Veterinary Parasitology: Regional Studies and Reports 17 (2019) 100290

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Immunohistochemical characterization of cutaneous leishmaniasis in cats from Central-west Brazil

Selwyn Arlington Headley[a,b,*], Luciano Anunciação Pimentel[c], Izabela Ferreira Gontijo de Amorim[d], Alexandre Mendes Amude[b], Nayara Emily Viana[a], Lívia Saab Muraro[b], Wagner Luiz Tafuri[d], Marcelo Diniz dos Santos[b]

[a] Laboratory of Animal Pathology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Paraná, Brazil
[b] Faculty of Veterinary Medicine and Programa de Pós-Graduação em Biociência Animal, Universidade de Cuiabá, Mato Grosso, Brazil
[c] Laboratory of Veterinary Pathology, Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Bahia, Brazil
[d] Laboratorio de Patologia das Leishmanioses, Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Amastigotes
Immunohistochemistry
Onychogryphosis
Ulcerative dermatitis

ABSTRACT

Feline leishmaniasis (FeL) is an emerging infectious disease of cats caused by *Leishmania infantum* with global distribution. This study investigated the cause of chronic progressive cutaneous lesions in two cats from Central-west Brazil by using cytological, histopathologic, and immunohistochemical (IHC) analyses. Clinically, both cats had ulcerative cutaneous lesions at the nasal planum and ear resulting in a tentative diagnosis of squamous cell carcinoma (SCC). Moreover, both cats had varying degrees of onychogryphosis. However, cytology revealed chronic inflammatory reactions associated with intralesional amastigotes; histopathology confirmed chronic ulcerative dermatitis associated with intralesional and intracytoplasmic parasitic organisms consistent with amastigotes of *Leishmania* spp. within histiocytes. The IHC assay demonstrated that the intralesional parasitic structures identified by cytology and histopathology were immunoreactive to antigens of *Leishmania* spp., confirming the participation of this infectious disease agent in the development of the cutaneous lesions of these cats. The observation of onychogryphosis must be highlighted, since this lesion is frequently observed in dogs with visceral leishmaniasis but is underreported in FeL. Collectively, the pathologic and IHC findings of the chronic cutaneous disease confirmed active infections due to *Leishmania* spp. in these cats. Additionally, FeL with associated lesions to the ear and nasal planum must be considered as differential diagnosis for SCC in cats.

1. Introduction

Feline leishmaniasis (FeL) is an emerging infectious disease of cats caused by *Leishmania infantum*, also known as *L. chagasi* (Pennisi and Persichetti, 2018; Soares et al., 2016) with global distribution (Soares et al., 2016), while cases have also been associated with other species including *L.* (*Viannia*) *brasiliensis* (Schubach et al., 2004), *L. amazonensis* (Souza et al., 2009), and *L. mexicana* (Minard et al., 2017; Trainor et al., 2010). Serological surveys done worldwide have determined that the prevalence of FeL varies from 4.2% in São Paulo, Brazil (Coelho et al., 2011) to 60% in Southern Spain (Martín-Sánchez et al., 2007). Although the possibility of cats being reservoirs for *Leishmania* spp. exists (Maia and Campino, 2011; Pennisi, 2015; Soares et al., 2016), this hypothesis remains controversial (Maia and Campino, 2011), but is gaining force worldwide due to the frequent reports of FeL from endemic regions (Pennisi, 2015), and a combination of factors that favours this animal as a potential reservoir host (Soares et al., 2016).

Most studies that have evaluated the occurrence of FeL worldwide used serological investigations (Almeida et al., 2012; Braga et al., 2014; Cardia et al., 2013; Coelho et al., 2010, 2011). Additional diagnostic methods included molecular characterization (Dahroug et al., 2011; Metzdorf et al., 2017), immunofluorescence assays (Souza et al., 2005), histopathology combined with immunohistochemistry, IHC (Navarro et al., 2010), and a combination of several diagnostic methods (Freitas et al., 2012; Vides et al., 2011). Serological studies are effective in confirming that the animal had contact with the infectious disease agent but does not necessarily imply that the animal is actively infected and thus clinically compromised. Alternatively, molecular

* Corresponding author at: Laboratory of Animal Pathology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR 445 Km 380, Campus Universitário, PO Box 10.011, 86057-970 Paraná, Brazil.
E-mail address: selwyn.headley@uel.br (S.A. Headley).

https://doi.org/10.1016/j.vprsr.2019.100290
Received 29 August 2018; Received in revised form 19 March 2019; Accepted 17 April 2019
Available online 19 April 2019

identification alone confirms infection and exposure to the disease agent (Maia et al., 2010), but does not necessarily confirm that the animal has a clinical disease. Therefore, molecular identification to confirm disease should be associated with histopathologic identification of typical findings in the affected animal, thereby confirming an active infection, since the results of molecular testing does not necessarily correlate with infection (Di Mattia et al., 2018).

Accordingly, we have developed an IHC assay for the identification of *Leishmania* amastigotes in formalin-fixed paraffin-embedded (FFPE) tissue sections of dogs by using an hyperimmune serum derived from naturally infected dogs (Tafuri et al., 2004). The efficiency of this method was then confirmed and ratified (Amorim et al., 2011). Histopathology associated with the IHC identification of intralesional *Leishmania* amastigotes efficiently characterizes the animal as being actively infected; thereby, correlating with the pathogenesis of infection (Di Mattia et al., 2018). This report presents the pathologic and IHC findings associated with active manifestations of FeL in cats from Mato Grosso, Central-west, Brazil.

2. Material and methods

2.1. Study location, animals, and clinical history

Two female, short hair domestic cats were seen at the Veterinary Teaching Hospital, Universidade de Cuiabá, Mato Grosso, Central-west Brazil due to multiple progressive cutaneous lesions. Both cats originated from the urban region of Cuiabá. This city has a moderate population (22.1%; 95/430) of dogs that are serologically reactive to *Leishmania* spp. (Almeida et al., 2012), and is located in the State of Mato Grosso where the number of cases of human visceral leishmaniosis (VL) has increased drastically between 2001 and 2014 (Reis et al., 2017). Cat #1 was 2 years of age, while cat #2 was 4 years of age. Both cats had reported histories of chronic (3–4 months), progressive unresolved dermatological disease and were clinically examined.

Based on the anatomical location of the lesions a presumptive diagnosis of squamous cell carcinoma (SCC) was established; samples from the ear and nasal planum were collected to confirm this hypothesis using a combination of cytological, histopathologic, and IHC diagnostic methods.

2.2. Cytologic, histopathologic, and immunohistochemistry analyses

Fine needle aspiration cytology (FNAC) was done on all cutaneous masses; imprints were obtained on all ulcerative lesions and submitted for cytological evaluation. The cutaneous masses at the nostrils and ears were surgically incised and submitted for routine histopathologic evaluation with the Haematoxylin and Eosin (H&E) stain.

Additionally, selected FFPE tissue sections of the auricular and nasal masses were submitted for the IHC detection of amastigotes of *Leishmania* spp. by using a hyperimmune serum derived from an experimentally infected dog, as the primary antibody (Tafuri et al., 2004). Positive controls consisted of FFPE tissue sections known to contain *Leishmania* spp. amastigotes from previous studies (Amorim et al., 2011; Tafuri et al., 2004); for negative controls the primary antibody was substituted by phosphate buffered saline. Positive and negative controls were included in each IHC assay.

3. Results

3.1. Clinical evaluation and gross findings

The clinical evaluation revealed that both cats had multiple cutaneous lesions, were dyspnoeic due to severe nasal ulcerations, febrile (39.8 °C), and tachypnoeic. Cat #1 had an erosive lesion at the nasal planum, a cutaneous nodule at the right ear, and erosive and erythematous masses at the extremities of the fore- and hindlimbs (Fig. 1A–B). Cat #2 had severe ulcerative lesions at the right ear and the nasal planum. Moreover, both cats had varying degrees of onychogryphosis and crusting lesions at the interdigital spaces of all members; other cutaneous lesions were not observed in both animals.

3.2. Cytologic, histopathologic, and immunohistochemical findings

Analysis of FNAC and cytologic imprints revealed chronic inflammatory reactions due to the severe accumulations of macrophages associated with intracytoplasmic accumulations of amastigotes within macrophages.

The histopathologic evaluation of all tissues from both cats was similar with mild differences between anatomical locations evaluated and consisted of chronic, severe, ulcerative dermatitis associated with intralesional parasitic structures consistent with *Leishmania* amastigotes (Navarro et al., 2010; Pennisi et al., 2015). However, the parasitic infection was more intense in the tissues of cat # 1. These structures were rounded to oval and were predominantly intracytoplasmic within histiocytes (Fig. 1C–D). By IHC, there was widespread positive immunoreactivity to all parasitic structures identified as *Leishmania* spp. amastigotes by H&E in tissues from both cats (Fig. 1G–H); amastigotes were intracytoplasmic within infected macrophages.

4. Discussion

The pathologic and immunohistochemical findings observed in these cats are consistent with previous descriptions of leishmaniasis in companion animals (Di Mattia et al., 2018; Freitas et al., 2012; Navarro et al., 2010). Moreover, the ulcerative lesions at the nasal planum and ear observed in these two cats were described in cases of FeL associated with *L. infantum* (Navarro et al., 2010; Savani et al., 2004; Vides et al., 2011), *L.* (*Viannia*) *brasiliensis* (Schubach et al., 2004), *L. amazonensis* (Souza et al., 2009), *L. mexicana* (Minard et al., 2017; Trainor et al., 2010), and in a cat with oral lesions associated with *L. infantum* (Migliazzo et al., 2015). However, during this investigation, the diagnosis of FeL was done by IHC using a polyclonal antibody (Tafuri et al., 2004), and as such, the species of *Leishmania* associated with these cats cannot be confirmed based on this diagnostic strategy. It must be highlighted that seropositivity of dogs to *L. infantum* associated with VL from the city of Cuiabá, within the state of Mato Gross, where these cats were housed, varied from 64.5% (40/62) in 1998 (Moura et al., 1999) to 22.1% (95/430) in 2010 (Almeida et al., 2012). Furthermore, *L. infantum* DNA was identified in an asymptomatic lion (*Panthera leo*) maintained in a zoological park within the same city (Dahroug et al., 2011). Additionally, in 1998 there was an epidemic of VL in the State of Mato Grosso, during which 2.6–11.2/100,000 cases occurred in humans and 9% (3600/40,000) of the dogs evaluated were seropositive for *L. infantum* (Mestre and Fontes, 2007). Furthermore, cats may be infected by the same species of *Leishmania* that occurs in humans and other animals within a specific geographical area (Pennisi et al., 2015), and FeL normally occurs in an endemic zone of zoonotic VL (Gramiccia, 2011). Consequently, we can postulate that these cats were probably infected by *L. infantum*, which is the type of *Leishmania* occurring in animals (Almeida et al., 2012; Dahroug et al., 2011; Moura et al., 1999) and humans (Mestre and Fontes, 2007; Reis et al., 2017) within this region.

The ulcerative lesions at the nasal planum and ear of the cats from this study, are frequent clinical manifestations associated with SCC in cats (Thomson, 2007), while the nasal lesions can be confused with sporotrichosis (Montenegro et al., 2014; Santos et al., 2007). Interestingly, in these cats the clinical diagnosis of SCC was based on the type of lesions and their anatomical locations, and a diagnosis of FeL was only established after cytological evaluation revealed parasitic structures and not neoplastic cells. This neoplastic growth is of utmost interest in cats residing in regions of constant sunshine since this malignant lesion is associated with exposure to UV rays; solar exposure in the

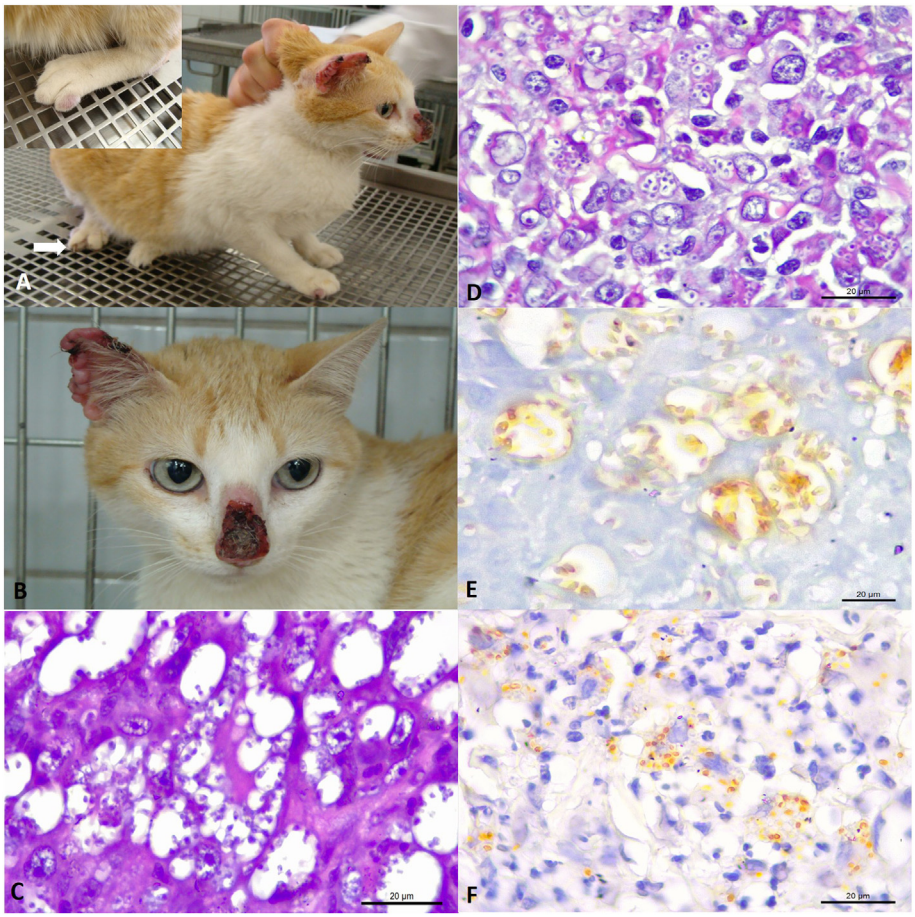

Fig. 1. Composite images of clinical, pathologic, and immunohistochemical findings associated with cutaneous leishmaniasis in cats. Observe the cutaneous lesions at the nasal planum and ear of cat # 1 (A-B), with onychogryphosis (white arrow) and the insert highlighting the erythematous nodule. Histopathological demonstration of chronic dermatitis with intralesional amastigotes from cats # 1 (C) and 2 (D). There is positive immunoreactivity to antigens of *Leishmania* spp. in ulcerative dermatitis of the ear cats #1 (E) and #2 (F). Haematoxylin and eosin stain, C-D, bar = 20 µm; Immunoperoxidase counterstained with Haematoxylin (E-F), bar = 20 µm.

city of Cuiabá is throughout the entire year (Nascimento et al., 2016). Moreover, a synergism between FeL and SCC was proposed in which the parasitic infection possibly initiates cellular transformation, while the development of the neoplasm is facilitated by the proliferation of the intralesional parasite (Soares et al., 2016). Sporotrichosis is an emerging mycotic zoonotic disease that produces gross lesions that cannot be easily differentiated from those of cutaneous leishmaniasis; this was demonstrated in a study where 55% (41/74) of dogs with ulcerative cutaneous lesions were in fact infected by *Sporothrix schenckii*, while three of these were seropositive for leishmaniosis (Santos et al., 2007). Therefore, cutaneous leishmaniasis in cats should be considered as a possible differential diagnosis for SCC, particularly for cats that are constantly exposed to solar radiation.

The cutaneous lesions and onychogryphosis observed in these cats were previously described in dogs with spontaneous visceral leishmaniasis (Freitas et al., 2012; Pennisi and Persichetti, 2018; Petersen, 2009). Onychogryphosis, as occurred in these cats was observed in 33.8% (Freitas et al., 2012) and 58.9% (Silva et al., 2017) of dogs naturally infected with *L. chagasi*, but dogs experimentally infected did not demonstrate this lesion even after five years of clinical observations (Abbehusen et al., 2017). Additionally, dogs with onychogryphosis are at elevated risk to develop visceral leishmaniasis (Silva et al., 2017). Alternatively, a review of FeL revealed that this common cutaneous feature of visceral leishmaniasis was not previously reported in FeL (Pennisi and Persichetti, 2018); while onychogryphosis was mistakenly associated with the clinical manifestations of feline leukaemia virus, FLV (Silva et al., 2010). Consequently, onychogryphosis should be included as a possible clinical manifestation of FeL, since the cat with onychogryphosis was FLV negative but contained nuclei acids of FeL in multiple organs by molecular testing (Silva et al., 2010). Additionally, both cats were febrile; elevated body temperature was described in more than 25% of the cases of FeL (Pennisi and Persichetti, 2018).

5. Conclusion

Cutaneous leishmaniasis was diagnosed in two cats from Central-west Brazil that were initially thought to have squamous cell carcinoma at the nasal planum and ear. However, FeL was only diagnosed due to a combination of cytologic, pathologic, and immunohistochemical findings. Both cats had varying degrees of onychogryphosis which facilitated a clinical diagnosis of cutaneous leishmaniasis. It is recommended that onychogryphosis be a potential indicator of FeL, particularity in endemic regions of this disease. Finally, epidemiologic data suggest that these cats were probably infected by *L. infantum*.

Conflict of interest

The authors declare that they have no conflict of interest relative to the preparation or publication of this manuscript.

Acknowledgements

Selwyn A. Headley and Wagner L. Tafuri are recipients of the National Council for Scientific and Technological Development (CNPq; Brazil) fellowships and grants. The authors thank the owners of the cats for permission to use their pets during this report.

References

Abbehusen, M.M.C., Almeida, V.d.A., Solcà, M.d.S., Pereira, L.d.S., Costa, D.J., Gil-Santana, L., Bozza, P.T., Fraga, D.B.M., Veras, P.S.T., dos-Santos, W.L.C., Andrade, B.B., Brodskyn, C.I., 2017. Clinical and immunopathological findings during long term follow-up in *Leishmania infantum* experimentally infected dogs. Sci. Rep. 7,

15914.
Almeida, A.B.P.F., Sousa, V.R., Cruz, F.A., Dahroug, M.A., Figueiredo, F.B., Madeira, M.F., 2012. Canine visceral leishmaniasis: seroprevalence and risk factors in Cuiabá, Mato Grosso, Brazil. Rev. Bras. Parasitol. Vet. 21, 359–365.
Amorim, I.F., Silva, S.M., Figueiredo, M.M., Moura, E.P., Castro, R.S., Lima, T.K., Gontijo Nde, F., Michalick, M.S., Gollob, K.J., Tafuri, W.L., 2011. Toll receptors type-2 and CR3 expression of canine monocytes and its correlation with immunohistochemistry and xenodiagnosis in visceral leishmaniasis. PLoS One 6, e27679.
Braga, A.R., Correa, A.P., Camossi, L.G., Silva, R.C., Langoni, H., Lucheis, S.B., 2014. Coinfection by *Toxoplasma gondii* and *Leishmania* spp. in domestic cats (*Felis catus*) in State of Mato Grosso do Sul. Rev. Soc. Bras. Med. Trop. 47, 796–797.
Cardia, D.F., Camossi, L.G., Neto Lda, S., Langoni, H., Bresciani, K.D., 2013. Prevalence of *Toxoplasma gondii* and *Leishmania* spp. infection in cats from Brazil. Vet. Parasitol. 197, 634–637.
Coelho, W.M., Lima, V.M., Amarante, A.F., Langoni, H., Pereira, V.B., Abdelnour, A., Bresciani, K.D., 2010. Occurrence of *Leishmania* (*Leishmania*) chagasi in a domestic cat (*Felis catus*) in Andradina, Sao Paulo, Brazil: case report. Rev. Bras. Parasitol. Vet. 19, 256–258.
Coelho, W.M., Amarante, A.F., Apolinario, J.C., Coelho, N.M., Lima, V.M., Perri, S.H., Bresciani, K.D., 2011. Seroepidemiology of *Toxoplasma gondii*, *Neospora caninum*, and *Leishmania* spp. infections and risk factors for cats from Brazil. Parasitol. Res. 109, 1009–1013.
Dahroug, M.A., Almeida, A.B., Sousa, V.R., Dutra, V., Guimaraes, L.D., Soares, C.E., Nakazato, L., Souza, R.L., 2011. The first case report of *Leishmania* (*leishmania*) *chagasi* in *Panthera leo* in Brazil. Asian Pac. J. Trop. Biomed. 1, 249–250.
Di Mattia, D., Fondevila, D., Abramo, F., Fondati, A., 2018. A retrospective histopathological, immunohistochemical and molecular study of the presence of *Leishmania* spp. in the skin of cats with head and neck ulcerative dermatitis. Vet. Dermatol. 29, 212–e276.
Freitas, J.C., Nunes-Pinheiro, D.C., Lopes Neto, B.E., Santos, G.J., Abreu, C.R., Braga, R.R., Campos Rde, M., Oliveira, L.F., 2012. Clinical and laboratory alterations in dogs naturally infected by *Leishmania chagasi*. Rev. Soc. Bras. Med. Trop. 45, 24–29.
Gramiccia, M., 2011. Recent advances in leishmaniosis in pet animals: epidemiology, diagnostics and anti-vectorial prophylaxis. Vet. Parasitol. 181, 23–30.
Maia, C., Campino, L., 2011. Can domestic cats be considered reservoir hosts of zoonotic leishmaniasis? Trends Parasitol. 27, 341–344.
Maia, C., Gomes, J., Cristovao, J., Nunes, M., Martins, A., Rebelo, E., Campino, L., 2010. Feline leishmania infection in a canine leishmaniasis endemic region, Portugal. Vet. Parasitol. 174, 336–340.
Martín-Sánchez, J., Acedo, C., Muñoz-Pérez, M., Pesson, B., Marchal, O., Morillas-Márquez, F., 2007. Infection by *Leishmania infantum* in cats: epidemiological study in Spain. Vet. Parasitol. 145, 267–273.
Mestre, G.L.C., Fontes, C.J.F., 2007. A expansão da epidemia da leishmaniose visceral no Estado de Mato Grosso, 1998-2005. Rev. Soc. Bras. Med. Trop. 40, 42–48.
Metzdorf, I.P., Costa Lima, M.S.J., Fatima Cepa Matos, M., Souza Filho, A.F., Souza Tsujisaki, R.A., Franco, K.G., Shapiro, J.T., Almeida Borges, F., 2017. Molecular characterization of *Leishmania infantum* in domestic cats in a region of Brazil endemic for human and canine visceral leishmaniasis. Acta Trop. 166, 121–125.
Migliazzo, A., Vitale, F., Calderone, S., Puleio, R., Binanti, D., Abramo, F., 2015. Feline leishmaniosis: a case with a high parasitic burden. Vet. Dermatol. 26, 69–70.
Minard, H.M., Daniel, A.K., Pool, R.R., Snowden, K.F., Levine, G.J., 2017. Pathology in practice. Cutaneous leishmaniasis. J. Am. Vet. Med. Assoc. 251, 57–59.
Montenegro, H., Rodrigues, A.M., Dias, M.A.G., Silva, E.A., Bernardi, F., Camargo, Z.P., 2014. Feline sporotrichosis due to *Sporothrix brasiliensis*: an emerging animal infection in São Paulo, Brazil. BMC Vet. Res. 10, 269.
Moura, S.T., Fernandes, C.G.N., Pandolpho, V.C., Rodrigues e Silva, R., 1999. Diagnóstico de leishmaniose canina na área urbana do município de Cuiabá, Estado de Mato Grosso, Brasil. Braz. J. Vet. Res. Anim. Sci. 36, 101–102.
Nascimento, C.S., Galiza, G.J.N., Mattos Carvalho, A., Rocha Guedes, K.M., Pimentel, L.A., Santos, M.D., Headley, S.A., 2016. Survey of cutaneous neoplastic and non-neoplastic lesions of horses from central-West Brazil. J. Equine Vet. Sci. 38, 48–53.
Navarro, J.A., Sanchez, J., Penafiel-Verdu, C., Buendia, A.J., Altimira, J., Vilafranca, M., 2010. Histopathological lesions in 15 cats with leishmaniosis. J. Comp. Pathol. 143, 297–302.
Pennisi, M.G., 2015. Leishmaniosis of companion animals in Europe: an update. Vet. Parasitol. 208, 35–47.
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., Miro, G., Oliva, G., Solano-Gallego, L., 2015. LeishVet update and recommendations on feline leishmaniosis. Parasit. Vectors 8, 302.
Petersen, C.A., 2009. Leishmaniasis, an emerging disease found in companion animals in the United States. Top. Companion Anim. Med. 24, 182–188.
Reis, L.L., Balieiro, A.A.S., Fonseca, F.R., Gonçalves, M.J.F., 2017. Changes in the epidemiology of visceral leishmaniasis in Brazil from 2001 to 2014. Rev. Soc. Bras. Med. Trop. 50, 638–645.
Santos, I.B., Schubach, T.M.P., Leme, L.R.P., Okamoto, T., Figueiredo, F.B., Pereira, S.A., Quintella, L.P., Madeira, M.F., Coelho, F., Reis, R.S., Schubach, A.O., 2007. Sporotrichosis - the main differential diagnosis with tegumentary leishmaniosis in dogs from Rio de Janeiro, Brazil. Vet. Parasitol. 143, 1–6.
Savani, E.S.M.M., Oliveira Camargo, M.C.G., Carvalho, M.R., Zampieri, R.A., Santos, M.G., D'Áuria, S.R.N., 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.
Schubach, T.M., Figueiredo, F.B., Pereira, S.A., Madeira, M.F., Santos, I.B., Andrade, M.V., Cuzzi, T., Marzochi, M.C., Schubach, A., 2004. American cutaneous leishmaniasis in two cats from Rio de Janeiro, Brazil: first report of natural infection with *Leishmania* (*Viannia*) *braziliensis*. Trans. R. Soc. Trop. Med. Hyg. 98, 165–167.
Silva, S.M., Rabelo, P.F., Gontijo Nde, 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, K.R., Mendonça, V.R.R., Silva, K.M., Nascimento, L.F.M., Mendes-Sousa, A.F., Pinho, F.A., Barral-Netto, M., Barral, A.M.P., Cruz, M.S.P.E., 2017. Scoring clinical signs can help diagnose canine visceral leishmaniasis in a highly endemic area in Brazil. Mem. Inst. Oswaldo Cruz 112, 53–63.
Soares, C.S., Duarte, S.C., Sousa, S.R., 2016. What do we know about feline leishmaniosis? J. Feline Med. Surg. 18, 435–442.
Souza, A.I., Barros, E.M.S., Ishikawa, E., Novaes Ilha, I.M., Barbosa Marin, G.R., Brandão Nunes, V.L., 2005. Feline leishmaniasis due to *Leishmania* (*Leishmania*) *amazonensis* in Mato Grosso do Sul state, Brazil. Vet. Parasitol. 128, 41–45.
Souza, A.I., Nunes, V.L.B., Borralho, V.M., Ishikawa, E.A.Y., 2009. Domestic feline cutaneous leishmaniasis in the municipality of Ribas do Rio Pardo, Mato Grosso do Sul state, Brazil: a case report. J. Venom Anim. Toxins Incl. Trop. Dis. 15, 359–365.
Tafuri, W.L., Santos, R.L., Arantes, R.M., Goncalves, R., Melo, M.N., Michalick, M.S., Tafuri, W.L., 2004. An alternative immunohistochemical method for detecting *Leishmania* amastigotes in paraffin-embedded canine tissues. J. Immunol. Methods 292, 17–23.
Thomson, M., 2007. Squamous cell carcinoma of the nasal planum in cats and dogs. Clin. Tech. Small Anim. Pract. 22, 42–45.
Trainor, K.E., Porter, B.F., Logan, K.S., Hoffman, R.J., Snowden, K.F., 2010. Eight cases of feline cutaneous leishmaniasis in Texas. Vet. Pathol. 47, 1076–1081.
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.