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Factors associated with *Leishmania infantum* infection in dogs from urban areas endemic for visceral leishmaniasis in Brazil

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ABSTRACT

There are gaps in the knowledge of the factors associated with canine visceral leishmaniasis (CVL). Moreover, there is a need for studies conducted with appropriate sample selection and with standardized diagnostic methods, to assess the current infection status of animals. This study analyzed factors associated with *Leishmania infantum* infection in dogs from urban areas endemic for CVL in Brazil. Data from four cities in different regions of the country (Fortaleza, Brasília, Palmas, and Bauru) were included, encompassing a large and heterogeneous sample group. Dogs were considered infected if they had positive results in parasitological tests (skin lesion culture, histological or immunohistochemical analysis of skin). Data analysis consisted of logistic regression with adjustment for the effect of cities as cluster variables, using generalized estimating equations. The canine positivity for L. *infantum* was 9.8%. Bauru had the highest percentage (18.7%), followed by Brasília (8.4%), Fortaleza (7.9%), and Palmas (4%). Male animals were twice as likely to be infected as females (Confidence Interval (CI): 1.5–2.8). Dogs living outdoors were 1.5 times more likely to be positive (CI: 1.4–1.6). Dogs living in households rearing chickens were 40% less likely to be infected (CI: 0.5–0.8). A significant multiplicative interaction indicated that a protective association between purebred and CVL was stronger among dogs older than seven years. These findings provide evidence for the role of individual and environmental factors on L. *infantum* infection in dogs from endemic urban areas. They may be used for identifying groups of animals at higher risk for delivering control interventions.

1. Introduction

American visceral leishmaniasis (VL) is a widely distributed zoonosis in Brazil and is a recognized public health problem (Ministério da Saúde, 2020; WHO, 2020). Transmission of *Leishmania infantum* infection, the etiological agent of the disease in the Americas, occurs through the bite of female sand flies of the genus *Lutzomyia*, among which *L. longipalpis* is the primary vector (Sousa-Paula et al., 2021). Currently, the disease is continually expanding in some state capitals and metropolitan regions in Brazil (Del Carro et al., 2020; Ministério da Saúde, 2020).

In domestic environments, dogs are considered the most important reservoirs of L. *infantum*. They have a role as amplifying the transmission in peridomestic cycles of the disease, which makes these animals one of the main targets in disease control actions (Gontijo and Melo, 2004;

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Nascimento et al., 2022). However, in Brazil, until 2021 the only control action directed at these animals was to conduct surveys to identify and euthanize dogs with a positive serodiagnosis (Ministério da Saúde, 2014). The serological tests usually employed in these surveys have limitations in terms of sensitivity, specificity, and reliability (Peixoto et al., 2015; Belo et al., 2017), making it difficult to use the results to investigate the factors associated with the infection and to define priority areas for targeting interventions. Furthermore, such control actions are considered scientifically and operationally limited (Romero and Boelaert, 2010; Werneck, 2016).

Over recent years, there has been an increase in studies about risk factors for VL in both humans and dogs (Coura-Vital et al., 2011; Silva et al., 2017; Azevedo et al., 2019). However, gaps in the knowledge of factors associated with canine visceral leishmaniasis (CVL) highlight the need for further research. Studies on associations between age, sex, breed, type of dog hair, presence of chickens near the home, and occurrences of infections have not produced consensual results (Rondon et al., 2008). A systematic review with meta-analysis revealed consistent associations of some factors with L. *infantum* infection in dogs. However, further research was recommended to expand knowledge about poorly investigated factors and to confirm the identified patterns. The review also emphasized the need for studies with the use of standardized diagnoses based on the current infection status in animals (Belo et al., 2013).

Thus, through a study carried out in different regions of Brazil, with a large and heterogeneous sample group and parasitological tests for diagnosing CVL, we sought to analyze factors associated with infection of dogs by L. *infantum* in urban areas endemic for VL in Brazil.

2. Material and methods

2.1. Study design and locations

This analytical cross-sectional multicenter study was carried out in four Brazilian municipalities considered endemic for VL by the Brazilian Ministry of Health (*Ministério da Saúde do Brasil*). The study formed part of a project developed by the Brazilian Ministry of Health, entitled "Construction of a canine serum panel for Ministry of Health use, for validation of diagnostic kits for visceral leishmaniasis". This research was carried out with the collaboration of the Municipal and State Health Departments, working in partnership with the field team of the Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation (IPEC/FIOCRUZ) (Schubach et al., 2014).

The areas chosen to carry out this study are in four different regions of Brazil (North, Northeast, Southeast and Central-West). The municipalities of Bauru (São Paulo), Brasília (Federal District), Palmas (Tocantins), and Fortaleza (Ceará) were selected considering the historical high level of CVL, which would provide an adequate number of infected and non-infected dogs to analyze factors associated with infection.

In 2019, the municipality of Bauru in the state of São Paulo (SP) had 376,818 inhabitants and an area of 667,684 km^2. The municipality of Brasília in the Federal District (DF) had 3,015,268 inhabitants and an area of 5,779,999 km^2. Palmas, in the state of Tocantins (TO), had 299,127 inhabitants and an area of 2,218,943 km^2. Lastly, the municipality of Fortaleza, in the state of Ceará (CE), had 2,669,342 inhabitants and an area of 314,930 km^2 (IBGE, 2019).

2.2. Animal samples

Areas in the municipalities with prevalence of CVL equal or higher than to 10% were selected for sampling. Dogs living in selected urban areas of the four municipalities were randomly selected through an active search for the collection of samples and data regarding explanatory variables using a veterinary field protocol. Similar numbers of animal samples were obtained from each of the four municipalities based on a sample size calculation performed in the study to validate diagnostic kits. Thus, samples were collected from 1597 dogs in the four municipalities, distributed as follows: Bauru (400), Brasília (369), Palmas (400), and Fortaleza (428). The sample assumed an expected prevalence of 10%, a confidence level of 95% and error of 3.0%. Multiple models were built with up to fifteen variables.

2.3. Eligibility criteria and missing data

The eligibility criteria for inclusion of the dogs in this study were the following: (1) live in the urban area of the municipality; (2) have been found through active search; (3) the dogs' owner agreed to participate in the study through signing a free and informed consent statement, regardless of the dog's clinical presentation; and (4) dogs aged 8 months of age or older. We excluded dogs whose owners had been established in the study region for <6 months; dogs with a physiological dysfunction that meant that the veterinarian responsible for the cases contraindicated the use of sedatives; aggressive dogs whose handling would jeopardize the safety of their owner or the field team; and dogs living in places with difficult access or that presented a risk to the safety of the health team. Dogs from which the material collected was insufficient and or contaminated constituted missing data.

2.4. Variables

2.4.1. Explanatory variables

Data obtained through the veterinary field protocol included general characteristics of the animal such as breed (mixed breed/purebred), sex (female/male), age (up to 7 years/over 7 years), fur (short/long), and weight (continuous variable); and data on living place (indoors/outdoors); presence of other animals (no/yes); and whether chickens, cats or other dogs were present in the household (no/yes). In addition, we investigated whether there was any history of a resident with VL (no/yes) and whether an animal had ever been identified as a suspected CVL case (no/yes). The following variables related to animal custody were also examined: care taken to the animal (none, minimum, good), vaccinations (none - no vaccines used / minimum - vaccination for rabies / good - vaccination for rabies and another vaccine), presence of ectoparasites (yes/no), and the dog owner' knowledge about VL and how it is transmitted (no/yes).

2.4.2. Outcome variable

Dogs with L. *infantum* infection were those presenting a positive result in one of the parasitological examinations (culture of intact skin or cutaneous lesion, histological analysis, or skin immunohistochemical analysis), according to previously described methodology (Schubach et al., 2014).

2.5. Statistical analysis

Differences in the distribution of variables in the four studied municipalities were analyzed using the chi-square test, at a significance level of 5%.

Associations between explanatory variables and the outcome were analyzed by logistic regression models. Models were adjusted considering the cities as cluster variables by means of generalized estimating equations (GEE) (Hardin and Hilbe, 2002).

The multiple logistic regression models were built using variables associated with CVL in the bivariate analyses at p-values < 0.20. Models were selected using a manual procedure, in which the least significant variables were removed step by step until the final, most parsimonious model was reached ($p < 0.05$). Multiplicative interactions between breed and sex, breed and age and age and sex were considered in the modeling. The age categorization aimed to divide the dogs into a group of young animals (up to 7 years old) and another consisting of elderly (>7 years old) in addition to increasing the statistical power of the

interaction analyses. The results were expressed as odds ratios (OR) with 95% confidence intervals (CI).

The data analysis was performed using the Stata statistical software, version 11.0 (Texas, USA).

2.6. Ethical issues

Sample collections from all the animals were authorized by their owners. The veterinarian responsible for the procedures provided identification upon arrival at the residence and explained the study objectives and the procedures to be performed on the animals by the technical team. Simultaneously, the dog owner was presented with a free and informed consent statement detailing these procedures and was invited to sign it if they agreed to participate in the research. The keeper of the dog needed to be of legal age. The study protocol describing all procedures had previously been submitted to the Ethics Committee for Animal Use (CEUA/FIOCRUZ) and approved under protocol L-038/08. All procedures followed the ethical guidelines for the use of animals in research as issued by the Conselho Nacional de Controle de Experimentação Animal (CONCEA).

3. Results

The overall positivity was 9.8% (156/1597), with Bauru presenting the highest percentage of canine positivity for L. *infantum* (75/400 = 18.7%), followed by Brasília (31/369 = 8.4%), Fortaleza (34/428 = 7.9%), and Palmas (16/400 = 4%). The difference in positivity between the municipalities was statistically significant.

Mixed-breed dogs comprised the largest group selected (78.1%), and most of the sample consisted of animals up to 7 years of age (92.9%). Regarding care provision, the majority of the owners had given minimal care to their dogs: most of the dogs had only received rabies vaccination (77.2%), while 60% of the dogs had undergone treatments for ecto-parasites. Most of the dogs lived outside the house (93.7%). Except for sex, all variables differed significantly between municipalities (Table 1).

In bivariate analysis, the greater chances of CVL were found among male and mixed-breed dogs; not reared with chickens; raised in a yard or with access to the streets; presenting ectoparasites; living in homes with previous cases of CVL; and cohabiting with another animal (Table 2). In the final multiple regression model, male sex and access to the streets were directly associated with CVL. Conversely, purebred animals and the presence of chicken were associated with a decreased chance of CVL.

The interaction between breed and sex was not significant (OR = 0.9; CI: 0.6–1.6). There were significant interactions between age and sex (OR = 0.5; CI: 0.3–0.9) and between breed and age in simple regression (OR = 0.16; CI: 0.4–0.7). In multiple regression only the interaction between breed and age (OR = 0.2; CI: 0.1–0.6) remained significant, indicating that the protective association of being a purebred dog was stronger among dogs older than 7 years (Table 3).

4. Discussion

Gaps in the knowledge of factors associated with L. *infantum* infection in dogs in high transmission urban areas still remain, leading to disagreements among researchers regarding the influence of these factors on increasing the risk of transmission. We therefore sought to evaluate a broad number of factors, using a population-based study in different endemic settings to explore patterns previously identified in other studies.

Male animals were twice as likely to be infected as females in the present study. Occurrence of greater chances of infection among males has also been identified in the literature (Belo et al., 2013; Shokri et al., 2017). However, the strength of this association in the present study was greater than usually reported. Differences in prevalence among sex may be due to different risks of exposure between male and female dogs (Zivicnjak et al., 2005; Penaforte et al., 2013) or to differences in host

Table 1
Distribution of the sample and of the variables in four urban endemic areas in Brazil.

Municipality - N (%)	*Bauru* - 400 (25)	*Brasília* - 369 (23)	*Palmas* - 401 (25)	*Fortaleza* - 428 (27)	Total - 1598 (100)	
Variables	N	N	N	N	N (%)	P-value*
Breed						
Mixed	257	249	335	339	1180 (78)	<0.001
Purebred	137	116	57	20	330 (22)	
Sex						
Female	183	160	185	178	706 (45)	0.36
Male	214	209	207	250	880 (55)	
Age						
Up to 7 years	338	329	347	335	1349 (93)	0.02
> 7 years	36	24	14	29	103 (7)	
Fur						
Long	136	123	79	257	595 (41)	<0.001
Short	239	226	276	131	872 (59)	
Weight						
1, 2 and 3 Quartiles	275	246	268	300	1089 (68)	<0.001
4 Quartile	91	106	100	56	353 (32)	
Care taken in relation to the animal						
Good	85	13	7	58	163 (11)	<0.001
Minimum	248	286	303	257	1194 (77)	
None	67	57	65	0	189 (12)	
Presence of ectoparasites						
No	141	80	181	215	617 (40)	<0.001
Yes	242	282	202	200	926 (60)	
Living place						
Inside the house	0	233	4	94	98 (6)	<0.001
Outside the house	399	132	388	325	1477 (94)	
Presence of other animals						
No	129	107	78	95	409 (29)	<0.001
Yes	203	258	214	333	1008 (71)	
Other dog(s) in the household						
No	190	183	170	126	669 (47)	<0.001
Yes	142	182	122	302	748 (53)	
Cat(s) in the household						
No	302	313	242	370	1227 (87)	0.03
Yes	30	52	49	58	189 (13)	
Chickens in the household						
No	324	280	223	342	1169 (82)	<0.001
Yes	8	85	69	86	248 (18)	

(continued on next page)

Table 1 (*continued*)

Municipality - N (%)	*Bauru* - 400 (25)	*Brasília* - 369 (23)	*Palmas* - 401 (25)	*Fortaleza* - 428 (27)	Total - 1598 (100)	
Variables	N	N	N	N	N (%)	P-value*
Any history of a resident with VL						
No	388	365	374	400	1527 (98)	0.03
Yes	3	3	13	3	22 (2)	
Previous CVL						
Yes	298	343	297	390	1328 (86)	<0.001
No	85	25	94	11	215 (14)	
Dog owner' knowledge about VL						
No	172	233	147	247	799 (57)	<0.001
Yes	139	113	237	121	610 (43)	

CVL = canine visceral leishmaniasis; VL = visceral leishmanisis; N = number; % = percentage.

* *P* values refer to the comparison of proportions between municipalities.

immune responses (Travi et al., 2002; Zafra et al., 2008; Maia and Campino, 2018). It is possible that these characteristics are more intense in the studied areas. Thus, male dogs can be prioritized in prevention and control actions.

We found that dogs kept in peridomestic areas and/or had free access to the streets were 1.5 times more likely to become infected. Belo et al. (2013) showed that the chances of acquiring *Leishmania* infection were lower among animals restricted/restrained to domestic areas. Adult dogs, if permitted, tend to stay outside most of the time and this can increase the chances of contact with the insect vector (Matos et al., 2006; Leça-Júnior et al., 2015). The higher prevalence among dogs that were living without restrictions reinforces the importance of educational awareness strategies about the proper ways of raising dogs (Melo et al., 2018; Soares et al., 2022).

Raising chickens presented a protective effect against *Leishmania* infection, such that in households where chickens were kept, dogs had 40% less chance of becoming infected. Other studies have also shown that the presence of birds can be a protective factor against occurrences of infection in dogs (Azevedo et al., 2008). In contrast, through a meta-analysis the presence of chickens or birds in the household was responsible for an increase in the chance of infection, but without a statistically significant association (Belo et al., 2013). This heterogeneity shows that although the role of poultry and the associated accumulation of organic matter in attracting sand flies is well established (Oliveira et al., 2010; Bray et al., 2014), the environment can modulate the direction of the association between chickens and occurrence of VL. Thus, control measures relating to raising chickens need to be specific to local contexts. Chickens attract sand flies but are refractory to *Leishmania* infection and can therefore function as zooprophylactic agents (Alexander et al., 2002; Rohousova et al., 2015). The evidence available so far does not confirm whether the presence of chickens and other domestic birds significantly increases the risk of infection in dogs and, consequently, in humans.

We also demonstrated a lower chance of infection in purebred dogs. Interestingly, the association was stronger in dogs over 7 years old. This result may be associated with genetic and immunological factors not evaluated, or even with the characteristics of the care provided by the owners to purebred or mixed-breed dogs (Urfer et al., 2019). Another hypothesis is that there may be a greater proportion of mixed breed dogs acquired as adults, coming to households from places where the chances of infection are greater, such as the street (Teixeira-Neto et al., 2014; Melo et al., 2018). The association between breed and CVL has been

Table 2
Factors associated with canine positivity for *Leishmania infantum* aggregated by municipalities in urban endemic areas in Brazil.

Variable	Infected/ Total (%)	Univariate analysis: Odds Ratio (95%CI)	Multiple regression: Odds Ratio (95% CI)
Breed[a]			
Mixed	118/1179 (10)		
Purebred	31/330 (9.4)	0.7 (0.5–0.9)	0.8 (0.7–0.9)
Sex[a, b]			
Female	54/705 (7.8)		
Male	101/880 (11.5)	1.6 (1.3–1.8)	2.0 (1.5–2.8)
Age			
Up to 7 years	138/1349 (10)	0.8 (0.3–2.3)	1.1 (0.5–2.4)
> 7 years	10/103 (9.7)		
Fur			
Long	59/595 (10)		
Short	85/871 (10)	1.0 (0.6–1.6)	
Weight (			
≤ 7 kg	41/386 (10)	(reference category)	
7–10 kg	42/406 (10)	0.9 (0.6–1.4)	
10–15 kg	29/297 (9)	0.9 (0.3–2.3)	
> 10 kg	29/352 (8)	0.7 (0.5–1.5)	
Care taken in relation to the animal[a]			
Minimum/good	133/1356 (9.8)		
None	20/189 (10.5)	1.1 (1.0–1.2)	
Presence of ectoparasites[a]			
No	51/617 (8.2)		
Yes	102/925 (11)	1.3 (1.0–1.7)	
Living place[a,b]			
Inside the house	5/98 (5.1)		
Yard/Acess to the street	148/1476 (10)	1.6 (1.4–1.8)	1.5 (1.4–1.6)
Presence of other animals			
No	38/409 (9.3)		
Yes	101/1008 (10)	1.3 (0.9–1.9)	
Other dog(s) in the household			
No	65/669 (9.7)		
Yes	74/748 (9.8)	1.1 (0.7–1.6)	
Cat(s) in the household			
No	125/1227 (10)		
Yes	14/189 (9.8)	0.8 (0.4–1.8)	
Chickens in the household [a,b]			
No	127/1169 (10.8)		
Yes	12/248 (4.8)	0.6 (0.5–0.7)	0.6 (0.5–0.8)
Any history of a resident with VL			
No	148/1526 (9.7)		
Yes	1/22 (4.5)	0.6 (0.1–2.2)	
Previous CVL[a]			
No	115/1327 (8.6)		
Yes	30/215 (13.9)	1.6 (0.8–3.0)	
Dog owner' knowledge about VL			
No	73/799 (9.1)		
Yes	60/609 (9.8)	1.2 (0.8–1.7)	

CVL = canine visceral leishmaniasis; VL = visceral leishmanisis; N = number; % = percentage.

[a] Statistically significant results in the univariate analysis.

[b] Statistically significant results in the multiple regression analysis.

Table 3
Multiplicative interaction between breed and age in the multiple regression model.

Variables*	Odds Ratio	(95% Confidence interval)
Mixed breed dogs and ≤7 years	1.0 (reference category)	
Purebred dogs and ≤7 years	0.8	0.7–0.9
Mixed breed dogs and > 7 years	1.1	0.5–2.4
Pure bred dogs and > 7 years	0.2	0.0–0.5

controversial and inconsistent in the literature (Belo et al., 2013). Our results reinforce the importance of evaluating it considering the characteristics of the animals and the environment in which they live and the need for proper care of dogs throughout their life cycle.

Among the limitations of the present research, we can highlight its cross-sectional design. Thus, the interpretation of associations needs to be made with caution because it may not represent causal parameters. In addition, the number of variables relating to the socioeconomic and environmental context was restricted. Moreover, samples were not assessed by molecular methods and parasitological tests were conducted using skin as the analysis material, and the sensitivity of these may have failed to identify infection in some animals. Nevertheless, this study makes important contributions. There are no records in the literature of nationwide multicenter study in Latin America, with such a heterogeneous and large sample. Moreover, the present study used parasitological tests that are little used in studies on factors associated with L. *infantum* infection in dogs, given that they are expensive and present logistical difficulties.

5. Conclusions

This study expands the understanding of the factors associated with L. *infantum* infection in dogs and confirms or refutes patterns previously identified. The percentage of canine positivity for L. *infantum* reveals the importance of studying CVL in animals in their localities, to highlight factors associated with occurrence of the infection. Male animals were twice as likely to be infected as females and the environment in which the animals were living increased the chance of infection. On the other hand, living in a place where chickens were reared and being a purebred dog were protective factors. The modification effect of breed, depending on the age of the dog, was novel and indicates that interaction analysis should be better explored in the literature. Such findings bring new evidence for studying factors associated with infection in dogs in urban endemic areas that may be used for identifying groups of animals at higher risk for delivering target interventions.

Data availability statement

The datasets employed and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of Competing Interest

None.

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