ARTICLE

PARASITE AND BACTERIAL CO-INFECTIONS WITH *LEISHMANIA* SPP. IN DOGS

VIRGINIA MARIA GÓES DA SILVA
BrainExperiments.com, 2436 Rue de l'Acajou, Montreal, Quebec, Canada

RITA ELIZABETH MOREIRA MASCARENHAS
Escola Bahiana de Medicina e Saúde Pública, Av. Dom João VI, 275, Brotas,40.290-000, Salvador, Bahia, Brazil

CANDACE MACHADO DE ANDRADE
Universidade Federal da Bahia, Instituto de Ciências da Saúde, Av. Reitor Miguel Calmon, S/N, Canela, 40.231-300, Salvador, Bahia, Brazil, candace.andrade@ufba.br

MANUELA DA SILVA SOLCÀ
Universidade Federal da Bahia, Escola de Medicina Veterinária, Av. Milton Santos, 500, Ondina, 40.170-110, Salvador, Bahia,Brazil

DANIELA FARIAS LARANGEIRAA
Universidade Federal da Bahia, Escola de Medicina Veterinária, Av. Milton Santos, 500, Ondina, 40.170-110, Salvador, Bahia,Brazil

GERALDO GILENO DE SÁ OLIVEIRA
Fundação Oswaldo Cruz, Instituto Gonçalo Moniz, R. Waldemar Falcão, 121, Candeal, 40.296-710, Salvador, Bahia, Brazil

WASHINGTON LUIS CONRADO DOS SANTOS
Fundação Oswaldo Cruz, Instituto Gonçalo Moniz, R. Waldemar Falcão, 121, Candeal, 40.296-710, Salvador, Bahia, Brazil

Abstract: Canine visceral leishmaniasis (CVL) is a major disease affecting dogs and is often associated with other illnesses. In this study, we investigated the distribution of helminths, ectoparasites and bacteria in dogs of an endemic urban area of CVL. A total of 71 dogs, uninfected or naturally infected with *Leishmania* spp. were studied. Splenic samples were cultured for *Leishmania* identification, and anti-Leishmania antibodies were measured in the serum. Helminths were diagnosed in the feces using flotation or spontaneous sedimentation techniques. Serum antibodies against six ectoparasite-transmitted pathogens were detected. Microbial growth from eyes, skin, urine, and blood samples were evaluated. To our knowledge, this is the first time that co-infections with *Leishmania* spp., parasites and bacteria together has been reported. Co-infections with *Leishmania* were observed in 89% of the animals with helminths and 95% with ectoparasites. Most dogs were positive for *Ehrlichia* spp. and *Anaplasma* spp. Coagulase-negative *Staphylococcus* was the most frequently isolated organism. It is found that *Leishmania* positivity dogs from endemic area in Brazil have a higher rate of co-infections with helminths, ectoparasites and bacteria. Therefore, effective treatment and public measures are needed to contain the spread of canine leishmaniasis and other infections.

Keywords: : bacteria, dog, ectoparasite, helminth, leishmaniasis.
Rev. Biol. Neotrop. / J. Neotrop. Biol., Goiânia, v. 21, n. 1, p. 1-10, jan.-jul. 2024

Coinfecções parasitárias e bacterianas com *Leishmania* spp. em cães

Resumo: A leishmaniose visceral canina (LVC) é uma doença importante que afeta os cães e está frequentemente associada a outras doenças. Neste estudo, investigamos a distribuição de helmintos, ectoparasitas e bactérias em cães de uma área urbana endêmica de LVC. Foram estudados 71 cães, não infectados ou naturalmente infectados por *Leishmania* spp. Amostras esplênicas foram cultivadas para identificação de *Leishmania*, e anticorpos anti-Leishmania foram mensurados no soro. Os helmintos foram diagnosticados nas fezes utilizando tecnicas de flutuação ou sedimentação espontânea. Foram detectados anticorpos séricos contra seis patógenos transmitidos por ectoparasitas. O crescimento microbiano dos olhos, pele, urina e amostras de sangue foram avaliados. Até onde sabemos, esta é a primeira vez que co-infecções com *Leishmania* spp., parasitas e bactérias juntas foram relatadas. Co-infecções com *Leishmania* foram observadas em 89% dos animais com helmintos e 95% com ectoparasitas. A maioria dos cães foi positiva para Ehrlichia spp.. Constata-se que cães positivos para *Leishmania* provenientes de área endêmica no Brasil apresentam maior índice de co-infecções com helmintos, ectoparasitas e bactérias. Portanto, um tratamento eficaz e medidas públicas são necessários para conter a propagação da leishmaniose canina e outras infecções.

Palavras-chave: bactéria, cão, ectoparasita, helminto, leishmaniose.

Introduction

Unsurprisingly, the fluctuations of human and canine visceral leishmaniasis are usually similar, although the proportion of dogs with parasitism remained relatively high, even in the periods of low incidence of human disease (Fraga et al., 2012). However not all infected hosts with *Leishmania infantum* (Nicolle, 1908) develop the disease (Ribeiro et al., 2011). On the other hand, some of them can develop a severe disease leading to death by bacterial co-infection, bleeding diathesis or both (Cortese et al., 2011; Gallo-Francisco et al., 2022).

It is not known, if *Leishmania spp*. is the primary cause for CVL, or whether other diseases are likely to contribute to the establishment of leishmaniasis (Mekuzas et al., 2009). Some authors have proposed that the immunosuppression induced by *L. infantum* could increase the susceptibility of dogs to parasites (Attipa et al., 2018; Ramos et al., 2022) or not (Costa Oliveira et al., 2021; Ribeiro et al., 2011). However, the impact of these co-infections is usually unknow. In fact, a protective action of *Wolbachia* limiting the seriousness of leishmaniasis was observed in dogs co-infected with *L. infantum* and *Dirofilaria immitis* (Leidy, 1856) (Tabar et al., 2013).

Mixed infections with different vector-borne pathogens are frequently in canines and may also potentiate disease pathogenesis and adversely influence prognosis (Suksawat et al., 2001; Cortese et al., 2011; Baxarias et al., 2018). Co-infections with the global pathogens, agents of leishmaniasis, ehrlichiosis and anaplasmosis, characterized by the presence of anti-platelet antibodies (Cortese et al., 2011), can aggravate the diseases (Mekuzas et al., 2009; Cortese et al., 2011).

In fact, dogs with CVL develop a variety of skin, eyes and other mucosal lesions (Saijonmaa-Koulumies & Lloyd, 1996; Parin et al., 2020; Goulli et al., 2023) potentially associated with primary or secondary bacterial and fungal infections, including bacteria 100% resistant to penicillin G and gentamicin (Saijonmaa-Koulumies & Lloyd, 1996; Parin et al., 2020).

For all these facts, we investigated *Leishmania* spp. co-infections in dogs with intestinal helminths, vector-borne pathogens, and bacteria in an endemic area, Jequié, Bahia Brazil.

Material and Methods

Ethics Statement

Procedures involving animals were conducted in accordance with Brazilian Federal Law on Animal Experimentation (Law 11794) (https://www.planalto.gov.br/ccivil_03/_ato2007-2010/2008/lei/l11794.htm), with the Oswaldo Cruz Foundation guidelines for research with animals (http://sistemas.cpqam.fiocruz.br/ceua/hiceuaw000.aspx) and with the manual for the surveillance and control of visceral leishmaniasis. This study was approved by the ethics committee for the use of animals in research (CPqGM-FIOCRUZ, Ceua, license N.040/2005).

Characterization of the study area

The study was carried out in the municipality of Jequié, located in the southwest region of the state of Bahia, Brazil. Jequié covers an area of 3,035 km^2, geographical coordinates are

11° 10' 50" N latitude and 40° 31' 6" W longitude at an altitude of 463 m above sea level.

The climate of Jequié is semiarid, characterized by a long dry season with torrential and irregular rains and periods of extreme water scarcity (Sherlock & Santos, 1964). The dry season typically extends from September to December and the rainy season usually corresponds to the period between January and March (Sherlock and Santos 1964). There are two transition regions, composed by the contact between Caatinga (tropical dry forest)-Cerrado (tropical savanna) and Caatinga-Atlantic Forest biomes. The average annual temperature is 24 °C. Higher temperature values are recorded during the summer, with maximum observed of 40 °C, while in winter the average is never below 18 °C.

Animals

A total of 71 mongrel stray dogs, naturally or uninfected infected with *L. infantum*, were collected from the avenues of the urban zone of Jequié, Bahia Brazil. This study was performed in collaboration with the Endemic Diseases Surveillance Program of the State Health Service as part of a program for the surveillance and control of visceral leishmaniasis. The dogs were identified with sequential numbers and were only included in the research if they had not been claimed by their presumed owners. After 48 hours in the kennel with free access to water and food, the canines were sedated using acepromazine (0.1 $mg.kg^{-1}$ iv, Acepran 1%, Vetnil, Brazil) plus sodium thiopental (15 $mg.kg^{-1}$ iv, Thiopentax 1 g, Cristália, Brazil) and euthanized with a saturated solution of potassium chloride (2 $mL.kg^{-1}$, iv). During the necropsy were determined the approximate age, sex, size, and skin injuries. The general characteristics of all animals were not determined, just as not all dogs were subjected to all tests.

Culture of the spleen aspirate for *Leishmania* identification

After the euthanasia, were collected splenic samples from each canine by puncture utilizing an 18 G x 38 mm gauge needle connected to a 20-mL syringe. Spleen cells were cultured in biphasic agar-blood-Schneider's medium, supplemented with 10% fetal bovine serum as previously described (Santos et al., 2008). Cultures were inspected weekly for identification of promastigotes, and examinations continued for up to 2 months when they remained negative.

Anti-*Leishmania* antibody activity

Approximately 10 mL of blood were obtained by punter with sterile syringe directly from heart during necropsy to obtain the serum. After clotting at room temperature for at least 30 minutes, the blood sample were centrifugated for 10 minutes at 1000 x g. Then, the serum was transferred to Eppendorf-type and assayed immediately or aliquoted and stored at -20 ºC. The anti-*Leishmania* antibody was detected by ELISA as previously described (Santos et al., 2008). Briefly, 96-well plates were adsorbed with crude antigen from *L. infantum*. The plates were washed, blocked with PBS supplemented with 10% of skimmed milk, and the serum of each dog was incubated at the dilution of 1:400, followed by an anti-dog total IgG peroxidase conjugate (Sigma). The chromogen tetramethyl benzidine (Sigma) was added. Values higher than the mean plus three standard deviation values of the results detected in healthy canines from non-endemic regions were considered positive.

Identification of helminths

One sample of feces was collected/dog during exercise for parasitological tests. Approximately 5 g of a cross-section of stool to include both surface and internal content were collected in a clean container and frozen on dry ice until the parasitological assays were performed on the same day. Feces were processed by flotation technique in sodium chloride saturated solution with 1.182 density (Willis, 1921) and spontaneous sedimentation in water (Hoffman et al., 1934). The eggs were counted under an optical microscope (magnification 40x).

Ectoparasite collection

The ticks, fleas and lice were morphological identified by veterinarians.

Anti-*Ehrlichia canis* (Donatien and Lestoquard, 1935) / *Ehrlichia ewingii* (Anderson et al. 1992), *Anaplasma phagocytophilum* (Foggie, 1949) / *Anaplasma platys* (Dumler et al. 2001), *Dirofilaria immitis and Borrelia burgdorferi* (Johnson et al. 1984) antibody activity. Serum samples were examined with the SNAP® 4Dx® Plus test (IDEXX Laboratories, Inc., Westbrook, Maine, USA) according to the manufacturer's information, with at least 98% specificity in all four diseases.

Microbiological assays

Samples from the eyes and skin were obtained using a sterile cotton-tipped swab. Samples from urine and blood were obtained by punter with sterile syringe directly from bladder and heart during necropsy. Samples from eyes, skin and urine were inoculated in blood agar (Tryptic Soy Agar medium, Merck KGaA, Darmstadt, Germany) and MacConkey agar (Difco-Becton Dickinson Microbiolgy Systems, Maryland, USA), then incubated at 37 ºC for 24 to 48 hours. Samples from blood were inoculated in Mueller-Hinton broth (Merck KGaA, Darmstadt, Germany) at 35 ºC for 12 days and inoculated in blood agar and MacConkey agar at 35 ºC du-

ring 24 to 48 hours on days 2, 5, 7 and 12, or with any turbidity or CO_2 production. The samples from blood that presenting turbidity or CO_2 production in Mueller-Hinton broth, without aerobic growth, were inoculated in Thioglycolate and Anaerisol agar (Probac, Bacteriological products Lt, Santa Cecília, São Paulo, Brazil), and incubated in aerobic conditions, in microaerophilic atmosphere (10% CO_2) and aerobic conditions with anaero kit (Probac, Bacteriological products Lt, Santa Cecília, São Paulo, Brazil) in anaerobic chamber. The microbial growth was evaluated by bacterial morphology and identification of specific assays. The morphology and Gram stain were analyzed. Further differentiation of the organisms by standard microbiological tests was done using catalase, coagulase and oxidase tests, hemolytic pattern, and subcultures on biochemical proofs (for identification of the Enterobacteriaceae family and non-fermentative Gram-negative bacillus, and anaerobic microorganisms) and chromogenic medium for yeast (Difco-Becton Dickinson Microbiolgy Systems, Maryland, USA).

Expression and significance of the results

The Chi-square test, the Fisher's exact probability test, or the Chi-square test for trends were used used for comparisons involving proportions (Kirkwood & Sterne, 2003).

Results

General canine characteristics

Leishmania spp. infection was detected in 80.3% (53/66) dogs, of which 29 and 3 animals had only positive serology or culture, respectively, 4 were serologically positive, but the culture was not performed, and 17 had both tests positive (data not shown). The main characteristics of these animals are shown in Tab. 1. There were no *Leishmania* culture or serology significant differences according to age, sex or size of the animals. However, most of them were 3 to 5 years old and all the animals older than 5 years were infected with *Leishmania* spp. Most dogs were small and medium sized.

Co-infections with *Leishmania*

Co-infections with *Leishmania* spp. were observed in 89% (33/37) of the dogs with helminths and 94,6% (35/37) with ectoparasites. Hookworm, *Dipylidium caninum* and *Toxocaracanis* were the most prevalent enteroparasites (Fig. 1A), and fleas and tick the most frequent ectoparasites (Fig. 1B). No statistically significant differences were observed between culture or serology for *Leishmania* spp. and the presence of parasites (Fig. 1).

Serological tests for *Ehrlichia* spp. and *Anaplasma* spp. were positive in most of the animalswith negative (culture = 85%, ELISA = 89%) or positive (culture= 100%, ELISA= 95%) results for *Leishmania* spp., and no significant difference between groups. Serology for *D. immitis* and *B. burgdorferi* were negative for all the tested animals.

Bacterial infection was detected by culture in most of the animals. Eyes and skin were the predominant sites of bacterial infection (Fig. 2). Although only 17 *Leishmania*-infected dogs showed skin injury from which bacterial cultures were collected. Coagulase-negative *Staphylococcus* was the most frequently isolated organism. However, there was not statistically difference in the distribution of infection between infected and uninfected animals (Fig. 2). *Staphylococcus* spp. and *Streptococcus beta haemolytic* were the most commonbacteria found in dog´s eyes, while only *Streptococcus* spp.

Tab. 1. Tab. 1. General characteristics of stray dogs collected from the streets of Jequié (Bahia State, Brazil), an area of endemic vis-ceral leishmaniasis.

Characteristic		*Leishmania* culture (n=47)		*Leishmania* serology (n=66)	
		Positive	**Negative**	**Positive**	**Negative**
Estimated age (years)	0 - 2	26%(5/19)	25%(7/28)	24%(12/49)	29%(5/17)
	3 - 5	68%(13/19)	54%(15/28)	55%(27/49)	59%(10/17)
	≥ 6	5%(1/19)	21% (6/28)	20%(10/49)	12%(2/17)
		***Leishmania* culture (n=60)**		***Leishmania* serology (n=67)**	
		Positive	**Negative**	**Positive**	**Negative**
Sex	M	60%(12/20)	52%(21/40)	50%(25/50)	71%(12/17)
	F	40%(8/20)	47%(19/40)	50%(25/50)	29%(5/17)
		***Leishmania* culture (n=58)**		***Leishmania* serology(n=65)**	
		Positive	**Negative**	**Positive**	**Negative**
Size	Small	39%(7/18)	20%(8/40)	31%(15/48)	18% (3/17)
	Medium	44%(8/18)	72%(29/40)	60%(29/48)	65%(11/17)
	Long	17%(3/18)	7%(3/40)	8%(4/48)	18%(3/17)

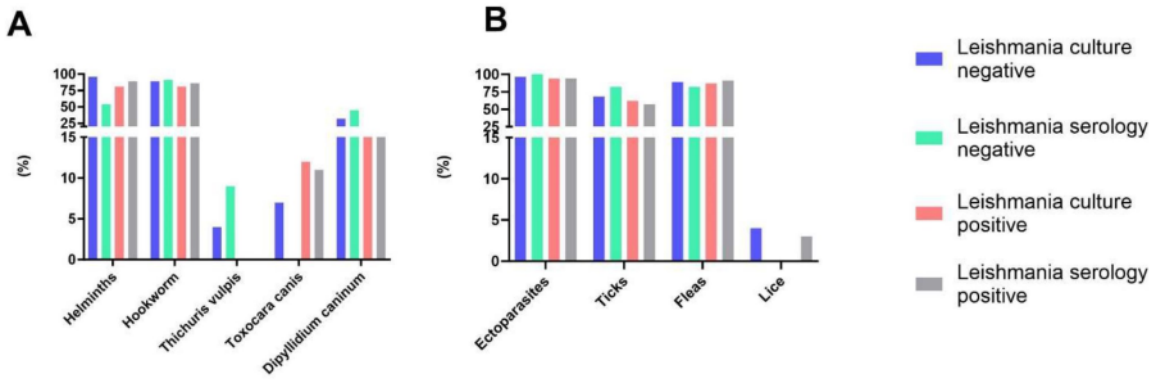

Fig 1. Distribution of *Leishmania spp*.culture and serology in dogs by helminths (A) and ectoparasites (B) co-infections. No statistically significant differences were observed.

was found in the urine of an animal serologically positive for *Leishmania*. Anaerobic bacteria were the most present in the blood of the animals. No statistically significant differences were observed between culture or serology for *Leishmania* spp. and the presence of bacteria (Fig. 2).

Discussion

This is the first report comparing *Leishmania* spp. co-infections with intestinal helminth, ectoparasite and bacteria. Our results endorsed that all dogs were positive for some infection.

In total, 80.3% (53/66) of the dogs

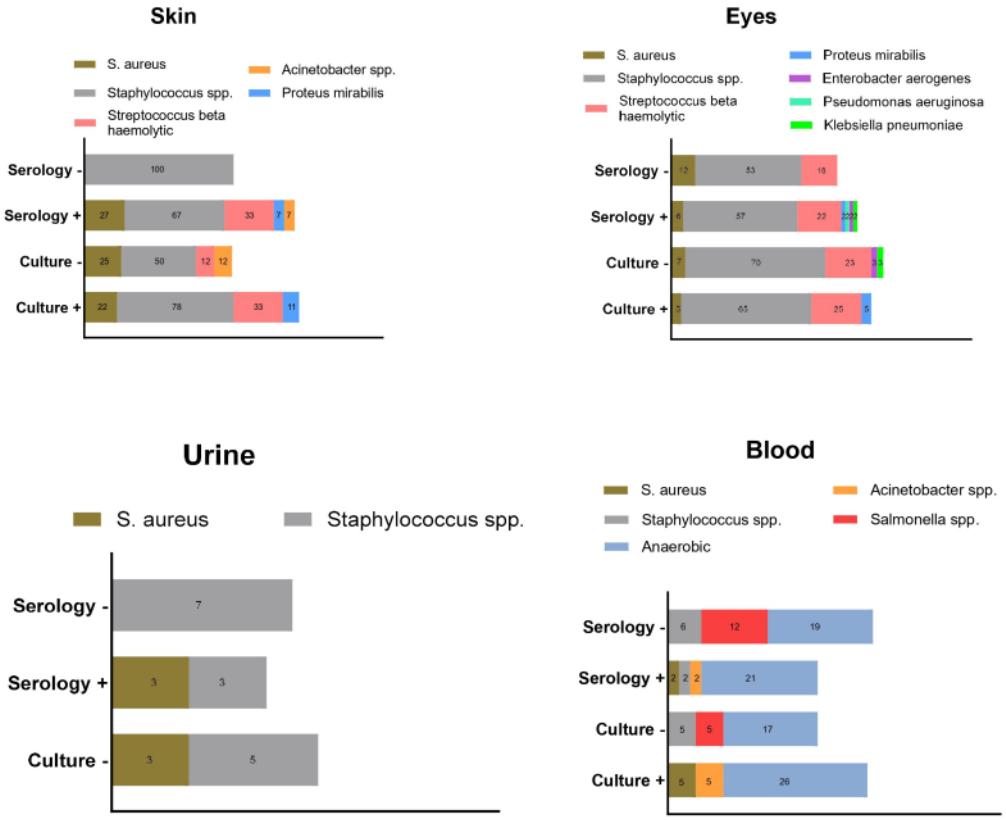

Fig. 2. Distribution of Leishmania spp. culture and serology in dogs by bacterial in skin (A); eye (B); urine (C) and blood(D) co-infections.No statistically significant differences were ob-served.

showed evidence of *Leishmania* spp. infection, indicating that Jequié conti-nuous to be an endemic region since the first report in 1998 (Paranhos-Silva et al., 1998). Jequié became a municipality of predominantly urban population (IBGE, 2010) with a clearly disordered growth. The narrow, unpaved streets and houses in close proximity make this city especially prone to flooding during the rainy season, consequently creating favorable conditions for vector diseases reproduction. Furthermore, organic and inorganic pollution of the Contas and Jequiezinho rivers favors the endemic character of vector diseases, such as leishmaniasis (Sherlock and Santos, 1964).

In addition, the highest frequency of dogs with only positive serology compared with negative spleen culture may be explained by the higher sensitivity of ELISA test (Gow et al., 2022). Also, 5.7% of the dogs were positive only by culture, suggesting the initial stage of the infection before the antibody response (Paranhos-Silva et al., 1998).

The high number of animals with *Leishmania* spp. evidence older than 3 years could be related to the cumulative increase of the exposure period to *Lutzomyia longipalpis* (Lutz & Neiva, 1912) vector over the years. In symptomatic dogs, the age distribution has been shown to be bimodal, with the highest prevalence of leishmaniasis occurring between 2 to 4 years of age and a second peak at seven years or older (Miranda et al., 2008). In the present work, not all animals showed CVL symptoms, and this factor was not correlated with the co-infections.

Almost 90% of animals infected or not with *Leishmania* spp. had some helminths infection, probably the dogs had been living outdoors without preventive measures against endoparasites and more exposed to the parasites. The most common *Leishmania* co-infection with helminths were hookworm, followed by *D. caninum*, roundworms and *Trichuris vulpis* (Froelich, 1789). Nevertheless, Saldanha-Elias et al. (2019), in captured street dogs in Brazil, described positive correlation between serology for *Leishmania* infection and *D. caninum* intestinal parasitism (Saldanha-Elias et al., 2019). Gastrointestinal signs are rarely attributed to infection with *Leishmania*, for this reason the high parasite loads of this protozoon in the intestinal mucosa with only mild pathological changes have allowed us to consider whether the protozoan obtains advantage of intestinal tolerance (Pinto et al., 2011).

Mixed infections with tick-borne pathogens are frequently in canines (Suksawat et al., 2001; Baxarias et al., 2018). In Jequié, almost 100% of dogs were parasitized by ticks and/or flea. Moreover, dogs were infected by E. canis and *A. phagocytophilum* transmitted by tick and *L. infantum* transmitted by fleas and phlebotomine sand flies (Suksawat et al., 2001; Baxarias et al., 2018; Gow et al., 2022). The high seroreaction dogs against *Ehrlichia* spp. and *Anaplasma* spp. evidence exposure of its vector-tick, *Rhipicephalus sanguineus* (Latreille, 1806), an extremely common ectoparasite in tropical and subtropical areas (Szabó et al., 2013), such as Brazil. On the other side, any dog showed serologic positive to *D. immitis*, probably because the geographical distribution of the vectors for this infection and *L. infantum* does not usually overlap (Tommasi et al., 2013). In the same way, the absence of seropositivity dogs for *B. burgdorferi* induces the idea that the presence of this spirochete is low or does not occur in the zone (Costa Oliveira et al., 2021).

In our work, dogs with or not *Leishmania* spp. evidence infection had positive serology for *Ehrlichia* spp. and *Anaplasma* spp. Corroborating our results, the high prevalence of co-infection between *Leishmania* and *Ehrlichia* spp. has already been demonstratedin different world regions such as northeastern Brazil, Rio de Janeiro, Spain and on the Côte d'Ivoire (Medkour et al., 2020; Montoya-Alonso et al., 2020; Costa Oliveira et al., 2021; Ramos et al., 2022). In fact, some studies have shown *Leishmania-Ehrlichia* co-infection could more severely affect the immunity in canines (Cortese et al., 2011; Mekuzas et al., 2009) potentiating the disease pathogenesis (Attipa et al., 2018; Baxarias et al., 2018). In the same direction, dogs with CVL had higher rate of co-infections with *Rickettsia conorii* (Brumpt, 1932), *Bartonella henselae* (Regnery et al., 1992) and *A. phagocytophilum*, associated with more marked clinico pathological abnormalities, for instance reduction in albumin or red blood cells rates or increase in globulins (Baxarias et al., 2018), which was not observed in the association with *Anaplasma* platys, *Hepatozoon* spp. and *Mycoplasma haemocanis* (Messick et al., 2002) (Attipa et al., 2018).

Interestingly, *L. infantum* seropositive dogs had more endo and ectoparasites, suggesting that CVL antibodies in dogs may increase their susceptibility to parasites. Correspondingly, the significant higher levels of anti-*Leishmania* antibodies have been observed in symptomatic dogs (Ribeiro et al., 2011). Furthermore, *Leishmania*-infected dogs were more infested by ectoparasites (ticks, fleas, or both) (Ramos et al., 2022).

Bacterial infection was detected by culture in most of the animals with culture or sero-

logy positives, predominantly in the eyes and skin. In fact, bacterial and mycotic may be identified in cutaneous lesions, especially in ear, dorsal and pectoral region, in CVL (Parin et al., 2020).

The opportunistic *Staphylococcus* was identified as one of the most frequent bacteria colonizing infectious agents in canine skin and eyes lesions. There is still controversy over which bacteria are resident or transient in canine skin, however pathological conditions favor *Staphylococcus* colonization and predispose to infection (Saijonmaa-Koulumies& Lloyd, 1996). In Turkey, Parin et al identified the higher incidence for *S. aureus*, followed by *Staphylococcus epidermidis* (Winslow and Winslow 1908) and *Bacillus cereus* (Frankland and Frankland, 1887) in lesions of *Leishmania*-seropositive dogs with CVL (Parin et al., 2020).

Considering the frequent ocular manifestations in dogs with leishmaniasis, the production of anti-*L. infantum* IgG initiated local and followed by antibodies from the bloodstream to the aqueous humor (Goulli et al., 2023), may aggravate immunopathogenic mechanisms with the deposition of immune complexes in the vessels (Pumarola et al., 1991).

The meibomian glands represent the glands most affected by inflammation in canines with leishmaniasis (Naranjo et al., 2005). When these glands are damaged, it is possible that bacteria from the normal skin flora in the periocular region, such as *Staphylococcus*, are favored and can infect the orbital cavity.

Also, *Staphylococcus* were isolated from urine samples from both *Leishmania* infected and non-infected dogs. Although the literature has described the occurrence of opportunistic cystitis in dogs infected by *L. donovani*, there is a knowledge gap concerning the microorganisms responsible for urinary tract infections in dogs with CVL (Santos et al., 2013). Studies have indicated the presence of cellular inflammatory infiltrate in the bladder of dogs with CVL (Santos et al., 2013; Silva et al., 2019).

In fact, many infectious diseases of humans and animals are caused by more than one microorganism from different kingdoms, genera, species, and by phenotypic variants of a single species (Bakaletz, 2004). In human, bloodstream polymicrobial infections often occur in patients with poor medical conditions (XU et al., 2023). The risk factors for these infections include diabetes, chronic liver diseases, cardiovascular diseases, immunosuppressive diseases, hematologic diseases, and tumors (XU et al., 2023). In our study, blood co-infection was identified among the canines with or without *Leishmania* infection, and many dogs showed poor health and nutritional conditions. Furthermore, we de-tected the growth of anaerobic bacteria concomitantly with facultative anaerobic or facultative aerobic bacteria. In this context, further studies should be conducted to better understand the clinical implications associated with the presence of bacteria in different organs of dogs with CVL.

Conclusion

Taken together, these data prove that it is necessary to investigate other co-infectious agents in *Leishmania*-infected dogs in endemic areas. Highlighting that several zoonoses have been identified and it is necessary an effective treatment and public measures to contain the spread of canine leishmaniasis and other infections.

References

Attipa, C., L. Solano-Gallego, K. Papasouliotis, F. Soutter, D. Morris, C. Helps, S. Carver, & S. 2018. Association between canine leishmaniosis and *Ehrlichia canis* co-infection: a prospective case-control study. Parasit Vectors, 11(1): 1-9. DOI: https://doi.org/10.1186/S13071-018-2717-8

Bakaletz, L. O. 2004. Developing animal models for polymicrobial diseases. Nat Rev Microbiol. 2(7): 552-568. DOI: https://doi.org/10.1038/nrmicro928

Baxarias, M., A. Álvarez-Fernández, P. Martínez-Orellana, S. Montserrat-Sangrà, L. Ordeix, A. Rojas, Y. Nachum-Biala, G. Baneth & L. Solano-Gallego. 2018. Does co-infection with vector-borne pathogens play a role in clinical canine leishmaniosis? Parasit Vectors, 11(1): 1-16. DOI: https://doi.org/10.1186/S13071-018-2724-9

Cortese, L., G. Terrazzano, D. Piantedosi, M. Sica, M. Prisco, G. Ruggiero & P. Ciaramella. 2011. Prevalence of anti-platelet antibodies in dogs naturally co-infected by *Leishmania infantum* and *Ehrlichia canis.* Vet J., 188(1): 118-121. DOI: https: // doi.org/10.1016/J.TVJL.2010.03.015

Costa Oliveira, V., A. A. V. M. Junior, L. C. Ferreira, T. M. Q. Calvet, S. A. Santos, F. B. Figueiredo, M. P. Campos, F. C. C.

Rodrigues, R. V. C. Oliveira, E. R. S. Lemos, T. Rozental, R. G. Silva, M. R. R. Amendoeira, R. Teles-De-Freitas, R. V. Bruno, F. N. Morgado, L. F. C. Miranda & R. C. Menezes. 2021**.** Frequency of coseropositivities for certain pathogens and their relationship with clinical and histopathological changes and parasite load in dogs infected with *Leishmania infantum*. PLoS ONE, 16(3). DOI: https://doi.org/10.1371/JOURNAL.PONE.0247560

Fraga, D. B. M., M. S. Solcà, V. M. G. Silva, L. S. Borja, E. G. Nascimento, G. G. S. Oliveira, L. C. Pontes-de-Carvalho, P. S. T. Veras & W. L. C. Santos. 2012**.** Temporal distribution of positive results of tests for detecting *Leishmania infection* in stray dogs of an endemic area of visceral leishmaniasis in the Brazilian tropics: A 13 years survey and association with human disease. Vet Parasitol,190(3-4): 591-594. DOI: https://doi.org/10.1016/J.VETPAR.2012.06.025

Gallo-Francisco, P. H., M. Brocchi & S. Giorgio. 2022**.** Leishmania and its relationships with bacteria. Future Microbiol, 17(3): 199-218. DOI: https://doi.org/10.2217/FMB-2021-0133

Goulli, A. F., L. Zribi, R. Sanhaji, A. Chabchoub, A., Bouratbine, M. Gharbi & H. Abdelmelek. 2023**.** Study of ocular manifestations and humoral immune response in eyes of dogs with leishmaniasis. Vet Med Sci, 9(2): 625-637. DOI: https://doi.org/10.1002/VMS3.982

Gow, I., N. C. Smith, D. Stark & J. Ellis. 2022. Laboratory diagnostics for human *Leishmania* infections: a polymerase chain reaction-focussed review of detection and identification methods. Parasit Vectors, 15(1) paginação eletronica. DOI: https://doi.org/10.1186/S13071-022-05524-Z

Hoffman, W. A., J. A. Pons & J. L. Janer, J. L. 1934. The sedimentation-concentration method in *Schistostomiasis mansoni*. J Public Health Puerto Rico, 9(3): 283-291.

Instituto Brasileiro de Geografia e Estatística. 2010. Portal Cidades e Estados: Jequié. Dísponível em: <https://cidades.ibge.gov/brasil/ba/jequie/pesquisa/23/25124>. Access in nov. 2023.

Kirkwood, B. R., & J. A. C. Sterne. 2003. Essential Medical Sta-tistics. 2 ed. Oxford, Blackwell Science.

Medkour, H., Y. Laidoudi, E. Athias, A. Bouam, S. Dizoé, B. Davoust, O. Mediannikov. 2020. Molecular and serological detection of animal and human vector-borne pathogens in the blood of dogs from Côte d'Ivoire. Comp Immunol Microbiol Infect Dis., 69:101412. DOI: https://doi.org/10.1016/j.cimid.2019.101412.

Mekuzas, Y., L. Gradoni, G. Oliva, V. F. Manzillo & G. Baneth. 2009. *Ehrlichia canis* and *Leishmania infantum* co-infection: A 3-year longitudinal study in naturally exposed dogs. Clin Microbiol Infect.15 (SUPPL. 2): 30-31. DOI: https://doi.org/10.1111/j.1469-0691.2008.02150

Miranda, S., X. Roura, A. Picado, L. Ferrer & A. Ramis. 2008. Characterization of sex, age, and breed for a population of canine leishmaniosis diseased dogs. Clin Microbiol Infect,85 (1): 35-38. DOI: https://doi.org/10.1016/J.RVSC.2007.09.003

Montoya-Alonso, J. A., R. Morchón, N. Costa-Rodríguez, J. I. Matos, Y. Falcón-Cordón & E. Carretón. 2020. Current Distribution of Selected Vector-Borne Diseases in Dogs in Spain. Front Vet Sci, 7: 564429. DOI: https://doi.org/10.3389/fvets.2020.564429

Naranjo, C., D. Fondevila, M. Leiva, X. Roura & T. Peña. 2005. Characterization of lacrimal gland lesions and possible pathogenic mechanisms of keratoconjunctivitis sicca in dogs with leishmaniosis. Vet Parasitol, 133(1): 37-47. DOI: https://doi.org/10.1016/J.VETPAR.2005.05.017

Paranhos-Silva, M., E. G. Nascimento, M. C. B. F. Melro, G. G. S. Oliveira, W. L. C. Santos, L. C. Pontes-De-Carvalho & A. J. Oliveira-Dos-Santos. 1998. Cohort study on canine emigration and leishmania infection in an endemic area for american visceral leishmaniasis. Implications for the disease control. Acta Trop., 69(1): 75-83. DOI: https://doi.org/10.1016/S0001-706X(97)00116-2

Parin, U., G. Erbas, K. Ural, S. Savasan, H. T. Yuksel, G. Balat & S. Kirkan. 2020. Investigation of bacterial and fungal agents from cutaneous lesions in canine *Leishmania*sis. Indian J. Anim. Res., 54(1): 96-100. DOI: https://doi.org/10.18805/IJAR.B-696

Pinto, A. J. W., M. M. Figueiredo, F. L. Silva, T. Martins, M. S. M. Michalick, W. L. Tafuri & W. L. Tafuri. 2011. Histopatho-

logical and parasitological study of the gastrointestinal tract of dogs naturally infected with *Leishmania infantum*. Acta Vet Scand., 53(1): 67. DOI: https://doi.org/10.1186/1751-0147-53-67

Pumarola, M., L. Brevik, J. Badiola, A. Vargas, M. Domingo & L. Ferrer. 1991. Canine leishmaniasis associated with systemic vasculitis in two dogs. J Comp Pathol.. 105(3): 279-286. DOI: https://doi.org/10.1016/S0021-9975(08)80196-X

Ramos, R. A. N., A. Giannelli, C. R. C. Ubirajara-Filho, C. A. N. Ramos, D. Betbder, M. A. Bezerra-Santos, F. Dantas-Torres, L. C. Alves & D. Otranto. 2022. Vector-borne pathogens in dogs from areas where leishmaniosis is endemic. Vet Parasitol Reg Stud Reports, 32: 100746. DOI: https://doi.org/10.1016/J.V-PRSR.2022.100746

Ribeiro, R. R., M. E. Silva, S. M. Silva, G. O. Fulgêncio, H. F. J. Pena, F. Frézard, M. S. M. Michalick & S. M. Gennari. 2011. Occurrence of anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibodies in dogs with visceral leishmaniasis. Pesq Vet Bras., 31(6): 527-532. DOI: https://doi.org/10.1590/S0100-736X2011000600012

Santos, W. L. C., E. E. Jesus, M. Paranhos-Silva, A. M. Pereira, J. C. Santos, C. O. Baleeiro, E. G. Nascimento, E. D. Moreira, G. G. S. Oliveira & L. C. Pontes-de-Carvalho. 2008. Associations among immunological, parasitological and clinical parameters in canine visceral leishmaniasis: Emaciation, spleen parasitism, specific antibodies and leishmanin skin test reaction. Vet Immunol Immunopathol., 123(3-4): 251-259. DOI: https://doi.org/10.1016/J.VETIMM.2008.02.004

Santos, J. P., L. C. Alves, R. A. N. Ramos, D. S. Pimentel, G. A. Carvalho, M. F. M. Monteiro & M. A. G. Faustino. 2013. Histological changes and immunolabeling of *Leishmania infantum* in kidneys and urinary bladder of dogs. Rev. Bras. Parasitol. Vet., 22 (3). DOI: https://doi.org/10.1590/S1984-29612013000300017

Saijonmaa-Koulumies, L. E & D. H. Lloyd. 1996. Colonization of the canine skin with bacteria. Vet Dermatol., 7(3): 153-162. DOI: https://doi.org/10.1111/J.1365-3164.1996.TB00240.X

Saldanha-Elias, A. M., M. A. Silva, V. O. Silva, S. L. A. Amorim, A. R. Coutinho, H. A. Santos, R. C. Giunchetti, R. W. A. Vitor & S. M. Geiger. 2019. Prevalence of Endoparasites in Urban Stray Dogs from Brazil Diagnosed with *Leishmania*, with Potential for Human Zoonoses. Acta Parasitol., 64(2): 352-359. DOI: https://doi.org/10.2478/S11686-019-00043-X/METRICS

Sherlock I.A. & A. C. Santos. 1964. Leishmaniose visceral na zona de Jequié estado da Bahia. Rev Bras Malariol D Trop., 16: 441-448.

Silva, J. L., V. V. G. Oliveira, L. A. M. T. Silva, R. P. E. Silva, L. C. Alves, M. P. Cavalcanti & V. A. Silva Júnior. 2019. Evaluation of Serum Biochemical Parameters, Structural Changes, Immunohistochemistry and Parasite Load in the Urinary System of Dogs Infected Naturally by *Leishmania* infantum. J Comp Pathol., 167: 26-31. DOI: https://doi.org/10.1016/j.jcpa.2018.11.007.

Suksawat, J., Y. Xuejie, S. I. Hancock, B. C. Hegarty, P. Nilkumhang & E. B. Breitschwerdt. 2001. Serologic and Molecular Evidence of Coinfection with Multiple Vector-Borne Pathogens in Dogs from Thailand. J Vet Intern Med., 15(5): 453-462. DOI: https://doi.org/10.1111/J.1939-1676.2001.TB01574.X

Szabó, M. P. J., A. Pinter & M. B. Labruna. 2013. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front Cell Infect Microbiol., 3: 27. DOI: https://doi.org/10.3389/FCIMB.2013.00027

Tabar, M. D., L. Altet, V. Martínez & X. Roura. 2013. Wolbachia, filariae and *Leishmania* coinfection in dogs from a Mediterra-nean area. J Small Anim Pract., 54(4): 174-178. DOI: https://doi.org/10.1111/JSAP.12041

Tommasi, A. S., D. Otranto, F. Dantas-Torres, G. Capelli, E. B. Breitschwerdt & D. Caprariis. 2013. Are vector-borne pathogen co-infections complicating the clinical presentation in dogs? Parasit Vectors, 6(1): 97. DOI: https://doi.org/10.1186/1756-

3305-6-97

Xu, J., Y. Yuan, B. Wang, Q. Zhang, J. Wang, S. Wang, Y. Li & W. Yan. 2023. Microbiological Analysis and Mortality Risk Factors in Patients with Polymicrobial Bloodstream Infections. Infect Drug Resist., 20(16): 3917-3927. DOI: https//doi.org/10.2147/IDR.S412669

Willis, H. H. 1921. A simple levitation method for the detection of hookworm ova. Med. J. Aust., 2(18): 375-376. DOI: https://-doi.org/10.5694/J.1326-5377.1921.TB60654.X

Editor Chefe / Editor en Jefe / Chelf Editor: Clever Gomes Cardoso, UFG, Brazil
Recebido / Recibido / Received:27.07.2023
Revisado / Revised: 15.01.2024
Aceito / Aceptado /Accepted: 15.01.2024
Publicado / Published: 11.03.2024
DOI: https://doi.org/10.5216/rbn.v21i1.76440
Dados disponíveis / Datos disponibles / Available data: not informed