Clinical and epidemiological aspects of feline leishmaniasis in Brazil

Aspectos clínicos e epidemiológicos da leishmaniose felina no Brasil

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Abstract

Tegumentary and visceral leishmaniasis are severe and unfortunately common parasitic diseases in Brazil. Among domestic animals, dogs are considered the main urban reservoir of the protozoan parasites, however, there is evidence that infected cats can also contribute towards the disease pool. The number of cats diagnosed with leishmaniasis has greatly increased in the last few years, highlighting the importance of thorough investigations on the role of the cat in the epidemiological cycle of the disease and in public health related issues. The main clinical manifestations of leishmaniasis suffered by cats, even when infected with *Leishmania chagasi*, a viscerotropic species, are skin abnormalities, which can be confounded with multiple other diseases. Indirect ELISA should be used as a screening test in epidemiological investigations for being a sensitive technique, followed by more specific laboratory tests. The standardization and validation of rapid, economical and reproducible diagnostic methods, to be employed in epidemiological surveillance, are still required.
Key words: *Leishmania* spp., cat, epidemiology, diagnosis, zoonosis, prevalence

Resumo

Leishmaniose tegumentar e visceral são doenças parasitárias graves e, infelizmente, comuns no Brasil. Entre os animais domésticos, o cão é considerado o principal reservatório urbano do parasito protozoário; no entanto, há indícios de que gatos infectados também possam contribuir para essas doenças. O número de gatos com diagnóstico de leishmaniose aumentou muito nos últimos anos, destacando a importância de investigações aprofundadas sobre o papel desse hospedeiro no ciclo epidemiológico da doença e em Saúde Pública. As principais manifestações clínicas da leishmaniose felina são anormalidades na pele, o que pode ser confundida com várias outras doenças, mesmo em casos de infecção por *Leishmania chagasi*, uma espécie viscerotrópica. ELISA indireto poderia ser usado como teste de triagem em investigações epidemiológicas, por ser um método sensível, seguido de exames laboratoriais mais específicos. A padronização e validação de métodos de diagnóstico rápidos, econômicos e reprodutíveis a serem empregados na vigilância epidemiológica ainda são necessárias.
Palavras-chave *Leishmania* spp., gato, epidemiologia, diagnóstico, zoonose, prevalência

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Introduction

Leishmaniasis is a protozoan disease caused by parasites of the genus *Leishmania*. Leishmaniasis is commonly classified in two clinical forms: tegumentary leishmaniasis (TL) or visceral leishmaniasis (VL), depending on the species of *Leishmania* causing the infection (CASTRO, 1996; DESJEUX, 2004; BRASIL, 2006a, 2006b).

The main route of VL transmission in America is through blood meals of the female *Lutzomyia longipalpis* and *Lutzomyia cruzi species*, which belong to the *Psychodidae* family (CHAGAS, 1940; SANTOS et al., 1998). Among domestic animals, dogs are the main urban reservoir of the protozoan parasite (ALENCAR et al., 1991). Hence, to decrease the spread of the protozoa and the incidence of human VL, this host is the target of stringent actions: early diagnosis and euthanasia of seropositive dogs (BRASIL, 2014).

Recently, reports of feline leishmaniasis increased dramatically, achieving a prevalence of up to 61% in certain cat populations (PENNISI et al., 2000). This phenomenon could be explained by three hypotheses: increase in the active investigation of the protozoan in this host, improvement of the diagnostic techniques, and current increase in the rate of disease prevalence in domestic cats.

The aim of this literature review is to describe the etiology, prevalence, clinical signs, and diagnosis of feline leishmaniasis, and highlight the importance of cats as a leishmaniasis host in Brazil.

Historical background

The first feline infection with *Leishmania* sp. was described in Algeria (SERGENT et al., 1912). In the subsequent years, researchers continued to actively investigate the presence of the protozoan in cats in Italy (GIORDANO, 1933), Spain (GIMENO-ONDOVILLA, 1933) and Brazil (DEANE, 1956).

In the 80s, after the cytological detection of *Leishmania* sp. in 20.5% of 78 samples of feline spleens and livers, the highest sample prevalence until then, diagnostic studies of leishmaniasis in cats were intensified (MORSY et al., 1980). In 1985, the first case of co-infection with HIV and *Leishmania donovani* was reported in humans, an event that led the World Health Organization to significantly increase *Leishmania* epidemiological surveillance (WHO, 2007). Therefore, in mid-2000, research on feline leishmaniasis was intensified both at international level (Table 1) and in Brazil (Table 2).

Table 1. Chronological order of the occurrence of feline leishmaniasis in several countries illustrated by description of cases (D) or epidemiological studies (I), using different diagnostic techniques.

Country	D/I	Technique[1]	Prevalence (%)	Reference
Algeria	D	-	1/1 (100)	(SERGENT et al., 1912)
Italy	I	Cytol., Hist.	0/120 (0.0)	(GIORDANO, 1933)
Spain	I	-	1/495 (0.2)	(GIMENO-ONDOVILLA, 1933)
Jordan	I	Cytol.	16/78 (20.5)	(MORSY et al., 1980)
Egypt	I	IH	3/80 (3.7)	(MICHAEL et al., 1982)
Egypt	I	IH	1/28 (3.6)	(MORSY et al., 1988)
France	I	IFAT	1/174 (0.6)	(BEZ; CHAUVE, 1992)
Egypt	I	IH	2/60 (3.3)	(MORSY; ABOU El SEOUD, 1994)
France	I	WB	12/97 (12.4)	(OZON et al., 1998)
Spain	D	Cytol.,RIFI	2/2 (100)	(HERVÁS et al., 1999)
Italy	I	PCR, IFAT	54/89 (60.6)	(PENNISI et al., 2000)

continuação

Country	D/I	Technique[1]	Prevalence (%)	Reference
Italy	I	IFAT	1/110 (0.9)	(POLI et al., 2002)
Spain	D	IFAT, PCR	4/4 (100)	(PENNISI et al., 2004)
Italy	I	IFAT	33/203 (16.3)	(VITA et al., 2005)
Spain	D	ELISA	1/1 (100)	(LEIVA et al., 2005)
Switzerland	D	Hist., PCR, ELISA	2/2 (100)	(RÜFENACHT et al., 2005)
Mediterranean	I	ELISA – protein A	28/445 (6.3)	(SOLANO-GALLEGO et al., 2007)
		ELISA – IgG	23/445 (5.3)	
Spain	I	IFAT	52/183 (28.3)	(MARTÍN-SÁNCHEZ et al., 2007)
		PCR-ELISA	47/183 (25.7)	
Israel	I	ELISA	7/104 (6.7)	(NASEREDDIN et al., 2008)
Spain	I	IFAT	3/233 (1.3)	(AYLLON et al., 2008)
		PCR	1/233 (0.4)	
Spain	I	PCR	3/100 (3.0)	(TABAR et al., 2008)
Portugal	I	IFAT	0/20 (0.0)	(MAIA et al., 2008)
		PCR	7/23 (30.4)	
Iran	I	IFAT, PCR, Cytol., Cult.	4/40 (10.0)	(HATAM et al., 2010)
Greece	I	ELISA	11/284 (3.9)	(DIAKOU et al., 2009)
Portugal	I	DAT	6/316 (1.9)	(CARDOSO et al., 2010)
		ELISA	9/316 (2.8)	
French Guiana	D	PCR	1/1 (100)	(ROUGERON et al., 2011)
Mexico	I	ELISA-CAG	17/95 (17.9)	(LONGONI et al., 2012)
		ELISA-Fe-SODe	34/95 (35.8)	

[1]Cytol.: cytology, Hist.: histopathology, IH: indirect hemagglutination, WB: western blot, RIFI: indirect fluorescence antibody test, PCR: polymerase chain reaction, ELISA: enzyme-linked immunosorbent assay, DAT: direct agglutination test, ELISA-CAG: crude antigen ELISA, Cult.: isolation and biological culture, ELISA-Fe-SODe: iron superoxide dismutase secreted antigen ELISA.

Table 2. Chronological order of the occurrence of feline leishmaniasis illustrated by description of cases (D) or epidemiological studies (I), using different diagnostic methods in Brazil.

D/I	Technique[1]	Prevalence (%)	Reference
I	Cytol.	1/202 (0.5)	(CHAGAS et al., 1938)
I	Cytol.	0/142 (0.0)	(DEANE, 1956)
I	Hist.	1/53 (1.9)	(SHERLOCK, 1996)
I	IFAT	0/53 (0.0)	(SHERLOCK, 1996)
I	ELISA	9/84 (10.7)	(SIMÕES-MATTOS et al., 2001)
D	Cytol.,PCR	1/1 (100)	(SAVANI et al., 2004)
D	Cult., Hist.	2/2 (100)	(SCHUBACH et al., 2004)
D	Cytol.	1/1 (100)	(de SOUZA et al., 2005)
I	IFAT	2/8 (25.0)	(da SILVA et al., 2008)
I	PCR	2/8 (25.0)	(SILVA et al., 2008)
D	IFAT, ELISA, PCR	1/1 (100)	(SERRANO et al., 2008)
D	Cytol.	1/1 (100)	(de SOUZA et al., 2009)
I	IFAT	0/43 (0.0)	(FIGUEIREDO et al., 2009)
I	ELISA	1/43 (2.4)	(FIGUEIREDO et al., 2009)
I	Cytol.	8/200 (4.0)	(COSTA et al., 2010)

continuação

D/I	Technique[1]	Prevalence (%)	Reference
I	ELISA	23/200 (11.5)	(COSTA et al., 2010)
I	Cytol.	2/283 (0.7)	(BRESCIANI et al., 2010)
I	IFAT	0/283 (0.0)	(BRESCIANI et al., 2010)
I	ELISA-CAG	26/113 (23.0)	(da SILVEIRA NETO et al., 2011)
I	ELISA-FML	15/113 (13.3)	(SILVEIRA NETO et al., 2011)
I	ELISA-rK39	18/113 (15.9)	(VIDES et al., 2011)
	ELISA	14/55 (25.4)	
	RIFI	6/55 (10.9)	
I	IH	9/55 (16.4)	(VIDES et al., 2011)
I	Cytol.	10/55 (18.2)	(COELHO et al., 2011)
	PCR	3/52 (5.8)	
I	Cytol.	30/302 (9.9)	(SOBRINHO et al., 2012)
	ELISA	39/302 (12.9)	(SOBRINHO et al., 2012)
I	RIFI	14/302 (4.6)	(CARDIA et al., 2013)
I	RIFI	2/386 (0.5)	

[1]Citol.: cytology, Histopathol.: histopathology, IH: indirect hemagglutination, DAT: direct agglutination test, IFAT: indirect fluorescence antibody test, IH: immunohistochemistry, ELISA: enzyme-linked immunosorbent assay, PCR: polymerase chain reaction, Cult.: isolation and biological culture, ELISA-CAG: crude antigen ELISA, ELISA-FML: fucose-mannose ligand antigen ELISA, ELISA-rK9: recombinant K39 antigen ELISA.

Etiology

The causative agent of leishmaniasis belongs to the kingdom Excavata, superphylum Discoba, phylum Euglenozoa, class Kinetoplastea, subclass Metakinetoplastina, order Trypanosomatida, suborder Trypanosomatina, family Trypanosomatidae, order Trypanosomatidae, family Metakinetoplastina, and genus *Leishmania* (ADL et al., 2012), which is divided in two subgenera, *Leishmania* and *Viannia*, according to the location of the protozoa in the digestive tract of the vector (WHO, 2010).

The *Leishmania* subgenus includes four complexes: *Leishmania donovani* (species: *L. donovani*, *L. archibaldi*, and *L. infantum* [syn. *L. chagasi*]), *Leishmania. tropica* (species: *L. tropica*, *L. killicki*, and *L. aethiopica*), *Leishmania. major* (species: *L. major, L. gerbilli, L. arabica*, and *L. turanica*) and *Leishmania mexicana* (species: *L. mexicana* [syn. *L. pifanoi*], *L. amazonensis* [syn. *L. garnhami*], *L. aristidesi*, *L. venezuelensis*, and *L. forattini*). Although *L. enrietti* belongs to this

subgenus, it is not yet included in any of these complexes. Among the mentioned species, only infections caused by the *L. mexicana* complex and *L. chagasi* species have been reported in the New World (SCHÖNIAN et al., 2010).

The *Viannia* subgenus, which only causes infections on the New World, includes two complexes: *Leishmania braziliensis* (species: *L. braziliensis* and *L. peruviania*) and *Leishmania guyanensis* (species: *L. guyanensis*, *L. panamensis*, and *L. shawi*). Other species, such as *L. naiffi*, *L. lainsoni*, *L. lindenbergi*, and *L. utingensis*, also belong to this subgenus, but have not yet been classified into any particular complex (SCHÖNIAN et al., 2010).

Five other *Leishmania* species (*L. colombiensis*, *L. equatoriensis*, *L. hertigi*, *L. herreri*, and *L. deanei* (SCHÖNIAN et al., 2010)), which due to their low homology do not belong to either of these subgenera, are known as Paraleishmania (CUPOLILLO et al., 2000).

Currently, the taxonomy of the *Leishmania* genus is highly debated. In the future, the classification of this protozoan would probably benefit from a molecular characterization and classification based on genome sequencing (FRAGA et al., 2010; SCHÖNIAN et al., 2010; VAN DER AUWERA et al., 2011).

Epidemiology and Public Health

Leishmaniasis occurs in 98 countries (WHO, 2010), affecting 1.2 million (tegumentary leishmaniasis) and 400 thousand (visceral leishmaniasis) people, thus leading to approximately 40 thousand deaths per year. TL is distributed worldwide but VL affects mainly (90% of cases) six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil (ALVAR et al., 2012).

Between 1980 and 2005, more than 59 thousand cases of VL infections were reported in Brazil, 83% of which were diagnosed in the Northeastern region of the country. In the 80s, human *Leishmania* infections were reported in 19 states of Brazil. In 2003, 45% of the infections were reported in the Northern region, predominantly in the states of Pará, Amazonas and Rondônia, 26% of the infections were reported in the Northeastern region, mainly affecting the states of Maranhão, Bahia and Ceará, 15% of the infections were reported in the Midwestern region, mainly affecting the state of Mato Grosso, 11% of the infections were reported in the Southeastern region, mainly affecting the state of Minas Gerais and 3% of the infections were reported in the Southern region, mainly affecting the state of Paraná. Currently, the disease is distrubuted throughout the entire national territory (BRASIL, 2006b; MAIA-ELKHOURY et al., 2008).

In Brazil, VL is caused by *L. chagasi* (BRASIL, 2014) while TL is caused by *L. braziliensis*, *L. guyanensis*, *L. amazonensis, L. lainsoni*, *L. naiffi*, and *L. shawi* (BRASIL, 2006a). Among these species, three have already been isolated in Brazilian cats: *L. braziliensis* (SCHUBACH et al., 2004), *L.*

amazonensis (SOUZA et al., 2005), and *L. chagasi* (VIDES et al., 2011), whereas *L. venezuelensis* (BONFANTE-GARRIDO et al., 1996) and *L. mexicana* (TRAINOR et al., 2010) infect cats in other countries.

Malnourished and immunosuppressed humans are more susceptible to severe forms of these diseases (ASHFORD, 2000; BRASIL, 2014; GONTIJO, MELO, 2004; DAHER et al., 2009; TRAINOR et al., 2010), which especially affects patients with Acquired Immunodeficiency Syndrome (WHO, 2010). As in humans, cats with Feline Immunodeficiency Virus (FIV) are more predisposed to develop leishmaniasis (PENNISI et al., 2012). Moreover, age is a predisposing factor for the disease, as cats over three years are more likely to be infected by *Leishmania* sp. (PENNISI et al., 2012), probably due to a longer exposure to infected vectors.

Cats infected by *L. donovani* and *L. infantum* may be urban reservoirs of visceral leishmaniasis, as they can transmit the protozoan to phlebotomine sandflies used in xenodiagnosis (SILVA et al., 2010; MAROLI et al., 2007), and present high parasitism in the skin as assessed by immunohistochemistry (VIDES et al., 2011). Interestingly, in 2004 an autochthonous case of feline visceral leishmaniasis was reported in Cotia – SP, where no cases of canine or human disease had been detected (SAVANI et al., 2004). Only four years later the city became endemic for canine visceral leishmaniasis (BEPA, 2008), but no cases of human infections have been reported in Cotia to date (CVE, 2013).

Biological cycle

Leishmaniasis is transmitted by phlebotomine sandflies: small insects of the order Diptera, belonging to the *Phlebotominae* subfamily. Sandflies can be classified in the genera *Phlebotomus*, *Sergentomyia*, and *Chinus* in the Old World and by *Lutzomyia* in the New World (WHO, 2010). There are approximately 900 known species of sandflies,

70 of which can be leishmaniasis vectors (READY, 2013). In Brazil, two species of these insects transmit VL: *Lu. longipalpis* and *Lu. cruzi* (BRASIL, 2014). TL vectors include the species *Lu. flaviscutellata, Lu. whitmani, Lu. umbratilis, Lu. intermedia, Lu. wellcome, Lu. migonei* (BRASIL, 2007) *Lu. neivai*, and *Lu. sallesi* (SARAIVA et al., 2009).

Leishmania spp. is primarily present in the invertebrate host as an extracellular and flagellate (promastigote) form or as an obligate intracellular form without external flagellum (amastigote) in the vertebrate host (BAÑULS et al., 2007). Phlebotomine sandflies ingest the protozoan while sucking blood from an infected host. The parasite then multiplies in the intestine of the vector, which become infectious between 8 and 20 days later (DESJEUX, 2004). When biting a vertebrate host, the infected vector inoculates metacyclic promastigotes, which are then phagocytosed by macrophages. Within these cells, they become amastigotes and multiply by binary fission. The increased number of intracellular pathogens causes the disruption of the infected macrophages, thus leading to the dissemination of the microorganism to other phagocytic cells, which may be subsequently ingested by another vector (BAÑULS et al., 2007).

The main route of transmission of canine visceral leishmaniasis is through the bite of vectors infected with *L. chagasi* (BRASIL, 2014), however, alternative routes of transmission have been proposed. Those are: transplacental transmission (ROSYPAL et al., 2005), blood transfusion (FREITAS et al., 2006), and venereal transmission (SILVA et al., 2009). In cats only the transmission via sandflies have been confirmed thus far.

Clinical signs

Skin changes (papules, nodules, ulcers, erythema, alopecia) are the most frequent clinical signs of Leishmaniasis in cats, regardless of the *Leishmania* species that caused the infection: dermatotropic or viscerotropic. The head and in particular, the external ears, nasal plane, snout, and peri-ocular regions are the most affected areas, presumably due to their higher exposure to the vectors as they are less covered by fur (BONFANTE-GARRIDO et al., 1996; SIMÕES-MATTOS et al., 2005; VIDES et al., 2011).

Systemic manifestations of leishmaniasis are less frequent, although anorexia, apathy, emesis, diarrhea, dehydration, weight loss, and stomatitis may occur (OZON et al., 1998; HERVÁS et al., 1999; POLI et al., 2002; LEIVA et al., 2005). Approximately 30% of the cats infected with *L. chagasi* present only dermatosis. The frequency of lymphadenopathy, commonly developed by dogs with visceral leishmaniasis (CÂNDIDO, 2007), varies in infected cats from 3% (PENNISI, 2002) to 53 % (VIDES et al., 2011). Ocular alterations (POLI et al., 2002) such as edema and corneal ulcers, purulent uveitis, and eyelid nodules (LEIVA et al., 2005) have also been reported in cats. Feline visceral leishmaniasis may assume an acute atypical form that causes the animal to die in a few weeks (OZON et al., 1998).

The incubation time and the clinical course of the disease in human and dogs are highly influenced by properties of the parasite and by the competence of the host immune system to develop a humoral or cellular immune response (GENARO, 1993; PINELLI et al., 1994). Although domestic cats are relatively resistant to *Leishmania* infection, mainly due to the fact that they produce a cellular immune response (SOLANO-GALLEGO et al., 2007), the incubation period in these animals is still unknown. After the experimental infection of cats with *L. braziliensis*, the clinical manifestations of the disease start to appear, on an average, within two weeks (SIMÕES-MATTOS et al., 2005). On the other hand, Simoes-Mattos et al. observed a regression of clinical symptoms, on an average, nine months after the inoculation. During this period, the animals still presented low antibody titers, which suggest a cellular immune response (SIMÕES-MATTOS et al., 2005).

Pennisi et al. (2012) investigated visceral leishmaniasis in cats from an endemic area and found that is possible to amplify, by polymerase chain reaction (PCR), the parasite DNA from the blood of animals negative to the indirect immunofluorescence reaction (RIFI), that is, with antibody titers below 1:80. According to the authors, this suggests the existence of a balance between the host and the parasite achieved by the cellular immune response. However, the animals of this experiment were not monitored over time regarding serology and possible appearance of clinical signs, nor the concentrations of interleukins or other variables related to the cellular immune response were measured. Therefore, studies aiming to better assess the immunity of cats infected with *Leishmania* sp. are still required.

For a long time, domestic cats were considered accidental hosts of the species causing TL infections, probably because most of the cats infected with *Leishmania* sp. present exclusively skin changes. Moreover, studies used mainly cytology or antibody detection as diagnostic techniques, which were restricted to identify the parasite genus (SOUZA et al., 2005, 2009; FIGUEIREDO et al., 2009; SILVEIRA NETO et al., 2011). Currently, feline leishmaniasis is assessed by PCR followed by sequencing and genetic alignment, a diagnostic tool that facilitates the identification of the protozoan species. This change in the scientific methodology demonstrated that the VL agent *L. chagasi* is also common in infected Brazilian cats, in spite of their symptoms being predominantly skin abnormalities (SAVANI et al., 2004; COELHO et al., 2011; VIDES et al., 2011; SOBRINHO et al., 2012).

Diagnosis

In cats, the diagnosis of VL or TL can be divided in: a) epidemiological surveillance and b) clinical and laboratory methods. In the epidemiological surveillance, there is the need for an improvement in the techniques to identify the cause of the clinical signs manifested by the cat, and to subsequently delineate the procedures that should be adopted to control the disease. In the rare cases where leishmaniasis is diagnosed in an epidemiological surveillance, the adoption of diagnostic techniques such as immunohistochemistry and PCR is recommended in the entire cat population, which requires significant logistic and financial resources. For this reason, epidemiological investigations should prioritize sensitive and low cost techniques, such as serological analysis assessed by ELISA or RIFI.

Studies aiming the standardization and validation of serological diagnostic in cats are scarce. One rare exception to this rule is the series of epidemiological studies performed in cats from municipality of Araçatuba, located in the Northwestern region of the state of São Paulo, which is an endemic area for canine and human visceral leishmaniasis (BEPA, 2008). Five years after the disease was first detected in dogs from Araçatuba (LUVIZOTTO et al., 1999), the first case of feline leishmaniasis was diagnosed in this municipality (SERRANO et al., 2008). The animal presented cutaneous injuries, apathy, dehydration, and diarrhea (SERRANO et al., 2008). This finding contributed to the implementation of the largest epidemiological investigation ever carried out in cats in Brazil (ROSSI, 2007). In these studies, the sample prevalence obtained by RIFI and ELISA varied from 0.5% to 23%, respectively (SILVEIRA NETO et al., 2011; CARDIA et al., 2013), which demonstrates that the serological technique employed may influence the diagnosis. Moreover, a poor correlation of the *Kappa* coefficient was observed among the techniques of direct detection, such as cytology and immunohistochemistry, and indirect detection, such as RIFI and ELISA (VIDES et al., 2011; SOBRINHO et al., 2012). In general, ELISA showed higher sensitivity when compared to RIFI (VIDES et al., 2011; SOBRINHO et al., 2012), confirming the findings of studies performed in other regions (FIGUEIREDO et al., 2009).

In contrast to infected dogs, cats do not

present hypergammaglobulinemia, which makes it necessary to use more concentrated serum in ELISA, such as 1:40 (FIGUEIREDO et al., 2009), 1:50 (SILVEIRA NETO et al., 2011), or 1:200 (VIDES et al., 2011, SOBRINHO et al., 2012), compared to a 1:400 dilution used to diagnose the disease in dogs (LIMA et al., 2005). Unfortunately, the use of highly concentrated sera in ELISA may increase the chance of nonspecific reactions, which would influence the reliability of the results.

Silveira Neto et al. (2011) observed very different sample prevalence between the paired sera tested by ELISA using crude antigens (CAG), recombinant K39 antigen (rK39) and fucose-mannose ligand (FML), with values of 23.0%, 15.9%, and 13.3%, respectively. Although no significant disagreement was observed between the tests, the correlation between them varied from low to moderate. Cross-reaction between antibodies against *Toxoplasmoa gondii* and the rK39 and FML antigens was detected in 6.7% of the samples. These results reinforced the hypothesis that ELISA is more sensitive than RIFI, and highlighted the cross-reactivity with antigens of other etiological agents that may lead to false positive results.

In infected dogs, serological techniques can be more sensitive than cytopathologic examinations, with a sensitivity that vary from 50% to 83% in bone marrow samples, from 30% to 85% in lymph nodes, and from 71% to 91% when both tissues of a single individual are assessed in a paired manner (KOUTINAS et al., 2001; MOREIRA et al., 2002). Vides et al. (2011) and Sobrinho et al. (2012) found that 48-50% of the cats that were seroreactive to ELISA tested negative to RIFI, immunohistochemistry and cytology. Vides et al. (2011) also found that, although amastigote forms of the parasite were identified in 37% of the infected cats through a biopsy by needle puncture and aspiration (BPA) of the lymphoid organs, 18.5% of the cats showed no seroreactivity or evident parasites in the lymphoid organs and were diagnosed only when performing immunohistochemistry of the skin

lesions. This reinforces the idea that in cats with clinical suspicion of the disease, several diagnostic techniques should be combined, including the cytopathological examination of the lymphoid organs, skin immunohistochemistry, and PCR.

There is disagreement among authors regarding the choice of the best lymphoid organ to perform BPA and subsequently cytopathological examination: whether it should be the bone marrow (VIDES et al., 2011) or the lymph node (COSTA et al., 2010). In dogs, it was already demonstrated that the bone marrow presents the higher density of *Leishmania* sp. during the clinical course of the disease (REIS et al., 2009). However, it is necessary to independently evaluate the BPA in both lymphoid organs to unequivocally define the best lymphoid organ for diagnosis.

PCR techniques are generally considered more specific than serology to diagnose leishmaniasis in cats (MARTÍN-SÁNCHEZ et al., 2007; AYLLON et al., 2008; MAIA et al., 2008; TABAR et al., 2008). However, it should be kept in mind that depending on the stage of the disease (which affects parasitemia), it might be necessary to perform a PCR in lymphoid organs or skin and not in blood samples. In a comparative study between diagnostic methods assessing VL in dogs, it was concluded that amplification of *L. chagasi* kDNA may be detected even before seroconversion (ASSIS et al., 2010), which presents a detection threshold of 10^{-1} promastigotes mL^{-1} in blood samples of dogs artificially contaminated with *L. chagasi*, thus confirming the high sensitivity in relation to the serology assessed by ELISA and RIFI (NUNES et al., 2007).

Hence, the low reproducibility of results between different research groups illustrates the need for more accurate and standardized diagnostic techniques (ROSSI, 2007; BRESCIANI et al., 2010; SILVEIRA NETO et al., 2011; VIDES et al., 2011; SOBRINHO et al., 2012; CARDIA et al., 2013).

Final Considerations

TL and VL in domestic cats is a daily reality in endemic areas and should never be underestimated in the clinical diagnosis following the observation of skin changes in cats. Although there is increasing evidence that cats can serve as reservoir for the parasite, its role in the epidemiological cycle of the disease and in public health issues requires further investigation. The standardization and validation of accurate and accessible diagnostic methods, which may be applied to epidemiological surveillance, are still required. With the combined evidences gathered thus far, the use of drastic measures, such as euthanasia of seropositive cats, may still be considered premature. However, random epidemiological studies in cats are highly recommended to form a better picture of the risks of cat leishmaniasis to public health.

Acknowledgements

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