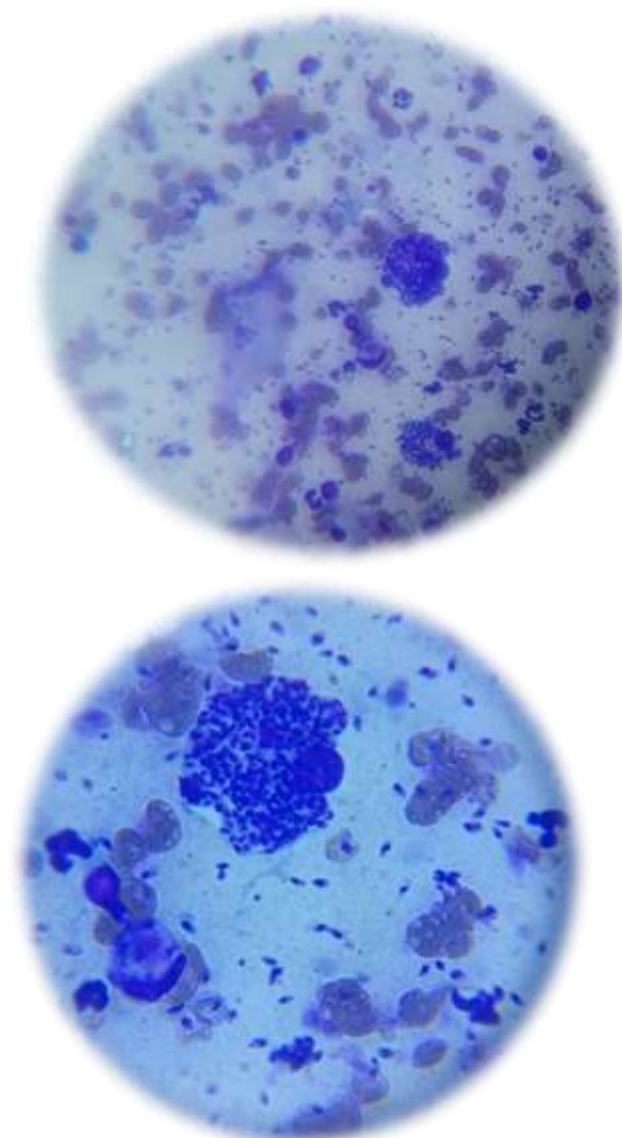
Figure 1 – Blade of scarring of skin with mineral oil (A), Blade of cytology of ear tip that had been blushed with Kit for Fast Panoptic Dying Laborclin (B).

The done blades were disposed in an optical microscope – a Nikon one – and the skin scarring (Figure 1- A) was evaluated under the presence of mites and directly evaluated; when, further, it was distilled a drop of violet dye on the material, and it was used acetate tape for the adherence of the material to the dye, making it possible the identification of cellular characteristics. The cytological blade, which had already been dyed (Figure 1- B), was soaked in a drop of immersion oil, and it was put in a cover slip; Further, it was evaluated regarding the cellularity.

Until the release of the requested exams, it prescribed the symptomatic treatment for the ophthalmological changes. Ophthalmological use of the following eye drops: Tobramycin 0,3% (1 drop in each eye, TID, for 10 days), Diclofenac sodium 0,1% (1 drop, QID, for 10 days), ethylenediaminetetraacetic acid 1% (1 drop, QID, for 10 days) and carmellose sodium 5mg/ml (1 drop, QID, for 10 days). For topical use, it was recommended the repellent collar on the basis of Deltamethrin. 1 g, in continuous use.

The microscopic evaluation of the lesion in ear tip, made it feasible the viewing of erythrocytes, inflammatory infiltration with the presence of rod cells, neutrophils, lymphocyte and macrophages with distended cytoplasm (Figure 2). In the objective of 40x, it was possible to observe macrophages full of amastigote forms of *Leishmania* spp (Figure 3), enabling the diagnosis of *Leishmaniasis*.

Figure 2 – Cytology of canine ear tip, in an optical microscope in the objective magnifying glass of 10X, with the presence of



red blood cells, leucocytes, rod cells, macrophages with distended and engorged cytoplasm of rounded cells of basophilic coloring and dispersed in the extracellular liquid.

Figure 3- Cytology of the lesion in canine ear tip in an optical microscope in the objective magnifying glass of 40X, shows centralized macrophage with amastigote forms of *Leishmaniasis*, promastigote forms of *Leishmaniasis*, extracellular promastigote, erythrocytes, rod cells and the presence of lymphocytes.

The evaluation of skin scarring was done through direct microscopy with the use of violet dye and acetate tape (Figure 4). In the material, it was observed the presence of fur with hair bulb, epithelial cells of enucleate flaking, presence of rounded bacteria violet coccoid, which are



suggestive of positive grams. It was also viewed the presence of fungi Fusiform macrolides, of thick wall and with the presence of septa, which are characteristic of the kind *Microsporum canis* (Figure 4).

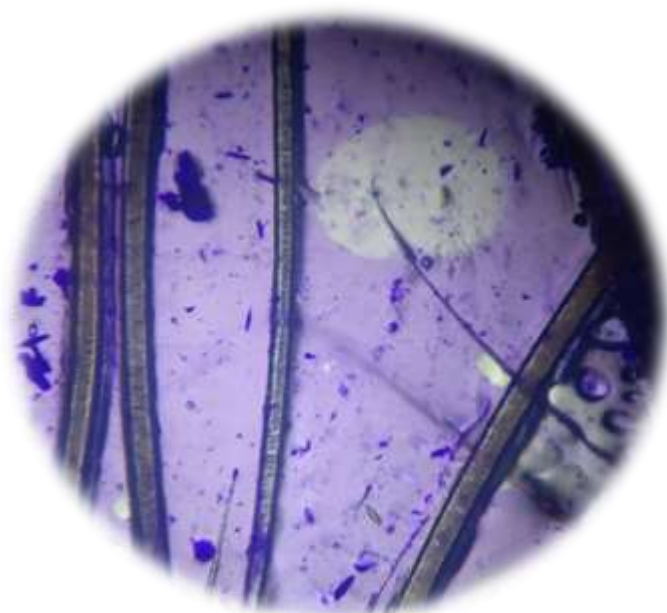


Figure 4 – Direct microscopy of scarring of canine skin, in the objective magnifying glass of 10X, using acetate tape and violet dye, identifying the presence of fur, enucleated flaking cells dyed of violet, fungi of the kind *Microsporum canis* and presence of bacteria of coccoid shape of positive gram.

The animal came from an urban area, in a wooded environment, which favors higher risk for contamination with diseases, once the phlebotomize is responsible is responsible for its transmission, and it proliferates in organic matter, in decomposition as fruits, leaves, waste, among others (WERNECK et al., 2002). The amastigote forms of *Leishmaniasis* spp are inserted in the vector when blood meal is held in a host that had been infected by the pathogen. Once ingested, the protozoan migrates to the gastrointestinal tract, and then it gets the form promastigote, and further it is directed to the salivary gland. During blood meal, the infected vector will inoculate the saliva in order to impede the vascular coagulation, and then, *Leishmania* will be inoculated in another host (ROCHA et al., 2020)

This way, macrophages in the host organism will phagocyte the amastigote form, in order to destroy the protozoan. Nevertheless, the macrophages cannot destroy the protozoans and, so, the pathogenic agent will replicate until it can destroy the cell, being released in the bloodstream, reaching other cells and tissues. According to the localization of the parasite, clinical signals will be attributed in the animal (CARVALHO and TAUIL, 2009; GOTO and LINDOSO, 2012).



According to Salzo (2008), the most frequent clinical symptoms are difficulties in the locomotion, anorexia, apathy, lymphadenomegaly, epistaxis, uveitis and conjunctivitis. Among the cutaneous manifestations that are commonly presented, there is the presence of cutaneous lesions, difficulties in scarring, onychogryphosis, hair loss, seborrhea and flaking, hyperkeratosis, among others (FEITOSA et al., 2000).

The most found lesions in cutaneous Leishmaniasis show self-limited, with ulcers, crusts and erythematous plaques, being them of slow scarring. Their etiological agents are endemic in the Middle East, but with immigration, these species were being dissipated through other regions (MATSUMOTO et al., 2021).

In the suspicion of *Leishmaniasis* investigation must take into consideration the patient's clinical evaluation, the characteristics of the lesions being observed, and the history of contact in endemic areas. The cytological diagnosis is confirmatory and it is done through direct microscopy, with the direct identification of the parasite, being that the serologic tests do not show reliability in the detection of *Leishmaniasis* (EIRAS et al., 2015).

In dogs, cutaneous *Leishmaniasis* can mainly be manifested with mucocutaneous lesions, formation of ulcers of difficult scarring and protruding edges, and they can appear in nasobucco pharyngeus and face (REIS et al., 2011). The lesions can appear in a localized or diffuse type (SILVA et al., 2007), being that the study animal showed cutaneous alterations in the face.

The confirmation of the cytological diagnosis is based on the identification of the characteristic morphology of the protozoan, usually seen inside the macrophages or in an extracellular form. As found in the cytology of ear tip, the amastigote forms of *Leishmania* spp., show rounded characteristic with more basophilic dyeing in its nucleus, and it can be viewed through microscopy in slide with biological colored material (ANTUNES et al., 2018).

Eguchi et al. (2017) held the cytology in a suspect animal for leishmaniasis, and it was held through aspirative puncture with a thin needle in a nodule of the ocular conjunctive, and he reported kind of easiness and efficiency in the execution. During the cytological evaluation, it was identified the presence of amastigote forms of *Leishmania*, which led to the diagnosis of nodular cerate- conjunctivitis, rather than Leishmania.

According to Pereira et al. (2021), by holding the cytology of felines' nasal lesion, it was possible to identify the presence of the *Leishmania* protozoan. Such authors describe that the cytological exam has high specificity and success in the parasitological diagnosis in relation to further laboratory analysis.

The animal in this study presented conditions of immune vulnerability due to the impairment of the *Leishmania* protozoan (SABATÉ et al., 2014). This way, its organism was more



sensitive to secondary contamination, with the lower of immunity there was the proliferation of the fungus *Microsporium canis*.

The fungus that had been identified through skin scarring, is one of the dermatophytes, which are constantly found in canines' skin; however, the presence in cytological evaluation is enough for the diagnosis of dermatophytosis that are of important evaluation, at the expense of being a zoonosis (NEVES *et al.*, 2011). Leishmaniasis affects the immune system so that there is the hyper production of immunoglobulins, and sharp lower of the cellular immunity (NETTO, 1989). Immunosuppressed animals are more susceptible to fungal manifestations because it is an appropriate reservoir for the process of development of pathogenic fungi (FRIAS; KOZUSNY-ANDREANI, 2008).

According to Vides (2010), animals that come from endemic areas of *Leishmaniasis* which show cutaneous alterations may be due to fungal impairment. In his study, it was possible to identify the colonization of various fungi species in felines' cutaneous lesions that were infected with *Leishmania*, and *Microsporium* was one of the highlighted agents, with higher predilection in the survey.

According to Oliveira (2017) the lesions of patients positive for *Leishmaniasis*, who are detected with opportunist fungi, take more time to epitalize and heal. Then, it was evidenced the negative influence of the fungal infection in the evolution of the treatment of the wounds in individuals who were contaminated with *Leishmania*.

According to Andrade et al. (2009) ocular changes are among the main signals that are present in dogs who are positive for Leishmaniasis, being that the most described ones are blepharitis, conjunctivitis, cerate conjunctivitis and cerate uveitis. Then, it also showed the possibility to diagnose the disease through the method of imprinting in animals with ocular lesions.

CONCLUSION

It is concluded that the exam of cytology shows efficacy in the direct detection of the protozoan of *Leishmaniasis*, being a technique of easy execution and low cost, and that presents high specificity and diagnostic precision.



REFERENCES

- ANDRADE, A. L.; SANTO, E. F. E.; SAKAMOTO, S. S.; LIMA, L. K. F.; LUVIZOTTO, M. C. R. Citologia de impressão da superfície ocular de cães infectados naturalmente por leishmaniasis (L.) chagasi. **Archives of Veterinary Science**, v. 14, n. 1, p. 9-16, 2009. <DOI: 10.5380/avs.v14i1.11951>.
- ANTUNES, T. R.; GODOY, K. C. S.; OLIVEIRA, G. G.; SILVEIRA, A. W.; RAMOS, C. A. N. R.; SOUZA, A. I. Técnicas de citologia aspirativa, biópsia e citobloco de medula óssea para identificação e determinação de intensidade parasitária na leishmaniose visceral canina. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 70, n. 5, p. 1362-1368, 2018. <DOI: 10.1590/1678-4162-9769>.
- ARRAES, S. M. A. A.; MARINI, M. T.; MARTELLO, D.; SILVEIRA, T. G. V.; LONARDONI, M. V. C.; NANNI, M. R. Investigação sorológica de casos subclínicos de leishmaniose tegumentar após um surto em uma localidade endêmica. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 41, n. 2, p. 205-208, 2008. <DOI:10.1590/S0037-86822008000200016>.
- BRASIL. Ministério da Saúde. **Manual de Vigilância e Controle da Leishmaniose Visceral**, v. 1, n. 5, p. 122, 2014.
- BRUM, L. C.; CONCEIÇÃO, L. G.; RIBEIRO, V. M.; HADDAD JUNIOR, V. Principais dermatoses zoonóticas de cães e gatos. **Clínica Veterinária**, v. 12, n. 69, p. 29-46, 2007.
- CARVALHO, M. S. L.; TAUIL, P. L. Estudo da transmissão da leishmaniose tegumentar americana no Distrito Federal. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 42, n. 6, p. 686-690, 2009. <DOI: 10.1590/S0037-86822009000600015>.
- DOTTA, S. C. N.; LOT, R. F. S.; ZAPPA, V. Métodos de diagnóstico da leishmaniose visceral canina. **Revista Científica Eletrônica de Medicina Veterinária**, v. 7, n. 12, p. 5, 2009.
- EGUCHI, G. U.; OLIVEIRA, G. G.; BABOTERRA, V. J.; SOUZA, A. I.; BARROS, R.; PALUMBO, M. I. P. Ceratoconjuntivite nodular em um caso de leishmaniose visceral canina: relato de caso. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 69, n. 6, p. 1480-1484, 2017. <DOI: 10.1590/1678-4162-9465>.
- EIRAS, D. P.; KIRKMAN, L. A.; MURRAY, H. W. Cutaneous leishmaniasis: current treatment practices in the USA for returning travelers. **Current treatment options in infectious diseases**, v. 7, n. 1, p. 52-62, 2015. <DOI: 10.1007/s40506-015-0038-4>.
- FEITOSA, M. M.; IKEDA, F. A.; LUVIZOTTO, M. C. R.; PERRI, S. H. V. Aspectos clínicos de cães com leishmaniose visceral no município de Araçatuba São Paulo, Brasil. **Clínica Veterinária**, v. 5, n. 28, p. 36-44, 2000.
- FRIAS, D. F. R.; KOZUSNY-ANDREANI, D. I. Isolamento e identificação de fungos associados à dermatofitose e dermatomicose em cães. **CES Medicina Veterinária y Zootecnia**, v. 3, n. 2, p. 58-63, 2008.
- GOMES, A. R.; MADRID, I. M.; MATOS, C. B.; TELLES, A. J.; WALLER, S. B.; NOBRE, M. O.; MEIRELES, M. C. A. Dermatopatias fúngicas: aspectos clínicos, diagnósticos e terapêuticos. **Acta Veterinaria Brasilica**, v. 6, n. 4, p. 272-284, 2012.
- GOTO, H.; LINDOSO J. A. L. Cutaneous and mucocutaneous leishmaniasis. **Infect Dis Clin North Am. PubMed**, v. 26, n. 2, p. 293-307, 2012. <DOI: 10.1016/j.idc.2012.03.001>.
- GREENE, C. E.; **Doenças infecciosas em cães e gatos**. 4. Ed. Rio de Janeiro: Guanabara Koogan, 2015.
- LACAZ, C. S.; PORTO, E.; MARTINS, J. E. C. **Micologia médica: fungos**,



actinomicetos e algas de interesse médico. 7. ed. São Paulo: Savier, 1998

MATSUMOTO, C. T.; OGAWA, M. M.; ENOKIHARA, M. M. S. S.; YARAK, S. Leishmaniose tegumentar por espécies não nativas no Brasil. **Journal of the American Academy of Dermatology**, v. 85, n. 3, p. 103, 2021. <DOI: [10.1016/j.jaad.2021.06.433](https://doi.org/10.1016/j.jaad.2021.06.433)>.

MEGID, J.; RIBEIRO, M. G.; PAES, A. C. **Doenças infecciosas em animais de produção e de companhia.** 1.ed. Rio de Janeiro: Roca, 2018.

NETTO, M. B. Imunossupressão mediada por soro na leishmaniose visceral. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 22, n. 2, p. 109-110, 1989. <DOI: [10.1590/S0037-86821989000200011](https://doi.org/10.1590/S0037-86821989000200011)>.

NEVES, R. C. S. M.; CRUZ, F. A. C. S.; LIMA, S. R.; TORRES, M. M.; DUTRA, V.; SOUSA, V. R. F. Retrospectiva das dermatofitoses em cães e gatos atendidos no hospital veterinário da universidade federal de mato grosso, nos anos de 2006 a 2008. **Ciência Rural**, v. 41, n. 8, p. 1405-1410, 2011. <DOI: [10.1590/S0103-84782011000800017](https://doi.org/10.1590/S0103-84782011000800017)>.

NOGUEIRA, J. L.; SILVA, M. V. M.; PASSOS, C. C.; AMBRÓSIO, C. E. A importância da leishmaniose visceral canina para a saúde pública: uma zoonose reemergente. **Revista Científica Eletrônica de Medicina Veterinária**, São Paulo, v. 7, n. 13, 2009.

OLIVEIRA, L. F. A. **A influência de infecção secundária e de outros fatores na cicatrização de lesões ulceradas de leishmaniose cutânea e esporotricose.** 2017. 125 f. Tese (Doutorado em Ciências da Saúde) - Instituto Nacional de Infectologia Evandro Chagas para obtenção do título de Doutora em Ciências da Saúde, Rio de Janeiro.

PEREIRA, A. A.; NOGUEIRA, A. F. S.; SANTOS, H. D.; LIMA, N. E. M. Leishmaniose felina no município de Araguaína-to. **Revista Multidisciplinar Em Saúde**, v. 2, n. 3, p. 33, 2021. <DOI: [10.51161/remis/1847](https://doi.org/10.51161/remis/1847)>.

REIS, H. R.; LOPES-MORI, F. M. R.; REIS, C. R.; FREIRE, R. L.; MARANA, E. R. M.; CHRYSSAFIDIS, A. L.; TEDIM, A. V.; RUFFOLO, B. B.; BUGNI, F. M.; CASTRO, E. A.; THOMAZ-SOCCOL, V.; NABUT, L. B.; NAVARRO, I. T. Soroprevalência da Leishmaniose Tegumentar Americana (LTA) canina e fauna de Flebotomíneos (Diptera: Psychodidae) em Bela Vista do Paraíso, Paraná State. **Semina: Ciências Agrárias**, v. 32, n. 3, p. 1083-1094, 2011. <DOI: [10.5433/1679-0359.2011v32n3p1083](https://doi.org/10.5433/1679-0359.2011v32n3p1083)>.

ROCHA, S. T. F.; SHIOSI, R. K.; FREITAS, A. B. M. Leishmaniose visceral canina -revisão de literatura. **Revista Científica de Medicina Veterinária**, São Paulo, v. 17, n. 34, p. 13, 2020.

SABATÉ, D.; LLINÁS, J.; HOMEDES, J.; SUST, M.; FERRER, L. A single-centre, open-label, controlled, randomized clinical trial to assess the preventive efficacy of a domperidone-based treatment programme against clinical canine leishmaniasis in a high prevalence area. **Preventive Veterinary Medicine**, v. 115, n. 1-2, p. 56-63, 2014. <DOI: [10.1016/j.prevetmed.2014.03.010](https://doi.org/10.1016/j.prevetmed.2014.03.010)>.

SALZO, P. S. Aspectos dermatológicos da leishmaniose canina. **Nosso clínico**, v. 11, n. 63, p. 30-34, 2008.

SAMPAIO, R. N. R.; GONÇALVES, M. C.; LEITE, V. A.; FRANÇA, B. V.; SANTOS, G.; SAMPAIO, R. N. R.; SALARO, C. P.; RESENDE, P.; PAULA, C. D. R. Leishmaniose tegumentar associada à AIDS: Relato de quatro casos. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 35, n. 6, p. 651-654, 2002. <DOI: [10.1590/S0037-86822002000600017](https://doi.org/10.1590/S0037-86822002000600017)>.

SILVA, F. S. Patologia e patogênese da leishmaniose visceral canina. **Revista Tropical -Ciencias Agrarias e Biologicas**, v. 1, n. 1, p. 20-31, 2007.

VIDES, J. P. **Infecção por leishmania chagasi em gatos com dermatopatias provenientes de área endêmica para leishmaniose visceral.** 2010. 91 f. Dissertação (Mestrado em Ciência Animal) -



Faculdade de Odontologia e Curso de Medicina Veterinária - Unesp, São Paulo.

WERNECK, G. L.; COSTA, C. H. N.; WALKER, A. M.; DAVID, J. R.; WAND, M.; MAGUIRE, J. H. The urban spread of visceral leishmaniasis: clues from spatial analysis. **Epidemiology**, v. 13, n. 3, p. 364-367, 2002. <DOI: [10.1097/00001648-200205000-00020](https://doi.org/10.1097/00001648-200205000-00020)>.

WHO. World Health Organization. **Estado de endemicidade da leishmaniose tegumentar Dados por país**. Disponível em: <<https://apps.who.int/gho/data/node.main.NTDLEISHCEND?lang=en>>. Acesso em: 08 out. 2021.