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Seroprevalence and Risk Factors of *Leishmania infantum* in Canine Shelters in Bucaramanga Metropolitan Area and Barrancabermeja, Andean Region Santander, Colombia

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ABSTRACT

Leishmaniasis is a protozoan disease caused by *Leishmania spp.* and transmitted by sand-flies to different hosts. In Colombia, *Leishmania* has been reported in dogs from rural and urban areas. The aims of the investigation were to define the risk factors of *Leishmania infantum* (*L. infantum*) in canine shelters in municipalities of the Bucaramanga metropolitan area, and Barrancabermeja municipality, and to determine the seroprevalence for *L. infantum*. An observational study was carried out. The samples were analyzed with ELISA antibody anti-*L. infantum* (n=282). Epidemiological data about the risk factors were achieved by conducting a survey in these dog shelters (14 questions). The data were analyzed to determine the association between the variables under study and the results of the ELISA test. The seroprevalence of *L. infantum* was 4.3% in the Bucaramanga metropolitan area and zero (0/48) in Barrancabermeja. The dogs sleeping outdoor was associated with the odds for *Leishmania* in Bucaramanga (P=0.04). We have demonstrated the seroprevalence of *L. infantum* in canine shelters in the Bucaramanga metropolitan area, possibly by the high contact of dogs with *Lutzomyia* spp. vectors. This data may help to identify risk factors in similar geographical areas, assisting to design new control strategies.

Key words: Risk factors, Seroprevalence, *Leishmania*.

INTRODUCTION

Leishmaniasis is a disease caused by the protozoa *Leishmania* spp. transmitted by the sand-flies *Lutzomyia* (Lu.) *longipalpis* and *Lu. evansi* in the Americas. The disease is ubiquitous in dogs and other hosts, including humans, being endemic in continents Asian, African, Europe, and the countries of the Latin American continent (LATAM) where vectors are found in suitable environmental conditions. *Leishmania* spp. cause visceral, mucocutaneous, and cutaneous leishmaniasis (Meleau and Hnilico 2006; Bowman 2014). A large number of canine infections in South America are produced by *L. infantum* and *L. braziliensis*, but their distribution is probably higher than it is recorded (Dantas-Torres 2009). In some LATAM countries, the canines are observed as the essential domestic accumulators of *L. infantum* having a high-risk exposure to *Leishmania* (Feliciangeli et al. 2005; Dantas-Torres and Brandão-Filho 2006; Fernandez et al. 2006; Dantas-Torres 2007; Romero et al. 2009; Barroso et al. 2015; Acosta et al. 2015; Rivero et al. 2018). Moreover, some other studies have observed that dogs may not play a major role in the epidemiology of the disease (Travi et al. 1992; Rosypal et al. 2007).

In Colombia, *L. infantum* has been reported in dogs from rural and urban areas. In Piedecuesta, *L. chagasi* was detected in dogs using a serological test (Márquez 2004). *L. infantum* antibodies were also observed in dogs from the Tolima Department (Romero et al. 2008; Romero et al. 2009). Fernández et al. (2002) and Fernández et al. (2006) have determined the seroprevalence of *L. infantum chagasi* from dogs from the Huila Department. Similar

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data were found from Neiva, determining a 6.1% prevalence of *L. infantum* in dogs (Zambrano et al. 2015). A study in Bogotá found a low (1.6%) *L. infantum* seroprevalence in dogs (Rosypal et al. 2007). Also, in a visceral leishmaniasis-endemic area of Cundinamarca, an investigation showed dogs that were serologically positive by an enzyme-linked immunosorbent assay (ELISA) which used soluble *Leishmania chagasi* (Travi et al. 2001).

In Bucaramanga, Colombia a dog was reported with *L. infantum* antibodies (Cáceres 2020). Serum samples of dogs stored at the Biobank of the National Institute of Health of Colombia from a previous outbreak of visceral leishmaniasis (VL) were found (100%) positive to *L. infantum* (Herrera et al. 2018; Herrera et al. 2019). In the Department of Sucre, *L. infantum* was determined in dogs by serology and PCR (Rivero et al. 2018; Rivero et al. 2020). Arbeláez et al. (2020) also reported an urban case of canine visceral leishmaniasis in Cali, using a primer-specific PCR diagnostic.

In Argentina, *L. infantum* was observed in a 6.2% of dogs (Acosta et al. 2015; Barroso et al. 2015). In Petrolina and Jequié, in the state of Bahia, Brazil, the seroprevalence of *L. infantum* was associated with the animal gender, breed, age group, and hair length (Moreira et al. 2003; Queiroz et al 2009; Pacheco et al. 2013; Barbosa et al. 2015; Araujo et al. 2016; Fujimori et al. 2016). In Venezuela, *L. infantum* was found in 2.1% of dogs by PCR detection (Feliciangeli et al. 2005; Rivas et al. 2020). *L. infantum* was also reported in Mexico from 6.1 to 11.9% in dogs (Arjona et al. 2012; López et al. 2012). Reports of the low incidence of *L. infantum* in dogs (IFA titers ≥1:128 and PCR) came also from the United States of America (Gaskin et al. 2002; Schaut et al. 2015; de Almeida et al. 2020).

Studies have been carried out in different regions of Colombia that have reported *Leishmania* species infecting humans and dogs in the same geographic area (Herrera et al. 2018; Herrera et al. 2019). Due to one health related problems of leishmaniasis, more epidemiological investigations should be carried out to determine *L. infantum* prevalence and associated risk factors in dog populations from Colombia (Rosypal et al. 2007). However, the routine surveillance of leishmaniasis in dogs is not regularly carried out in the country, and therefore, the assessment of the spread of the infection has not yet been fully elucidated (Herrera et al. 2018). In this way, the identification of risk factors of *Leishmania* disease in dogs is important to establish and carry out adequate prevention strategies in the dog population that could lead to improved and successful control programs of zoonotic visceral leishmaniasis (Moreira et al. 2003). Despite the above situation, there are no scientific reports that have assessed the epidemiological situation of this disease in the Andean region of Santander. The aims of investigation were to define the risk factors of *Leishmania infantum* in canine shelters in the municipalities of the Bucaramanga metropolitan area, and Barrancabermeja, Department of Santander. An additional, objective was the determination of seroprevalence for *Leishmania infantum*.

MATERIALS AND METHODS

Ethical Considerations

The present investigation was approved by the Committee of Ethics and Research of the University of Santander, Colombia (protocol no. CIF0308-19). Owners of the dogs participating in the project were informed of the research objectives and signed the 'Informed Consent Form' before all sample collections.

Study Design

A cross-sectional and descriptive investigation was carried out during September to December 2019 in dog shelters located in the Bucaramanga (7°07′07″N-73°06′58″W), Floridablanca (7°04′11″N-73°05′52″W), Girón (7°04′23″N-73°10′05″W), Piedecuesta (6°59′19″N-73°03′01″W) and Barrancabermeja (7°4' 1" N- 73° 52' 1" W) (Fig. 1) municipalities Santander Department, Colombia (Gobernación de Santander 2017). The environmental characteristics of the Department of Santander are similar, with an average temperature of 25°C and little climatic variation during the year. It is located between 600 and 1700 meters above sea level and with an average annual rainfall of 1040 mm, and 78% relative humidity. This Department has a geographical area of 1479 km2. Rainfall is more frequent during the months of October to December (IDEAM 2019).

The period was characterized as rainy with a constant presence of flies and mosquitos (A. Florez Muñoz, Personal communication).

Laboratory Analysis

Blood Sampling

Serum samples were taken applying simple random sampling in each canine shelter and Leishmaniasis was determined using an ELISA antibody anti-*Leishmania infantum.* Serum samples from canines older than 6 months and living in the shelters for at least 6 months were taken into account. A sample population of 282 was determined applying the model for a known frequency (Thrusfield 2007) with a projected canine seroprevalence of 23% (Le Pape 1992) and a confidence interval of 95%. The samples were randomly distributed (approx. 30 samples/shelter) with respect to the canine population reported in the municipalities under study (Ministerio de Salud de Colombia 2019).

Serological Analysis

A 6mL whole blood sample was taken by puncture of the cephalic vein in a sterile vacutainer tube with anticoagulant from each canine. These blood samples were centrifuged at 3000rpm for 10min, and the serum was transported to 1.5mL Eppendorf tubes and stored at -80°C. The prevalence of leishmaniasis was determined using a commercial indirect enzyme-linked immunosorbent assay (ELISA) test to identify antibody anti-*Leishmania infantum* (Ingezim LEISHMANIA, Madrid, Spain). The mean of two readings was used to classify samples as positive (ODPK=0.468), negative (ODNK=0.401), or inconclusive (between 0.401 and 0.468). The cut-offs were established following the product guide. All sera with positive results were retested at least once.

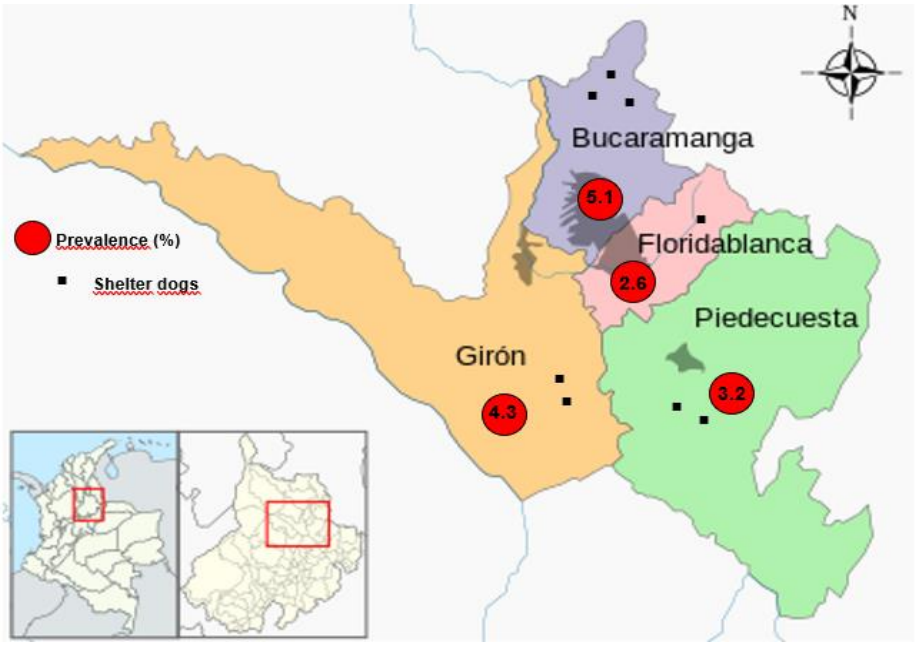

Fig. 1: Map of Bucaramanga metropolitan area where the regions under study, shelters dog visited and the overall prevalence of *L. infantum* are observed. In the Barrancabermeja municipality, one shelters dog was visited and seroprevalence was zero [Source: original map https://es.wikipedia.org/wiki/%C3%81rea_metropolitanadeBucaramanga]

Risk Factors for *Leishmania* (Questionnaire)

Epidemiological data about the risk factors were achieved by conducting a survey in these dog shelters (14 questions) and at the same time, blood samples were taken. The following variables from the survey were considered: municipalities (Bucaramanga, Floridablanca, Girón, Piedecuesta, and Barrancabermeja), gender, dog breed (pure breed/cross breed), age group (<1 year, 1 to 5 years, >5 years), hair length (short/long hair), mosquito control (yes/no), ectoparasite treatment (yes/no), presence of wild animals (yes/no), dogs sleeping outdoors (yes/no), contact with other animals (yes/no), presence of green areas/trees (yes/no), presence of mosquitoes (yes/no), knowledge of leishmaniasis (yes/no), and the presence of visible clinical signs of leishmaniasis (onychogryphosis, alopecia, mucocutaneous ulcers, and cachexia) (yes/no). A general clinical examination of each dog was performed by veterinarians, taking greater attention to mucocuta-neous problems and the general condition of the patient.

Statistical Study

The data were analyzed to determine the association between survey variables and results of test ELISA (presence or absence of antibodies of *Leishmania infantum*) through the Chi-square test. The probability of risk odds ratio (OR) and the confidence intervals of the risk factors were reached employing a univariate logistic regression study, taking as the reference category the one with the lowest probability of risk and leaving the others as study categories (Aguayo and Lora Monge, 2007). The statistical probability level for the study was P<0.05. The data evaluation was carried out using the SPSS 21 program (SPSS 2012).

RESULTS

Seroprevalence of *L. infantum* was 4.3% in canine shelters from Bucaramanga, Floridablanca, Girón, Piedecuesta municipalities (Table 1), no statistical association was observed. In the Barrancabermeja municipality the seroprevalence of *L. infantum* was zero (0/48). Regarding the results, antibody anti-*Leishmania infantum* were observed in all municipalities in the Bucaramanga area. No significant difference (P>0.05) was observed between presence or absence of antibodies to *L. infantum* and the intrinsic variables gender, dog breed, age group, hair length, and extrinsic variables mosquito's control, ectoparasite treatment, wild animal presence, contact with other animals, presence of mosquitoes, presence of green areas/ trees and knowledge of leishmaniasis. Dogs sleeping outdoors was the only variable that showed a statistical association (P=0.04). The prevalence was higher but not statistically significant (P>0.05) among females, with an approximate age range of 1 to 5 years old, and short haired dogs (Table 2).

Table 1: *Leishmania infantum* seroprevalence in dogs by the municipality in the Bucaramanga metropolitan area, Colombia

Variable	Total Dogs	Dogs (+)	Prevalence (%)	P-value	OR
Municipality					
Bucaramanga	118	6	5.1		1
Piedecuesta	31	1	3.2		0.59
Floridablanca	38	1	2.6		0.87
Girón	47	2	4.3	0.91	0.68
Overall	234	10	4.3		

1=Reference category. OR=odds ratio. Statistical significance (P<0.05).

Clinical manifestations were observed in eight seropositive dogs (Fig. 2, 3), while two seropositive dogs were not observed for any clinical signs (Table 5). Table 4 shows the clinical manifestations in the eight seropositive dogs for *L. infantum* antibodies. There were statistically significant differences (P<0.01) between seropositivity and the clinical signs of the disease, where 2.8% of seropositive dogs (8/282) showed specific clinical signs. Seroprevalence of *L. infantum* was higher in dogs with clinical symptoms (2.8%) than in those without any clinical signs (0.7%). Alopecia and ulcers were the most common clinical manifestations. In 83% (233) of the examined dogs no apparent clinical signs were suggestive of leishmaniasis.

Table 2: Logistic regression for risk factor associated with *Leishmania infantum* seroprevalence in dogs in the Bucaramanga metropolitan area, Colombia (Intrinsic variables)

Variable	Total Dogs	Dogs (+)	Prevalence (%)	P Value	OR
Gender					
Male	81	3	3.7		1
Female	153	7	4.6	0.75	1.2
Age group					
<1 year	13	0	0		1
1–5 years	117	6	5.1		1.3
>5 years	104	4	3.8	0.65	0.1
Dog breed					
Pure-breed	43	0	0		1
Cross-breed	191	10	5.2	0.12	1.05
Hair length					
Long hair breed	49	1	2		1
Short hair breed	185	9	4.9	0.38	0.4
Overall	234	10	4.3		

1=Reference category. OR=odds ratio. Statistical significance (P<0.05).

Table 3: Logistic regression for risk factor associated with *Leishmania infantum* seroprevalence in dogs in the Bucaramanga metropolitan area and Barrancabermeja, Colombia (Extrinsic variables)

Variable	Total dogs	Dogs (+)	Prevalence (%)	P-value	OR
Mosquito's control					
No	148	4	2.7		1
Yes	134	6	4.5	0.65	1.3
Wild animal presence					
No	36	0	0		1
Yes	246	10	4.0	0.23	1.04
Dogs sleep outdoor					
No	83	0	0		1
Yes	199	10	5.0	0.04	2.05
Contact with other animals					
No	78	1	1.3		1
Yes	204	9	4.4	0.20	0.02
Ectoparasite treatment					
No	58	1	1.7		1
Yes	224	9	4.0	0.43	2.3
Presence of mosquitoes					
No	13	0	0		1
Yes	269	10	3.7	0.47	0.99
Presence of green area/ trees					
No	41	0	0		1
Yes	241	10	4.1	0.1842	0.95
knowledge of leishmaniasis					
No	165	5	3.0		1
Yes	117	5	4.3	0.55	1.4
Overall	282	10	3.6		

1=Reference category. OR=odds ratio. Statistical significance (P<0.05).

Table 4: Frequency and prevalence of clinical signs from seropositive dogs for *Leishmania infantum* antibodies from the Bucaramanga metropolitan area, Colombia

Clinical Manifestations	No. Total Dogs	No. Seropositive	Sero-prevalence (%)
Alopecia	22	3	14
Cachexia	18	2	12
Diarrhea	2	0	0
Respiratory sigs	2	0	0
Onychogryphosis	3	1	33
Mucocutaneous ulcer	2	2	100

Fig. 2: Onychogryphosis observed in dog.

Fig. 3: Mucocutaneous ulcer in the nose in dog.

Table 5: Serological prevalence by ELISA according to the presence or absence of clinical manifestations associated with leishmaniasis

Clinical status	Positive dogs	Negative dogs	Total dogs
Apparently healthy	2	231	233
Clinically suspect	8	41	49
Total	10	272	282

DISCUSSION

The overall seroprevalence of *L. infantum* found in our study was 4.2% in canine shelters from Bucaramanga, Floridablanca, Girón, Piedecuesta municipalities and zero from Barrancabermeja. These results are comparable to surveys from Bogota (1.6%) and Neiva (6.1%) in Colombia (Rosypal et al. 2007; Zambrano et al. 2015), and reports from Argentina (6.22%), Brazil (0.4%), Mexico (6.1%) and Venezuela (2.1%) (López et al. 2012; Pacheco et al. 2013; Acosta et al. 2015; Rivas et al. 2020).

The above results describe the situation in nonendemic areas as not all infected dogs develop the disease (Baneth et al. 2008). In the case visceral leishmaniasis is starting its expansion, infected dogs may not yet have serum antibodies (Pacheco et al. 2013). This situation could be happening in the study geographic area showing low and even zero prevalence of the disease and the parasite could be widely distributed, showing a serious public health complication.

In other investigations, the prevalence was higher, as in the study of Márquez (2004) who reported a very high (39%) seroprevalence of *L. chagasi* in dogs from Piedecuesta. Romero et al. (2008, 2009) found a seroprevalence of 68.5 and 50.2% in dogs in two occasions from the Department of Tolima and by Fernández et al. (2002, 2006) who found a seroprevalence of 17.2 and 19.1% for *L. chagasi* from Huila. Picon et al. (2020) determined 12% of visceral leishmania in dogs in the Tolima and Huila Departments. Also, our data differ from what was reported from Brazil with 10.3, 11.3, 23, and 37.3% IgG antibodies for *L. infantum chagasi* in dogs (Queiroz et al. 2009; Barbosa et al. 2015; Araujo et al. 2016; Fujimori et al. 2016). The local epidemiology of visceral leishmaniasis might differ widely as this difference may be associated with environmental changes, population migration, urbanization, and rural exodus. These factors may also reduce the geographic area of this parasite, increasing epidemic foci. These modifications may affect the introduction of the agent that causes leishmaniasis disease in free areas, as well as the insertion of susceptible individuals in endemic areas (Dantas-Torres and Brandão-Filho, 2006; Barbosa et al. 2015; Fujimori et al. 2016).

Although the general seroprevalence of L. infantum found in this study was low, other more sensitive diagnostic tests should be considered to increase the possibility of finding true positive dogs for L. infantum in the study area, considering that when performing the ELISA test some samples were inconclusive and were negative when repeating the test, according to Wolf et al. (2014), compared different diagnostic tests for L. infantum antibodies in dogs, among which the ELISA used in our study, which presented the lowest sensitivity. Therefore, some of the samples with a negative result could correspond to animals with the true presence of the disease. The lack of a gold standard is one of the main difficulties in the diagnosis of canine leishmaniasis (Wolf et al. 2014). It is important to highlight that the canine positive samples may serve as reservoirs of parasites and a source of infection of insects. This would increase the risk of disease transmission in human populations (Fernández et al. 2006; Paternina et al. 2016).

Regarding the risk factors associated with *L. infantum* canine seroprevalence, the variables gender, dog breed, age group, hair length, mosquito control, ectoparasite treatment, presence of wild animals, dogs sleeping outdoors, contact with other animals, presence of green areas/trees (yes/no), presence of mosquitoes (yes/no), knowledge of leishmaniasis were not statistically different ($P>0.05$) and odds ratio was <1. These data are similar to reported by Arjona et al. (2012), Barbosa et al. (2015), and Fujimori et al. (2016). Conversely, Araujo et al. (2016), found that variables mongrel breed, male gender and presence of green area/trees were the highest risk factors. In other studies, short fur was found to be the highest risk factor for the transmission of canine leishmaniasis (França et al. 2003; Moreira et al. 2003; Fujimori et al. 2016). With regard to seropositivity to *L. infantum* and the clinical signs of the disease, there were statistically significant differences. This agrees with a study carried out by Araujo et al. (2016) in dogs from the municipality of Petrolina Brazil, where clinical manifestation was significantly associated with antibodies for *L. infantum*, but other studies did not show a statistical association with clinical signs (Barbosa et al. 2015). The clinical manifestations reported in this study are similar to other reports (Queiroz et al. 2009; Barbosa et al. 2015; Araujo et al. 2016; Fujimori et al. 2016). In the present investigation, the only variable that turned out to be a risk factor was dogs sleeping outdoors. This result is similar to those reported by Martín et al. (2009), as the authors considered that dogs that sleep outside may have greater exposure to insect bites. Sandoval et al. (2006) showed the disease presence in the Department of Santander. This study is the first epidemiological description of *Leishmania infantum* in shelter dogs in Bucaramanga metropolitan area and Barrancabermeja municipality.

In the Bucaramanga, Floridablanca, Girón and Piedecuesta municipalities, there were appropriate conditions for the development of the biological cycle of the parasite. The area includes areas with trees and mountains and is crossed by the Oro and Surata rivers in the municipalities of Girón and Bucaramanga (Esteban-Mendoza et al. 2020). Mosquito vectors of leishmaniasis disease have been informed in these municipalities (Sandoval et al. (2006). Therefore, the probability of transmission of zoonotic diseases transmitted by canine vectors could increase due to the vectors' presence (mosquitoes and ticks) in the owners' homes, a frequent situation due to the successful adaptation of these arthropods in domestic areas (Otranto et al. 2009).

The diagnosing of leishmaniasis is challenging, due to a complex clinical presentation and a high proportion of asymptomatic dogs (Rosypal et al. 2007; Queiroz et al. 2009). Considering that two seropositive dogs to *Leishmania* did not show any signs suggestive of Leishmaniasis, this situation is a matter of public health. Molina et al. (1994) and Laurenti et al. (2013) demonstrated that asymptomatic dogs can play an active role in the transmission of *Leishmania*, resulting in a complex situation as possible sources of transmission of the parasite.

Conclusion

We have demonstrated the seroprevalence of *L. infantum* in canine shelters in the Andean cities of Bucaramanga, Floridablanca, Girón, and Piedecuesta, for the first time. The samples from dogs of the Barrancabermeja municipality were seronegative to *L. infantum*. Dogs sleeping outdoors showed to be the most important risk factor for *L. infantum* positive dogs.

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Authors' Contribution

AAFM, JP, LEQ AR designed the study, interpreted the data, and drafted the manuscript, assisted in the collection of data and also contributed to manuscript preparation. MBM and JT were involved in interpretation of the data, drafting of the manuscript and manuscript preparation. AAFM and JP took part in preparing and critical checking this manuscript.

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