PARASITE AND BACTERIAL CO-INFECTIONS WITH Leishmania spp. IN DOGS

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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.
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 técnicas de flotação 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-infeções com Leishmania spp., parasitas e bactérias juntas foram relatadas. Co-infeções com Leishmania foram observadas em 89% dos animais com helmintos e 95% com ectoparasitas. A maioria dos cães foi positiva para Ehrlichia spp.. Consta-se que cães positivos para Leishmania provenientes de área endêmica no Brasil apresentam maior índice de co-infeções 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 unknown. 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 (Sukasawat 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).

CHARACTORIZATION 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 km², 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 puncter 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 °C. 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 morphologically identified by veterinarians.

Ant*-Ehrlichia canis* (Donatien and Lestouuard, 1935) / *Ehrlichia ewingii* (Anderson et al. 1992), *Anaplasma phagocytophilum* (Foggie, 1949) / *Anaplasma platys* (Dumler et al. 2001), *Dirofilaria immitis* and *Borrelia burgdorferii* (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 puncter 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 Microbiology Systems, Maryland, USA), then incubated at 37 °C for 24 to 48 hours. Samples from blood were inoculated in Mueller-Hinton broth (Merck KGaA, Darmstadt, Germany) at 35 °C for 12 days and inoculated in blood agar and MacConkey agar at 35 °C du-
ring 24 to 48 hours on days 2, 5, 7 and 12, or with any turbidity or CO₂ production. The samples from blood that presented turbidity or CO₂ production in Mueller-Hinton broth, without aerobic growth, were inoculated in Thioglycolate and Anaerisol agar (Probac, Bacteriological products Lt, Santa Cecilia, São Paulo, Brazil), and incubated in aerobic conditions, in microaerophilic atmosphere (10% CO₂) and aerobic conditions with anaero kit (Probac, Bacteriological products Lt, Santa Cecilia, 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 Microbiology 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 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 *Toxocara canis* 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 animals with 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 common bacteria found in dog’s eyes, while only *Streptococcus* spp.

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Leishmania</em> culture (n=47)</th>
<th><em>Leishmania</em> serology (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Estimated age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 2</td>
<td>26% (5/19)</td>
<td>25% (7/28)</td>
</tr>
<tr>
<td>3 - 5</td>
<td>68% (13/19)</td>
<td>54% (15/28)</td>
</tr>
<tr>
<td>≥ 6</td>
<td>5% (1/19)</td>
<td>21% (6/28)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Leishmania</em> culture (n=60)</th>
<th><em>Leishmania</em> serology (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>60% (12/20)</td>
<td>52% (21/40)</td>
</tr>
<tr>
<td>F</td>
<td>40% (8/20)</td>
<td>47% (19/40)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Leishmania</em> culture (n=58)</th>
<th><em>Leishmania</em> serology (n=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>39% (7/18)</td>
<td>20% (8/40)</td>
</tr>
<tr>
<td>Medium</td>
<td>44% (8/18)</td>
<td>72% (29/40)</td>
</tr>
<tr>
<td>Long</td>
<td>17% (3/18)</td>
<td>7% (3/40)</td>
</tr>
</tbody>
</table>

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

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**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.

**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 observed.
showed evidence of *Leishmania* spp. infection, indicating that Jequié conti-nuous to be an en-
demic region since the first report in 1998 (Pa-
ranhos-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 ri-
vers 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 nega-
tive 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 (Pa-
ranhos-Silva et al., 1998).

The high number of animals with *Leish-
mania* spp. evidence older than 3 years could be
related to the cumulative increase of the expo-
sure period to *Lutzomyia longipalpis* (Lutz &
Neiva, 1912) vector over the years. In symp-to-
matic 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 infec-
tion, probably the dogs had been living outdoors
without preventive measures against endopara-
sites and more exposed to the parasites. The most
common *Leishmania* co-infection with hel-
minths were hookworm, followed by *D. cani-
um*, roundworms and *Trichuris vulpis* (Froelich,
1789). Nevertheless, Saldanha-Elias et al.
(2019), in captured street dogs in Brazil, descri-
bled positive correlation between serology for
*Leishmania* infection and *D. caninum* intestinal
parasitism (Saldanha-Elias et al., 2019). Gas-
trointestinal signs are rarely attributed to infec-
tion with *Leishmania*, for this reason the high
parasite loads of this protozoan in the intestinal
mucosa with only mild pathological changes ha-
ve allowed us to consider whether the protozo-
an obtains advantage of intestinal tolerance
(Pinto et al., 2011).

Mixed infections with tick-borne patho-
gen 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 phlebo-
tomine sand flies (Suksawat et al., 2001; Baxarias
et al., 2018; Gow et al., 2022). The high sero-
reaction dogs against *Ehrlichia* spp. and Ana-
plasma 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. Corrobo-
rating our results, the high prevalence of co-infec-
tion between *Leishmania* and *Ehrlichia* spp. has
already been demonstrated in different world re-
gions such as northeastern Brazil, Rio de Janei-
ro, 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*-Ehr-
lchia co-infection could more severely affect the
immunity in canines (Cortese et al., 2011; Me-
kuzas 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 pathologi-
cal abnormalities, for instance reduction in al-
bumin 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 hae-
nocanis* (Messick et al., 2002) (Attipa et al.,
2018).

Interestingly, *L. infantum* seropositive
dogs had more endo and ectoparasites, sug-
gest ing that CVL antibodies in dogs may in-
crease their susceptibility to parasites. Correspond-
ingly, the significant higher levels of anti-Leish-
mania antibodies have been observed in
symptomatic dogs (Ribeiro et al., 2011). Fur-
thermore, *Leishmania*-infected dogs were
more infested by ectoparasites (ticks, fleas, or
both) (Ramos et al., 2022).

Bacterial infection was detected by cultu-
re 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 (Sajjonmaa-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 periorcular 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 detected 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**


