Leishmania Seroprevalence in Dogs: Comparing Shelter and Domestic Communities

Paulo Afonso , Ana Cláudia Coelho * , Hélder Quintas , Luís Cardoso

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Article

Leishmania Seroprevalence in Dogs: Comparing Shelter and Domestic Communities

Paulo Afonso [1,2,3,4], Ana Cláudia Coelho [1,2,5,*], Hélder Quintas [3,4] and Luís Cardoso [1,2,5]

[1] CECAV – Animal and Veterinary Research Centre, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal
[2] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
[3] Agrarian School, Polytechnic Institute of Bragança (IPB), Bragança, Portugal
[4] Mountain Research Center (CIMO), IPB, Bragança, Portugal
[5] Department of Veterinary Sciences, UTAD, Vila Real, Portugal
* Correspondence: accoelho@utad.pt

Simple Summary: Shelter dogs are considered more susceptible to infection with *Leishmania infantum* than domestic dogs due to the living conditions they are subjected to. These two populations of dogs were compared in an area where leishmaniosis is endemic, and shelter dogs were found less infected than domestic dogs. Statistically significant difference were also found between age groups and clinical statuses. Monitoring, preventing and treating canine leishmaniosis is crucial in reducing this zoonosis among animals and humans, under the scope of One Health.

Abstract: Canine leishmaniosis (CanL) is a chronic, systemic and often severe disease. The main causative agent of CanL is a protozoan parasite, *Leishmania infantum*, with phlebotomine sand flies acting as vectors. In Europe and other continents, *L. infantum* is also responsible for leishmaniosis in other animals, such as cats, horses and humans. In Portugal, animal and human leishmanioses are endemic, and high prevalence levels of infection and disease have been reported in dogs. There is a prejudice against stray animals and also those housed in shelters, assuming they have higher levels of infection with vector-borne pathogens, including *L. infantum*, when compared to domestic animals. In north-eastern Portugal, serum samples were obtained from March to May 2022 in three shelters (n = 179) and 13 veterinary clinics (n = 164), resulting in 343 dogs being analysed for antibodies to *Leishmania* spp. by the direct agglutination test (DAT). The overall seroprevalence was 9.9%, with 15.2% seroprevalence in domestic dogs and 5.0% in the shelter ones (p = 0.003). The fact that shelter dogs had a lower seroprevalence could be explained by more regular veterinary care provided in shelters regarding preventive measures, including insecticides with an antifeeding effect, in comparison with domestic dogs.

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1. Introduction

Although the infection can be subclinical, the disease canine leishmaniosis (CanL) caused by the protozoan parasite *Leishmania infantum* is a chronic, systemic and severe illness, which can often be fatal if not treated [1]. Apart from infecting dogs, which are its primary reservoir, *L. infantum* is also responsible for leishmaniosis in other animals, such as cats [2], horses [3] and humans [4], with phlebotomine sand flies acting as vectors [1]. Zoonotic visceral leishmaniosis (VL) is endemic in many geographic regions, including Europe, with a particular occurrence in southern Europe and the Mediterranean basin, where it is a primary veterinary and public health concern [5]. A review by Franco et al. described an overall seroprevalence of 23.2% (median: 10%) in 504,369 dogs from Italy, France, Spain and Portugal tested between 1971 and 2006 [6]. Human VL caused by *L. infantum* is a notifiable disease in Portugal, with around 80 cases officially notified from 1999 to 2021 [6,7]. There has been a growing interest in understanding the seroprevalence of leishmaniosis in dogs housed in shelters [8–12] in order to understand their role in the epidemiology of CanL and to develop more effective control strategies [12]. CanL has been assumed to be particularly problematic in stray and

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sheltered animals, which may have poor living conditions, health and nutritional needs, and overcrowding could increase the risk of transmission associated with potentially less preventive measures [8,13,14]. Consequently, there is a prejudice against these animals, assuming they would have a higher prevalence of infection and disease than their domestic counterparts [12–15]. The prevalence and distribution of CanL have been studied in Portugal by several authors [13,14,16–21]. Recent results suggest a duplication of seroprevalence in domestic dogs over a 10-year time frame [13]. However, studies in Portugal have yet to include shelter animals, a circumstance that represents a knowledge gap and could lead to a biased evaluation of the prevalence of infection. The present work aimed at assessing the seroprevalence of antibodies to *Leishmania* spp. in two groups of dogs living in northern Portugal, where CanL is endemic. In addition, the association between seropositivity to *Leishmania* and potentially associated risk factors was evaluated.

2. Materials and Methods

Geography

This study was conducted in the district of Bragança, located in northeastern Portugal, which is part of the historical province of Trás-os-Montes e Alto Douro. This district is made up of 12 municipalities: Alfândega da Fé, Bragança, Carrazeda de Ansiães, Freixo de Espada à Cinta, Macedo de Cavaleiros, Miranda do Douro, Mirandela, Mogadouro, Torre de Moncorvo, Vila Flor, Vimioso and Vinhais. The geographical area spans 6608 km2 and has a resident human population of 122,804 inhabitants, according to the 2021 census [22].

The region has a wide temperature range, with extreme values reaching 40 degrees Celsius in summer and minus 10 degrees Celsius in winter [23]. The total average rainfall ranges from 15.4 mm in July to 121.6 mm in December, not exceeding an annual average of 772.7 mm [23].

Animals and samples

This study was approved by the Ethics Committee of UTAD (process reference: Doc6-CE-UTAD-2022). In addition, legal detainers or owners signed informed consent for the inclusion of dogs in this study.

A total of 179 dogs were sampled from the three official shelters (CRO, Official Collection Centre) of the district of Bragança. This represents the number of dogs available for sampling at the shelters. In addition, an equivalent number of domestic dogs was sampled at 13 veterinary medical centres (CAMV, Veterinary Medical Care Centre) covering 10 of the 12 municipalities: Alfândega da Fé (n = 1), Bragança (n = 3), Carrazeda de Ansiães (n = 1), Macedo de Cavaleiros (n = 1), Miranda do Douro (n = 1), Mirandela (n = 2), Mogadouro (n = 1), Vila Flor (n = 1), Vimioso (n = 1), and Vinhais (n = 1). The distribution of dogs per municipality was carried out in adjustment with the resident human population (according to the 2021 census [22]). A number of 164 domestic dogs were sampled from the veterinary medical centres. The surplus of sera from blood samples collected in routine procedures at shelters and veterinary medical centres was used. Serum was subsequently stored at a temperature of -20°C until use. Data available on sex, age, breed, hair, habitat, clinical status (clinical manifestations related to CanL), municipality of origin, and use of ectoparasiticides (with action against phlebotomine sand flies) were registered for each dog (Table 1).

All dogs were clinically examined for signs or manifestations compatible with CanL, including alopecia, anaemia, anorexia, apathy/depression, arthritis/polyarthritis, ascites, muscle atrophy, cachexia, lameness, conjunctivitis, mucopurulent nasal discharge, skin desquamation, diarrhoea, pain, epistaxis, splenomegaly, ulcerative stomatitis, glomerulonephritis, haematochezia, hepatomegaly, liver disease, cutaneous hyperpigmentation, hyperkeratosis, hyperthermia, jaundice, chronic renal failure, lymphadenopathy, melena, meningitis, cutaneous nodules, onychogryphosis, osteitis, panophthalmitis, weight loss/slimming, pyoderma, pneumonia, polyuria/polydipsia, purpura, keratoconjunctivitis sicca, renomegaly, rhinitis, cough, skin ulceration, uveitis and vomiting [1].

Detection of antibodies to Leishmania spp.

The direct agglutination test (DAT) was used to titrate IgG antibodies specific to *Leishmania* spp. based on a standard freeze-dried antigen at a concentration of 5 × 107 promastigotes per millilitre in the direct agglutination test (DAT) to measure the titration of IgG antibodies specific to *Leishmania* spp. (Amsterdam University Medical Centres, Academic Medical Centre at the University of Amsterdam, Department of Medical Microbiology, Section Experimental Parasitology, Amsterdam, The Netherlands) [24].

Canine sera were serially diluted two-fold from 1:100 to 1:102,400 in saline solution (0.9% NaCl) containing 0.1 M β-mercapto-ethanol in V-shaped microtitre plates (Greiner, Germany). Plates were incubated at 37 ◦C for 1 hour. After incubation, 50 uL of reconstituted DAT antigen was added to each well containing 50 uL of diluted serum. The positive control for DAT was a serum sample from a dog with leishmaniosis and a DAT titre ≥102,400. The negative control serum used was from a dog living in a geographical region where CanL is not endemic. Results obtained with DAT are expressed as an antibody titre, i.e. the reciprocal of the highest dilution at which agglutination (large diffuse blue mats) is still clearly visible after 18 h incubation at room temperature. To enhance sensitivity and specificity, a cut-off titre of 400 was selected [24].

Data analysis

To compare the proportions of positivity among different categories of independent variables, the chi-square test and Fisher's exact test (FET) were used, with a significance level set at $p \leq 0.05$. In addition, exact binomial 95% confidence intervals (CI) were established for the values of general or overall seroprevalence and also for the values of seroprevalence related to the "origin" variable (i.e. shelter or domestic dogs). Stemstat, IBM SPSS Statistics 26® and WinEpi software tools were used for the analyses.

3. Results

A total of 343 dogs were sampled, adding the 179 dogs from shelters and the 164 domestic dogs. The age range was between 1 month and 19 years, with an average of 55.9 months. With an expected prevalence of approximately 16% [13] and a confidence level of 95%, the estimated absolute error is approximately 5.4% and 5.6% for shelter (n = 179) and domestic dogs (n = 164), respectively. The sample population was sex balanced (Table 1).

The overall seroprevalence was 9.9% (CI: 6.7-13.1%). Prevalence was significantly different (p = 0.003) between domestic dogs (15.2%; CI: 10.1-21.7%) and shelter ones (5.0%; CI: 2.3-9.3%) (Table 1). When comparing results among different age groups, the prevalence was significantly different between young and adult dogs (0.0% [CI: 0.0-5.7%] versus 10.6% [CI: 6.7-15.8%]; p = 0.045) and between young and senior dogs (0.0% [CI: 0.0-5.7%] versus 15.2% [CI: 8.1-25.0%]; p = 0.009) (Table 1). The seroprevalence o antibodies to *Leishmania* was significantly different between apparently healthy (8.6%; CI: 5.7-12.2%) and sick dogs (25.0%; CI: 10.7-44.9%) (p = 0.013) (Table 1). Furthermore, no statistically significant differences were found between the categories of the independent variables, sex, breed, habitat, use of ectoparasiticides, hair, municipality and vaccination. (Table 1).

Table 1. Seroprevalence of *L. infantum* infection in dogs from north-eastern Portugal by sex, breed, age group, habitat, use of ectoparasiticides, hair, clinical status, origin, municipality, and vaccination.

Variable	Title	Dogs tested (*n*)	Relative distribution (%)	DAT-positive (n)	Seropositive (%)	95% CI
Origin (p = 0.003)	Shelter	179	52.2	9	5.0	2.3–9.3
	Domestic	164	47.8	25	15.2	10.1–21.7
Sex (p = 0.721)	Female	182	53.1	17	9.3	5.5–14.5
	Male	161	46.9	17	10.6	6.3–16.4
Breed (p = 0.702)	Defined	111	32.4	12	10.8	5.7–18.1

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	Mongrel	232	67.6	22	9.5	6.0–14.0
Age group (p = 0.016)	Young[a,b]	63	18.4	0	0	0.0–5.7
	Adult[a]	198	57.7	21	10.6	6.7–15.8
	Senior[b]	79	23.0	12	15.2	8.1–25.0
Habitat (p = 0.092)	Access to outdoors	92	26.8	14	15.2	8.6–24.2
	Totally indoors	30	8.7	1	3.3	0.1–17.2
	Totally outdoors	221	64.4	19	8.6	5.3–13.1
Ectoparasiticides (p = 0.332)	No	58	16.9	8	13.8	6.2–25.4
	Yes	285	83.1	26	9.1	6.1–13.1
	Total	343	100	34		
Hair (p = 0.959)	Long	44	12.8	4	9.1	2.5–21.7
	Medium	75	21.9	8	10.7	4.7–19.9
	Short	224	65.3	22	9.8	6.3–14.5
Clinical status (p = 0.013)	Apparently healthy	315	91.8	27	8.6	5.7–12.2
	Sick	28	8.2	7	25.0	10.7–44.9
	Total	343	100	34		
Municipality (p = 0.854)	Medium	128	37.3	12	9.4	4.9–15.8
	Small	215	62.7	22	10.2	6.5–15.1
Vaccination (p = 1.0)	No	333	97.1	33	9.9	6.9–13.6
	Yes	10	2.9	1	10.0	0.3–44.5
Total	All	343	100	34	9.9	7.0–13.6

[a] p = 0.045; [b] p = 0.009. Bonferroni's correction has been incorporated by multiplying a previously significant pairwise p-value (0.015) by 3. Only statistically significant differences are shown for pairwise comparisons of age group categories (i.e. Young and Adult or Young and Senior, respectively).

4. Discussion

This study is an inaugural epidemiological investigation conducted on *Leishmania* infection in shelters in northern Portugal and is also the most extensive study carried out on the subject in the entire country. A lower seroprevalence was found in shelter dogs compared to domestic dogs. Despite our hypothesis considering shelter dogs more prone to infection and disease, our results revealed discordant results. Besides, a significant different seroprevalence was found in adults and in senior dogs and sick dogs.

The few studies that have compared shelter to domestic dogs found mostly a higher seroprevalence in shelter dogs [8,12,14]. In Portugal few studies have compared these two populations of dogs regarding seroprevalence. Cortes et al. [14] analysed 374 dogs in Lisbon urban area dogs between December 2002 and December 2003 with IFAT and found a seroprevalence of 18.4% (51/277) in domestic dogs and 21.6% (21/97) in shelter dogs with no statistical difference (p = 0.48) [14]. In Argentina, the seroprevalence of antibodies to *Leishmania* was significantly higher in shelter dogs (38.6%) compared to domestic dogs (20.1%) [8]. In Brazil, a survey conducted in 17 shelters found a seroprevalence of 33.7% (211/627) in sheltered dogs, ranging from 25.0% to 41.2%, contrasting with 3.4 to 9.6% found in previous studies involving domestic dogs [12].

Few studies have revealed results similar to those of the present report [25,26]. Colella et al. found a seroprevalence of 31.6% in domestic dogs contrasting with the 14.6% in shelter dogs [26]. On the other hand, Tamponi et al., found a higher seroprevalence in domestic dogs (27.2%) than in shelter dogs (10.6%) [25].

While some authors have not thoroughly examined the causes of seroprevalence variation between populations of shelter and domestic dogs, there are several possible explanations that have been proposed for the differences. Possible reasons for the higher seroprevalence of leishmaniosis in shelter dogs compared to domestic dogs include the lack of preventive measures [8], more favourable conditions for ectoparasite growth, due to organic material and blood meals [12], and limited access to veterinary care [27].

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In the opposite direction, the higher seroprevalence in domestic animals may be explained by the sedentary lifestyle of domestic dogs and the pronity to be bitten by vectors and consequently infected [25]. In contrast, shelter animals are more likely to receive prophylactic measures due to the commitment of animal shelters compared to what occurs in domestic animals as no preventive treatment was reported in 15.2% of domestic dogs in southern Italy [25]. Furthermore, the lack of veterinary care and failure to use ectoparasiticides have been associated with higher seroprevalence levels in domestic dogs [27].

Anecdotally, the lower and higher seroprevalence numbers of *Leishmania* infection in shelter animals have been attributed to the use [25] or the absence of prophylactic measures [8]. However, it is essential to note that these levels may reflect the context of each shelter and the specific application of preventive measures, rather than a broad generalization applicable to all shelter animals.

Other studies have focused only on studying seroprevalence in shelters. In Italy, a seroprevalence of 5.0% was detected in a canine shelter [11]. In another study conducted in kennels, *L. infantum* had a prevalence of 2.5% [28]. In another study in Italy seroprevalences levels of 1.8% and 10.0% were found in two shelters [10]. The authors point the differences in environment to justify the lower prevalence in the former shelter with windy area, absence of ravines and dry-stone walls. Despite the presumption that a shelter is more suitable biotope for the vector and in consequence places more susceptible to infection, no information about preventive measures (including ectoparasiticides) was given.

In the present study dogs older than 12 months showed a higher seroprevalence (Table 1), discordant with the bimodal age distribution of the disease that suggests a higher prevalence in younger and older dogs [29–31]. However, in accordance with others that claim that the risk of infection rises with age [27,28,32,33]the main explanation for this may be the outdoor lifestyle thar older dogs have, which increases possible contact with vectors [27].

The prevalence of *Leishmania* was significantly different between apparently healthy (8.6%) and sick dogs (25.0%) (p = 0.013) (Table 1), with apparently healthy dogs representing 79.4% (27/34) of seropositive dogs. Sauda et al. found contrary results, with higher representation of clinically suspected dogs 62.5% (10/16) [28]. Seemingly, healthy dogs represent animals at risk of contracting infection and suffering from disease and also a reservoir of *Leishmania*, with potential transmission to other animals and humans, making early detection crucial [34]. In the present study, of those dogs with clinical signs (7/34; 20.6%), the most frequently detected manifestations were apathy/depression (n = 2), arthritis/polyarthritis (n = 2), lameness (n = 2), and onychogryphosis (n = 2), a situation which is in line with Otranto et al. [15]. Other clinical manifestation less observed were localised alopecia, anaemia, anorexia, diarrhoea, hepatopathy, lymphadenopathy (localised and generalised), melena, mucopurulent nasal discharge, muscular atrophy, nasal hyperkeratosis, pain, purple, skin desquamation, skin ulceration, uveitis, and vomiting.

The wide variety of clinical manifestations can be explained by the broad spectrum of manifestations that the disease can present [34]. Also, subclinical infection with *L. infantum* is more common than clinical disease in areas of endemicity [1]. Although most dogs infected with *Leishmania* spp. appear healthy or show no evident clinical signs [13], some of them an still transmit the parasite to the phlebotomine sand flies [35]. This situation perpetuates the *Leishmania* life cycle and puts humans at risk of infection, making leishmaniosis a significant veterinary and public health concern [36] and making an early diagnosis of *L. infantum* infection mandatory for correct management [37].

No statistical difference was noted between sex, breed, habitat, use of ectoparasiticides, hair, municipality and vaccination (Table 1), suggesting a uniform distribution among the surveyed populations.

Several factors may contribute to the presence and spread of leishmaniosis in the Bragança district. One of the most important factors is the climate, which is characterised by hot and dry summers and mild winters[23], providing suitable conditions for the survival and reproduction of sand flies [18,38]. Another factor is the presence of wildlife reservoirs, such as foxes, and rodents [39], which can serve as sources of infection for sand flies and contact with stray dogs previously to their entrance into shelters.

The current climate changes with increasing temperature and humidity contributes to the northern spreading of the expansion of the vector niche [40–42]. In addition, the rural landscape of Bragança district, with its traditional agricultural practices and livestock farming, can also contribute to the spread of leishmaniosis. Domestic dogs that live in rural areas may be more likely to come into contact with sand flies, as they may be more exposed to the natural habitats of these insects [43], such as forests, riverbanks or farms. Moreover, dogs used for hunting or herding may have a higher risk of exposure and infection as they spend more time outdoors and are more likely to get bitten by sand flies [34].

Finally, the lack of effective preventive measures, such as regular veterinary care and vaccination programs, may also contribute to the prevalence of leishmaniosis in the Bragança district. As a result, it is essential for dog owners, veterinarians and public health officials to be aware of the risks associated with this disease and to take appropriate measures to prevent its spread. These measures could include the use of insect repellents, the implementation of vaccination programs and the promotion of good hygiene and sanitation practices.

Official shelters are entities that generally provide a high standard of veterinary care to the animals they foster, including preventive measures to control infectious diseases. These measures include vaccinating the animals and implementing rigorous hygiene protocols to reduce the spread of diseases. Therefore, adopting an animal from an official shelter can be a safe choice, as these entities usually follow high standards of quality in relation to the health and welfare of the animals they shelter.

By adopting an animal from a shelter, especially an official one, people can have greater confidence that they are receiving an animal in good condition and with good health.

5. Conclusions

The study is the first of its kind in the region and is representative of the canine population density of the municipality in the area. This study suggests that the seroprevalence of *Leishmania* infection among domestic dogs has doubled in 10 years in the Bragança district. Furthermore, this study challenges some assumptions about higher seroprevalence in shelter/stray dogs compared to domestic dogs, possibly due to the access to veterinary care provided in shelters, including prophylaxis against leishmaniosis, and found significant difference in seroprevalence between shelter and domestic dogs, which contradicts previous assumptions about higher seroprevalence in the shelter and stray dogs due to inadequate living conditions, poor health, and overcrowding. Overall, this study suggests the importance of regular screening, prevention and even treatment of leishmaniosis in both sheltered and domestic dogs, as they play a crucial role in transmitting *L. infantum* to other animals and humans. In addition, by improving dogs' management and health, we can promote public health and well-being in their communities.

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Institutional Review Board Statement: This study was approved by the Ethics Committee of UTAD (process reference: Doc6-CE-UTAD-2022).

Informed Consent Statement: Legal detainers or owners signed informed consent for the inclusion of dogs in this study.

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required.

Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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