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Original Article

Serological and molecular survey of *Leishmania* infection in dogs from Venezuela

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ABSTRACT

Venezuela is a country where human and canine leishmaniosis due to *Leishmania infantum*, *Leishmania braziliensis* and other *Leishmania* spp. is endemic. However, only limited data is available on canine *Leishmania* infection in Venezuela. The aim of this cross-sectional study was to evaluate the prevalence of *Leishmania* infection in dogs ($n = 152$) from the states of Lara ($n = 91$) and Yaracuy ($n = 61$) in Venezuela by means of serological and molecular methods. Physical examination was performed and blood samples were collected from all dogs. Serology for antibodies reactive with *L. infantum* and *L. braziliensis* antigens was assessed by the enzyme-linked immunosorbent assay (ELISA) and detection of *Leishmania* DNA from blood samples was evaluated by kinetoplast *Leishmania* real-time polymerase chain reaction (RT-PCR). In addition, *Leishmania* internal transcribed spacer (ITS-1) RT-PCR was performed on the samples positive by kinetoplast RT-PCR. The prevalence of *Leishmania* infection based on serological and/or molecular techniques was 11.8%. The seroprevalence for *L. infantum* and *L. braziliensis* antigens were 2.1% (3/144) and 8.3% (12/144), respectively. All dogs from the state of Yaracuy were serologically negative to *L. infantum* while 4.6% (4/86) of the dogs were reactive to *L. braziliensis* antigen. Fourteen percent (8/58) of the dogs from the state of Lara were positive to *L. infantum* and 5.2% (3/58) to *L. braziliensis* antigen. Three dogs were positive to both *Leishmania* spp. antigens. By RT-PCR, 6.5% (4/61) and 4.4% (4/91) of the dogs were positive for infection in the states of Lara and Yaracuy, respectively. The RT-PCR product of one dog from the state of Yaracuy was sequenced revealing a 100% identity with *L. infantum*. However, all RT-PCR positive dogs were seronegative to both *Leishmania* spp. antigens. In conclusion, the positivity for *Leishmania* spp. infections observed indicates that dogs are frequently infected by *L. infantum*, *L. braziliensis* or related *Leishmania* spp. in Venezuela.

1. Introduction

Canine leishmaniosis is widespread in South America and is among the most important canine vector-borne diseases in this region, mainly because of its major zoonotic relevance (Dantas-Torres et al., 2012). Leishmaniosis constitutes a considerable public health problem in many areas of tropical and subtropical Latin America (Grimaldi and Tesh, 1993).

The primary mode of transmission of *Leishmania* parasites from dog to dog is through the bites of infected phlebotomine sand flies. In South America, the vector sand flies of *Leishmania* spp. belong to the genus *Lutzomyia* (López-Céspedes et al., 2012). Moreover, secondary modes of transmission might be involved and could be relevant for the establishment of new foci of canine leishmaniosis in non-endemic areas (Dantas-Torres, 2009). Secondary modes of transmission include transplacental transmission (Rosypal et al., 2005), blood transfusion (De Freitas et al., 2006), and venereal transmission (Rotureau et al., 2006; Silva et al., 2009).

Several *Leishmania* spp. have been isolated or molecularly characterized from dogs in South America: *L. amazonensis* (Tolezano et al., 2007), *L. braziliensis* (Falqueto et al., 1991), *L. colombiensis* (Pace, 2014), *L. infantum* (syn. *L. chagasi*) (De Andrade et al., 2006), *L. mexicana* (Hashiguchi et al., 2018), *L. panamensis* (Pace, 2014), *L. peruviana* (Hashiguchi et al., 2018) and *L. pifanoi* (Pace, 2014).

Leishmania braziliensis and *L. infantum* are the most widespread species responsible for infecting dogs in South America. The distribution of both species is probably wider than actually reported (Dantas-Torres, 2009). Importantly, there is considerable epidemiological

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and experimental evidence indicating that dogs are the main reservoir hosts for infection with *L. infantum* in Latin American countries (Dantas-Torres, 2009; Reithinger and Davies, 1999). Therefore, the domestic dog plays an important role in the epidemiology of zoonotic human visceral leishmaniosis due to *L. infantum* in these countries (Dantas-Torres, 2007). However, the dog is most likely only an incidental host for *L. braziliensis* and its role in the zoonotic cycle of its transmission is probably negligible (Dantas-Torres, 2007).

Most information on the prevalence of *L. infantum* and *L. braziliensis* infections among dogs in South America is from serological and molecular surveys conducted in Brazil (Carvalho et al., 2015; Segatto et al., 2012) while a small number of studies originates from other countries such as Argentina (Barroso et al., 2015), Colombia (Ramírez et al., 2016) and Venezuela (Jorquera et al., 2005; Panamerican Health Organization, 2018).

In Venezuela, cutaneous human leishmaniosis is most abundant in rural and suburban areas and is mostly prevalent in people with scarce economic resources including farmers. The disease is widely distributed in the whole country, especially in the Andean region of Venezuela from which about 60% of the total cases are reported by the National Registry of Health (De Lima et al., 2002). The *Leishmania* spp. that can cause tegumentary leishmaniosis in humans in Venezuela are *L. amazonensis, L. braziliensis, L. colombiensis, L. garnhami, L. panamensis, L. pifanoi* and *L. venezuelensis* (Oletta and Saùl-Peña, 2011). Human visceral leishmaniosis caused by *L. infantum* is of increasing importance in Venezuela (Romero et al., 2012). However, limited data is available regarding *Leishmania* infections in canines in this country (Aguilar et al., 1984; Feliciangeli et al., 2005).

The aim of this cross-sectional study was to evaluate the prevalence of *Leishmania* infection in a convenience sample of dogs from the states of Lara and Yaracuy in Venezuela by means of serological and molecular techniques.

2. Material and methods

2.1. Study area and sample and data collection

2.1.1. Dogs

A convenience sample of dogs from urban areas in the states of Yaracuy (n = 91) and Lara (n = 61) in Venezuela that were admitted to veterinary clinics for routine health evaluation and vaccination from March 2014 to January 2016 were included in the study. A full physical examination was performed before blood sampling by venepuncture from the dogs (n = 152). Breed, age, sex and full clinical history were also recorded.

The blood samples (n = 152) were collected into ethylenediaminetetraacetic acid (EDTA) for DNA extraction and real-time polymerase chain reaction (RT-PCR) and into plain tubes centrifuged to obtain sera samples (n = 144) for the enzyme-linked immunosorbent assay (ELISA). All samples were stored at −20 °C before use. The geographical distribution of the dogs sampled is depicted in Fig. 1. Dogs were sampled from two cities in Lara state (Santa Rosa and Tamaca) and one city in Yaracuy state (Yumare).

Dogs that did not present evidence of systemic clinical signs or dermatological lesions were classified as apparently healthy (n = 134). Dogs with evidence of systemic clinical signs (lymphadenomegaly, anorexia, pale mucous membranes; n = 2) or dermatological lesions (exfoliative, ulcerative or pustular dermatitis, alopecia and erythema of scrotum; n = 16) were classified as sick (n = 18). Hematological and biochemical profiles were not performed due to the unavailability of reliable hematological or biochemical analyzers for dogs in the surveyed areas.

Dogs were classified according to age into two groups: young dogs if aged equal or less than 12 months, and adults if older than 12 months of age. In Yaracuy state, both sexes were represented by

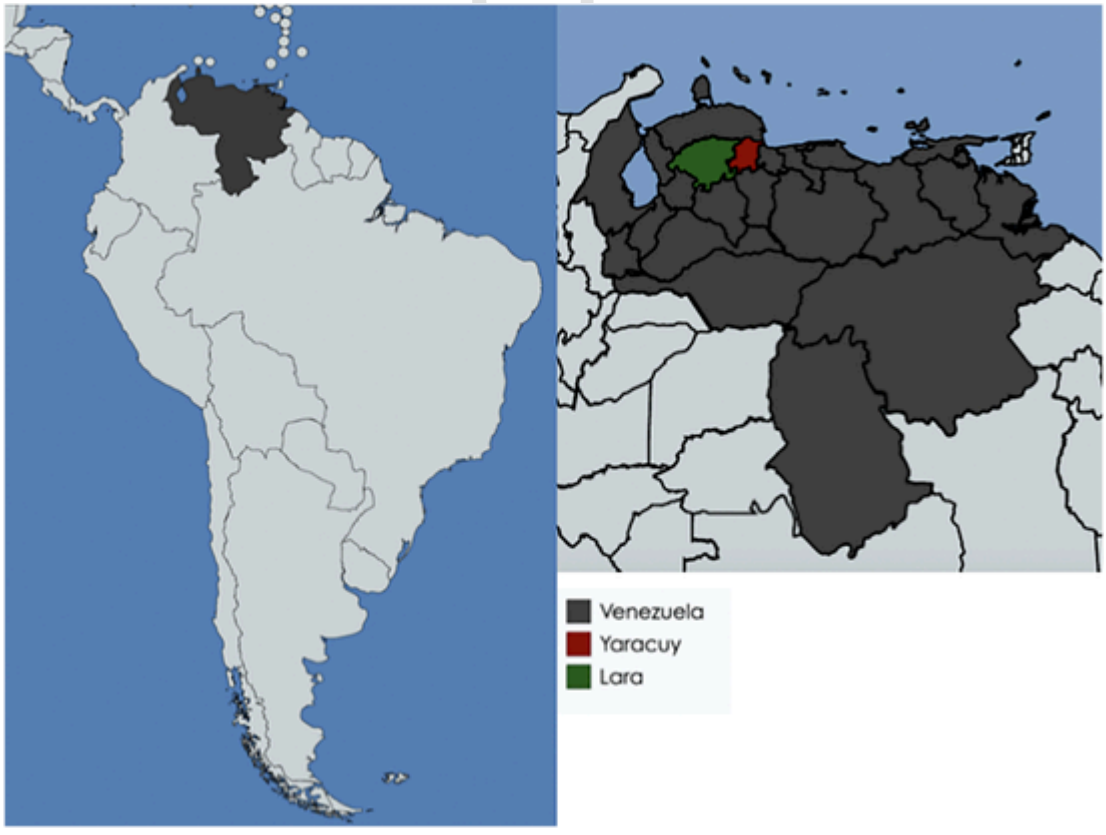

Fig. 1. A map of Venezuela highlighting the states of Yaracuy and Lara where canine sampling was performed.

30 females [young (n = 15) and adult (n = 15)] and 61 males [young (n = 40) and adult (n = 21)]. Dog breeds included Pitbull (n = 6), Pinscher (n = 5), Poodle (n = 5), Siberian husky (n = 5), Rottweiler (n = 3), Dachshund (n = 2), Dalmatian (n = 2), German shepherd (n = 2), Basset hound (n = 1), Beagle (n = 1), Cocker spaniel (n = 1), Doberman (n = 1), Golden retriever (n = 1) and Mastiff (n = 1). Fifty-six dogs were mixed-breed.

In Lara state, both sexes were represented by 16 females [young (n = 4) and adult (n = 12)] and 45 males [young (n = 18) and adult (n = 27)]. Dog breeds included Dalmatian (n = 3), Pitbull (n = 3), Poodle (n = 3), Rottweiler (n = 3), Dachshund (n = 2), German Shepherd (n = 2), Golden retriever (n = 2), Mastiff (n = 1) and Weimaraner (n = 1). The rest were mixed-breed (n = 40).

2.2. Diagnostic techniques

2.2.1. Detection of antibodies against L. infantum and L. braziliensis antigens by the quantitative enzyme-linked immunosorbent assay (ELISA)

Serology was based on a *L. infantum* in house ELISA protocol previously described for dogs (Solano-Gallego et al., 2014) which was slightly modified. *L. infantum* (MHOM/MON-1/LEM-75) and *L. braziliensis* (MHOM/BR/88/BCN-25) antigens were used in different wells in the same ELISA plate as described for cats (Rivas et al., 2018).

Dog sera were diluted to 1:800 in phosphate-buffered saline (PBS) with 0.05% Tween 20 (Sigma Aldrich, St. Louis Missouri, USA) containing 1% dry milk and incubated for 1 h at 37 °C with sonicated crude *L. infantum* or *L. braziliensis* antigens (20 μg/mL) in plates divided so that half of the wells contained one antigen while the other half contained the other antigen. The plates were then washed with 0.05% Tween 20 in PBS (PBS Tween 20) and incubated with Protein A conjugated to horseradish peroxidase 1:30.000 dilution for 1 h at 37 °C. Plates were washed again with 0.05% PBS Tween20 and developed by adding the substrate solution ortho-phenylene-diamine and stable peroxide substrate buffer (SIGMAFAST OPD, Sigma Aldrich, St. Louis, Missouri, USA). The reaction was stopped with 50 μl of 2.5 M H_2SO_4 and absorbance values were read at 492 nm by an automatic microELISA reader (ELISA Reader Anthos 2020, Cambridge, United Kingdom).

All plates included serum from a sick 9 year old intact female Yorkshire dog with confirmed *L. infantum* infection as a positive control (≈300 EU) and serum from a healthy one year spayed female Beagle dog confirmed by serology and PCR as a negative control (≈5 EU). All samples were analysed in duplicate. The samples were measured again by ELISA if disparate results were obtained. The results were quantified in ELISA units (EU) related to a positive canine serum used as a calibrator (optical density of approximately 1) for both *Leishmania* antigens and arbitrarily set at 100 EU (Solano-Gallego et al., 2014).

The cut-off value was established at 35 EU (mean + 4 standard deviation (SD) of values from 80 dogs from the United Kingdom, a non-endemic area) for *L. infantum*. Sera were classified as positive; when the results were equal or higher than 35 EU and negative when lower than 35 EU. The cut-off for *L. braziliensis* was established at 8.5 U (mean + 4 SD of values of 80 dogs from the United Kingdom). Sera were classified as positive for *L. braziliensis* antigen when equal or higher than 8.5 EU and negative when lower than 8.5 EU. ELISAs were performed at *Universitat Autònoma de Barcelona* (UAB).

2.2.2. Blood DNA extraction and Leishmania kinetoplast real-time polymerase chain reaction (RT-PCR)

DNA was extracted using the Gen Elute blood genomic DNA kit (Sigma Aldrich, Missouri, USA) and following the manufacturer's instructions with minor modification. Forty microliters of proteinase K solution were added to all samples. Four hundred microliters of whole blood were used for DNA extraction from the blood samples. The other steps were performed as described in the manufacturer's protocol. Blood from a clinically healthy non-infected dog was used as a control for DNA contamination in each DNA extraction performed (Solano-Gallego et al., 2016).

The presence of *Leishmania* spp. DNA was analysed by amplification of kinetoplast DNA sequence by a real-time polymerase chain reaction (RT-PCR). Each amplification was performed in triplicate in 10 μLreactions and included 15 pmol of forward primer (CTTTTCTGGTCCTCCGGGTAGG), 15 pmol of reverse primer (CCACCCGGCCCTATTTTACACCAA), 50 pmol of the labelled TaqMan probe (FAM-TTTTCGCAGAACGCCCCTACCCGC- TAMRA) and 5 μL of the tested DNA sample.

Amplification and detection were performed using the ABI Prism 7900 system (Applied Biosystems, Foster City, CA, U.S.A.) in two-step temperature (94 and 55 °C) over 45 cycles. Positive controls (DNA from *L. infantum* MHOM /ES /04 /BCN-61) and negative controls were included in each RT-PCR analysis (Martín-Ezquerra et al., 2009; Rivas et al., 2018).

2.3. Leishmania internal transcribed spacer (ITS-1) RT-PCR by high resolution melting analysis and sequencing

The identification of *Leishmania* species was performed only for *Leishmania* spp. RT-PCR positive dogs. A 265 bp fragment of the *Leishmania* internal transcribed spacer 1 (ITS1) region of the *L. infantum* rRNA operon was amplified by RT-PCR using primers ITS-219 F (AGCTGGATCATTTTCCGATG) and ITS-219R (ATCGCGACACGTTATGTGAG) and then evaluated by high resolution melting (HRM) analysis as previously described (Talmi-Frank et al., 2010).

DNA samples extracted from cell cultures of *L. infantum*, *Leishmania major*, and *Leishmania tropica* were used as positive controls for each corresponding PCR reaction and DNA from colony-bred dogs negative by PCR for vector-borne pathogens was used as negative control. A non-template control (NTC) with the same reagents described above but without DNA was added to each PCR to rule out contamination.

All positive RT-PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Center for Genomic Technologies, Hebrew University of Jerusalem, Israel. DNA sequences were evaluated with the ChromasPro software version 2. 1.1 (Technelysium Pty Ltd., Australia) and compared for similarity with sequences available in GenBank®, using BLAST program.

2.4. Statistical analysis

Statistical analysis was performed using the SPSS 22.0 software for Windows (SPSS Inc., Armonk, USA). A non-parametric Mann-Whitney *U* test was used to compare continuous variables among different groups. The non-parametric Wilcoxon signed-rank test was used to compare paired continuous variables. Fisher's exact test was used to compare frequencies among different groups. Cohen kappa agreement was used to compare diagnostic techniques. Differences were considered significant with a 5% significance level ($p < .05$).

3. Results

3.1. Serological test (ELISA)

The results of serological tests performed are summarized in Table 1 and Supplementary Table S1. The total percentages of *L. infantum* and *L. braziliensis* seropositive dogs were 2.1% (3/144) and 8.3% (12/144), respectively. The levels of antibodies reactive with *L. infantum* and *L. braziliensis* antigens had a mean ± SD of 39.1 ± 42.5 EU and 15.6 ± 11.6 EU, respectively (Wilcoxon signed-rank test, p = .001).

All dogs from Yaracuy state were serologically negative to *L. infantum* antigen, while 4.6% (4/86) of the dogs were seroreactive to

L. braziliensis antigen. Of the dogs from Lara state, 13.8% (8/58) were seroreactive to *L. braziliensis* antigen and 5.2% (3/58) to *L. infantum* antigen. Three dogs were seroreactive to both *Leishmania* antigens. However, higher antibody levels were found for *L. infantum* when compared with *L. braziliensis* (Wilcoxon signed-rank test, p = .001, see Supplementary Table S1).

The antibody response was significantly higher to both *L. infantum* and *L. braziliensis* antigens (Mann-Whitney U test, $p = .005$ and $p = .077$, respectively) in Lara state (*L. infantum*: mean ± SD of 101.6 ± 63.7 EU; *L. braziliensis*: mean ± SD = 18.1 ± 13.2 EU) when compared to the response of seropositive dogs from Yaracuy state (*L. infantum*: mean ± SD = 21.3 ± 8.3 EU; *L. braziliensis*: mean ± SD = 11.1 ± 3.8 EU). A fair agreement (Cohen kappa agreement of 0.35) was found between *L. infantum* and *L. braziliensis* antibody levels.

A significant association was found between *L. infantum* ELISA and being a female (Fisher's exact test, $p = .0272$; Table 1). No other significant associations were encountered.

3.2. Molecular test (RT-PCR)

The PCR results are listed in Table 1 and Supplementary Table S1. The total positivity of *Leishmania* infection in blood samples by means of *Leishmania* kinetoplast RT-PCR was 5.3% (8/152) with 6.5% (4/61) in Lara state and 4.4% (4/91) in Yaracuy state. No significant association was found between positivity by RT-PCR and age, sex, breed, geographical location or clinical status of dogs (Table 1). All the dogs positive for *Leishmania* by kinetoplast RT-PCR were seronegative by ELISA serology to both *Leishmania* antigens (Supplementary Table S1).

The identification of *Leishmania* species was attempted by ITS-1 RT-PCR for all dogs positive by *Leishmania* kinetoplast RT-PCR. However, the DNA sample from only one adult male dog from Yaracuy state resulted positive by the ITS-1 RT-PCR. The amplicon from this dog (ID:Cve50) was sequenced and 100% identity was found with *L. infantum* isolate MCAN/IL/2010/NT1 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence (ID GenBank®: KM677128®) (Yasur-Landau et al., 2016).

3.3. Combination of diagnostic techniques

The overall results based on serological and molecular techniques are displayed in Table 1. The total prevalence of *Leishmania* infection based on serological and molecular techniques was 11.8% (95% confidence interval of 7.5–18.0). No other significant associations were found.

4. Discussion

This study evaluated infection with *Leishmania* spp. in dogs in areas that are endemic for human leishmaniosis due to *L. infantum* (Feliciangeli et al., 2005), *L. braziliensis* (Bonfante-Garrido et al., 1992) and *L. venezuelensis/ mexicana* (Kato et al., 2011) in Venezuela. Due to the fact that several *Leishmania* spp. co-exist in Venezuela, two ELISAs with *Leishmania* antigens (*L. infantum* and *L. braziliensis*) were used for the detection of antibodies reactive with these antigens. To the best knowledge of the authors, this is the first serological investigation of *L. braziliensis* infection in dogs from Venezuela.

In total, 2.1% of the sera reacted with *L. infantum* antigens and 8.3%, with *L. braziliensis* while *Leishmania* spp. infection detected by *Leishmania* kinetoplast RT-PCR in blood samples was 5.3%. However, the PCR-positive dogs were all seronegative by ELISA. Interestingly, *L. braziliensis* was previously diagnosed in cutaneous lesions from several infected mammal species in Venezuela (Aguilar et al., 1984).

A study on dogs from the city of Ilhéus in north-eastern Brazil based mainly on blood conventional PCR showed a much higher prevalence of *Leishmania* infection (54.7%) when compared with the present study based on serological and molecular techniques (Carvalho et al., 2015). It is important to highlight that *L. major* antigen was employed for serological testing and not *L. braziliensis* antigen in the study from Brazil (Carvalho et al., 2015). In this Brazilian study, only *L. braziliensis* was diagnosed in dogs while *L. infantum* infection was not documented (Carvalho et al., 2015). Furthermore, no correlation between the serological and molecular test results was found in the Brazilian study (Carvalho et al., 2015) in agreement with the current study from Venezuela. In a study performed in dogs naturally infected by *L. braziliensis* from Paraná in Brazil, the parasite was detected by PCR in the blood, intact skin and internal organs in dogs despite their healthy appearance (de Marquez et al., 2017). This agrees with findings from our study in which 5.3% of the clinically healthy dogs were positive for *Leishmania* spp. by blood RT-PCR.

Studies on risk factors associated with *L. infantum* infection performed in South America are limited in number (Alves et al., 2016) when compared with Europe (Cardoso and Solano-Gallego, 2013). Several possible risk factors for *Leishmania* spp. infection were investigated in the present study. These included age, sex, breed, clinical status and geographical location. Interestingly, a significant association was only found between *L. infantum* seropositivity by ELISA and being a female dog. So far, the association of sex with *Leishmania* infection in dogs remains controversial and the majority of studies performed did not find differences between infection rates in female and male dogs in Europe (Cardoso and Solano-Gallego, 2013) and South America (Carvalho et al., 2015, Lima et al., 2012).

Although significant differences in infection values were not encountered between geographical locations in this study, a trend of a higher seroprevalence for both antigens was evident in dogs from the state of Lara when compared with dogs from the state of Yaracuy. A previous study conducted in Lara State (El Brasilar, Curarigua) described isolation of the parasite from bone marrow samples of two seropositive dogs and species identification by PCR based on telomeric sequences which detected *L. infantum* (Feliciangeli et al., 2005). This study concluded that, El Brasilar was an active focus of canine leishmaniosis due to *L. infantum*, mostly children were affected by the disease and the infection appeared to be increasing in prevalence (Feliciangeli et al., 2005). The only dog infected with *L. infantum* based on blood PCR in the current study and confirmed by ITS1 RT-PCR and sequencing was from the state of Yaracuy. To the authors' best knowledge, the present study documented canine *L. infantum* infection in the state of Yaracuy for the first time. Since dogs are considered the main reservoir for *L. infantum* infection worldwide, domestic dogs probably represent the main source of infection to humans also in Venezuela as found in other areas in the world (Sanchez et al., 2004).

Unfortunately, we did not manage to speciate the *Leishmania* sp. infecting all dogs that were positive by *Leishmania* kinetoplast RT-PCR in blood.

Leishmania mexicana was not diagnosed in the present study in apparently healthy dogs differently from the results found in sick cats and dogs with cutaneous lesions in Venezuela where *L. mexicana* or a closely –related species included in the *L. braziliensis* species complex was diagnosed (Rivas et al., 2018; Mondolfi et al., 2019).

The ELISA used in this study for the diagnosis of canine leishmaniosis in Venezuela does not determine the species of *Leishmania* infecting the seropositive dog. It is well recognized that serological cross-reactivity exists between different *Leishmania* spp. and also with *Trypanosoma* spp. (Cervantes-Landín et al., 2014). This supports the recommendation for combined use of serological and molecular techniques in canine studies on leishmaniosis (Trevisan et al., 2015). The application of PCR together with serology not only helps in determining the extension of subclinical infections but also allows estimation of the number of dogs to be targeted for control measures. A greater number of in-

Table 1
Summary of serological and molecular results from dogs living in the states of Yaracuy and Lara (Venezuela).

		Risk factors												
Diagnostic tests		Number (%, 95% CI) of positive dogs												
		Age		*P*-value	Sex		P-value [a]	Breed		P-value	Location [b]		P-value	Clinical status	
		Young	Adult		Male	Female		Pure breed	Mixed breed		Lara	Yaracuy		Healthy dogs	S
		(*n* = 77)	(*n* = 75)		(*n* = 106)	(*n* = 46)		(*n* = 56)	(*n* = 96)					(n = 134)	(
ELISA (n = 144)	*L. infantum*	0	3		0	3		0	3		3	0		3	0
		0	(3.8%,0.9–11.3)	0.2448	0	(6.1%,1.5–17.2)	**0.0272**	0	(3%,0.7–8.8)	0.2973	(5.2%, 1.2–14.7)	0	**0.0633**	(2.1%, 0.5–64.5)	0
	L. braziliensis	2	6		8	4		6	6		8	4		10	2
		(2.5%,0.2–9.2)	(7.8%,3.3–16.3)	0.2750	(7.4%,3.6–14.1)	(8.2%,2.7–19.7)	1.0000	(10.5%,4.5–21.5)	(6%,2.5–12.7)	0.3585	(13.8%,6.9–25.2)	(6.6%, 2.1–16.1)	**0.0670**	(7.2%,3.8–12.9)	(1
Kinetoplast RT-PCR [c] (n = 152)		4	4		5	3		4	4		4	4		8	0
		(5.1%, 1.6–13.0.)	(5.3%, 1.6–13.3)	1.0000	(4.7.%, 1.8–10.8)	6.5%, 1.6–18.1)	0.6987	(7.0%, 2.3–17.4)	(4.0%, 1.3–10.5)	0.4672	(6.5%, 2.1–16.1)	(4.4%, 1.3–11.1)	0.7144	(6.5%, 2.8–11.5)	0
L. infantum or *L. braziliensis* ELISA or RT-PCR [d]		6	13		13	10		10	13		15	8		21	2
		(7.7%, 3.3–16.2)	(17.3%, 10.2–27.5)	**0.0893**	(12.2.%, 7.2–19.9)	(21.7%,12.0–35.7)	0.1457	(17.8%, 9.8–30.0)	(13.5%, 7.9–21.9)	0.4894	(17.4%, 10.7–26.9)	(8.7%, 4.3–16.6)	0.1172	(15.6%, 10.4–22.8)	(1

Abbreviations: CI, confidence interval; RT-PCR, real-time polymerase chain reaction.
[a] p value <0.05 close to 0.05 is highlighted in bold.
[b] Sera and blood samples studied from dogs living in Yaracuy state were 58 and 61 and from Lara state were 86 and 91, respectively.
[c] one adult male dog from Yaracuy state was confirmed as infected with *L. infantum* by partially sequence ITS1 with 100% of identity.
[d] Calculated based on number of positive dogs based on *L. infantum* ELISA and/or *L. braziliensis* ELISA and/or RT-PCR without repetition of dogs if positive to more than one technique.

fected dogs can be detected when serological and molecular techniques are employed (Rennó et al., 2014). PCR was able to detect subclinical canine infection by *L. infantum* in the present study and previous studies (De Andrade et al., 2006), as well as dogs infected sub-clinically with *L. braziliensis* (Carvalho et al., 2015).

The ELISA and RT-PCR discordant results in the present study can be attributed to the inherent differences between serological testing and molecular methods, and therefore, PCR from blood appears not to have a high sensitivity (Silva et al., 2011). Another study from urban areas in Brazil showed that the prevalence of *L. infantum* infection in dogs determined by blood PCR and restriction fragment length polymorphism (RFLP) (24.7%) was higher than that detected by serology (15.9%), and demonstrated discordant results between serological and molecular tests (Veloso et al., 2011), in agreement with our study result. Sampling of other tissue such as skin, mucosal areas and lymph nodes was likely to improve the percentage of diagnosis of *Leishmania* infection in the present study as described elsewhere (de Marquez et al., 2017; Pace, 2014).

Another important point to remark is the fact that significantly higher antibody levels (EU) were found by the *L. infantum* ELISA when compared with the *L. braziliensis* ELISA in dogs from Venezuela. This finding is not surprising due to the fact that it is well known that humans produce low antibody levels against *L. braziliensis* (Fagundes-Silva et al., 2012) and the same appears to occur in canines (Figueiredo et al., 2009).

The limitations of this study include the small number of dogs and provinces studied from Venezuela, the lack of testing for other pathogens such as *Trypanosoma cruzi* and *Trypanosoma caninum*, and the type of tissue sampled used (blood) (Persichetti et al., 2017; Solano-Gallego et al., 2014). Other limitations are the absence of more in depth clinical data on the dogs in the study including a CBC and biochemical profile.

5. Conclusion

We conclude that the serological and PCR prevalences of *Leishmania* spp. infection observed indicate that dogs might be frequently infected by *L. infantum, L. braziliensis* or closely related species in areas where leishmaniosis is endemic in Venezuela.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2020.100420.

Ethics approval and consent to participate

An ethical approval was not required because blood from dogs was taken for a health check as part of a veterinary and human health program (veterinary consultation) in several cities. Residual samples were used for all the testing described in the present study. The program was performed by the Department of Animal Medicine and Surgery, Veterinary School, University of Centroccidental Lisandro Alvarado, Barquisimeto, Venezuela. Owner consent was obtained prior to blood sampling.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Funding

Not applicable.

Authors' contributions

LSG designed the research study. LSG, CR, RF and GB supervised technical work. LSG, AKR, and PMO contributed with data analysis and interpretation. LSG and AKR wrote the manuscript. AKR examined and collected samples from dogs living in Venezuela. PMO, MA and AKR performed serological testing. AKR, MA, SM, GB and YNB performed the molecular diagnosis in this study. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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