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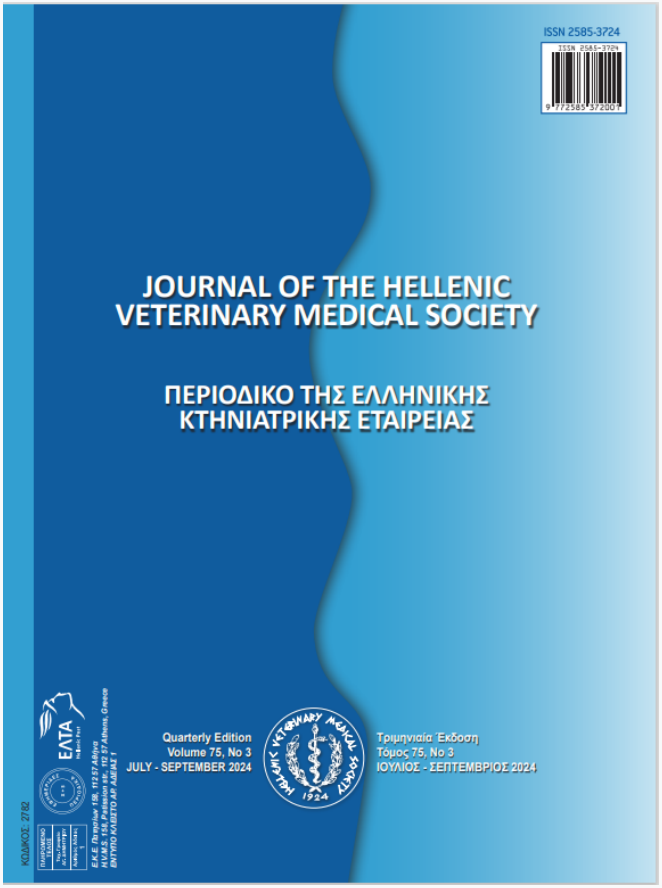

Occurrence of canine and human leishmaniosis between 2020 and 2022 in the Alto Tâmega region (Portugal): a descriptive study of seropositivity in clinically suspected dogs, a status report on the occurrence of cases in humans and perception of the disease by pet tutors

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Occurrence of canine and human leishmaniosis between 2020 and 2022 in the Alto Tâmega region (Portugal): a descriptive study of seropositivity in clinically suspected dogs, a status report on the occurrence of cases in humans and perception of the disease by pet tutors

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ABSTRACT: Zoonotic visceral leishmaniosis is a severe neglected zoonotic parasitic disease caused by *Leishmania infantum* with dogs as the major reservoir hosts. Although the European Centre for Disease Prevention and Control (ECDC) reported, in 2022, no human or animal cases in the Alto Tâmega region between 2009-2020, the truth is that there were notified cases. The present study main objective was to determine the occurrence ofCanL infection among 217 clinically suspected dogs subjected to serological tests in a Reference Veterinary Clinic in the Alto Tâmega region and among human patients of the Public Health Unit of Alto Tâmegae Barroso actuation area, in northern Portugal. In addition, a questionnaire was presented to 100 of the clinic's clients during the last fortnight of October 2022. The majority of sampled dogs had no defined breed (43.7%), they were mainly males (56.7%) and under 10 years of age (29.5%). Between January 2020 and September 2022, there were 64 seropositive dogs among those subjected to anti-*Leishmania* antibody detection, indicating that canine leishmaniosis (CanL) had 29.5% of occurrence within the sample of the population studied. Among the seropositive dogs, 44.4% were mongrel dogs and 56.52% were males. There were no reported human cases between 2020 and 2022. The majority of the questionnaire respondents believed that leishmaniosis does not affect humans (39.0%) and that is not a public health issue (33.0%) and a considerable part of the sample does not have a position about those questions (33.0% and 42.0%, respectively). Based on the survey sample collected, most people may not associate the disease with a zoonosis and, therefore, there may be a risk of underdiagnosis and underreport, as there is no demand for treatment and medical diagnosis directed towards some suspected cases or because human cutaneous leishmaniosis is not a notifiable disease. An improvement in education of the population in areas of endemicity is needed to reach the objective of minimizing the impact of leishmaniosis, perform a proper diagnosis and treatment, and ensure a safer bond between animals and humans.

Keywords: *dog; human; leishmaniosis; public health; veterinary medicine*

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INTRODUCTION

Leishmania spp. are parasitic protozoa transmitted via infected female sand flies (Phlebotominae) endemic in several countries of the Mediterranean Basin (Mihalca et al., 2019; Cardoso et al., 2021; Maia et al., 2022). As estimated by the World Health Organization (WHO, 2022), leishmaniosis, a severely neglected zoonotic parasitic disease, caused by *Leishmania* spp., is considered to lead to more than 1 million new human cases per year around the world (Azami-Conesa et al., 2020).

Cutaneous, mucocutaneous and visceral diseases are the clinical forms of human leishmaniosis (Gonzalez-Lombana et al*.,* 2013). Visceral leishmaniosis (VL) caused by parasites of *Leishmania donovani* complex is a severe human disease, being domestic dogs (*Canis familiaris*) the major host of *Leishmania infantum*, one of the species of that complex, and the main reservoir host for zoonotic human infection (Campinoand Maia, 2018; Velez andGállego, 2020). Importation of dogs from endemic areas and climate changes are the key factors to the expansion of CanL (Morales-Yuste et al., 2022). Some studies have related genetics, breed and dog's sex as factors of susceptibility to infection (Campinoand Maia, 2018). Among surveillance, treatment of infected animals and prophylaxis, early detection is an important step regarding the disease control.Enzyme-linked immunosorbent assay (ELISA) is widely used for laboratory diagnosis and is a technique that provides a good sensitivity (86 to 99%) and also specificity (Morales-Yuste et al., 2022).

Portugal is a country where leishmaniosis is endemic, with the Trás-os-Montes e Alto Douro, Lisbon, and Algarve geographic regions as the principal foci of the disease (ECDC, 2022).According to the report of notifiable diseases (2013-2016) by the Portuguese Directorate-General for Health (DGS, 2017), 3% of the notified VL human cases were reported from the Alto Tâmega region. Relating to the health statistics in the Transparency Portal from the National Health Service (SNS, 2018; INE, 2020), between 2014 and 2018, 4% of the reported VL cases in Portugal were recorded in the Alto Tâmega area, although the report commissioned by the European Centre for Disease Prevention and Control (ECDC, 2022) concluded that there were no reported cases of human or animal leishmaniosis between 2009 and 2020. In this present study, in a Reference Veterinary Clinic (RVC), located in the Alto Tâmega region, just in 2020, the prevalence of CanL cases was about 31% in dogs subject to a serological test. There is a lack of information about the prevalence and incidence of the disease in this Portuguese region, which lies very close to the northern border with Spain.

The present study main objective is to determine the occurrence of infection and disease among the dogs subjected to serological tests in anRVC in the Alto Tâmega region and among human patients. Another objective was to determine the knowledge on leishmaniosis among a sample of 100 clients of the clinic in the month of October 2022 and to see where the lack of information takes place, in order identify methods of communication to reach a best acquaintance of the population who owns at least one animal in a leishmaniosis endemic region.

MATERIAL AND METHODS

The present study was carried out in anRVC in the municipality of Chaves, Alto Tâmega, northern Portugal, which has about 13 000 clients. From the baseline of analytical results, with data from January 2020 to mid-September 2022, results from 217 clinically suspected dogs subjected for the first time to analysis for detection of anti-*Leishmania* antibodies, by means of Leiscan® (Ecuphar, Spain), were selected. Leiscan® is a serological ELISA test for the detection antibodies to *Leishmania*, which has a reported sensitivity and specificity of 95.3% and 99.8%, respectively.

Dogs were considered clinically suspected if the animals presented one or more of the following clinical signs compatible with CanL: weight loss, generalised lymphadenomegaly, exacerbated nail growth (onychogryphosis), decreased appetite, periorbital alopecia, nasal hyperkeratosis, exfoliative dermatitis, mucocutaneous ulcerative lesions, hepatosplenomegaly, epistaxis, polydipsia/polyuria, and laboratory results compatible with proteinuria. It should be noted that among these signs, there are clinical signs that may be also compatible with other conditions. The collected data included sex, age, breed, qualitative result, the reason of the serum sample (Rz), a quantitative result and the location of origin of the seropositive dogs.

It is noteworthy that the submission of animals to this type of diagnosis depended on the clinical condition that may lead to the suspicion of infection, not forgetting the financial availability of the owners or tutors. In general, the animals tested were animals

with clinical signs and whose tutors agreed to pay for the analysis.

Additionally, a small questionnaire was carried out to about 100 clients of the RVC in the last 15 days of October 2022.

To obtain epidemiological data on human leishmaniosis in the Alto Tâmega region, the Public Health Unit of Alto Tâmega e Barroso, based in Chaves, was contacted.

A descriptive statistical analysis was performed in the Excel Office 2019.

RESULTS

Characterization of the canine sample

Dogs clinically suspected of being affected by CanL(n = 217) were subjected to a serological test for the first time to detect anti-*Leishmania* antibodies, between January 2020 and mid-September 2022.

In terms of sex, there were 43.3% female and 56.7% male dogs (Table 1), 29.5% of the total dogs had less than 10 years old, 18.9% more than 10 years and there was no age data available for the remaining 51.6% (Table 1). Regarding breed, 43.7% had no defined breed, 42.8% had a defined breed and there was no information for 13.5% (Table 1).

In total, 67.7% of the samples were seronegative and 29.5% were seropositive (Table 1).

In 2020, 5.2% were low positive, 6.5% were positive, 11.7% were highly positive, and 6.5% were very highly positive. In 2021, 12.5% were low positive, 2.8% were positive, 12.5% were highly positive and 2.8% were very high positive. In 2022, between January and the mid of September, 2.9% were low positive, 8.8% were positive, 8.8% were highly positive and 7.4% were very high positive (Table 1).

Table 2 represents the descriptive statistics of the samples' Rz values between 2020 and 2022.

The majority of the seropositive dogs were males, representing 56.5% of the seropositive dogs in 2020, 54.6% in 2021, and 70.0% in 2022 (Table 3).

In the seropositive group, the majority of the dogs had no defined breeds (44.4%) (Table 4). For 16.4% of the seropositive dogs there was no information about their breed (Table 4).

Table 1. Absolute and relative frequency (%) of the tested dogs according to sex, age, breed, and qualitative serological results.

		2020		2021		2022		2020-2022	
		No. Cases	No. Cases (%)	No. Cases	No. Cases (%)	No. Cases	No. Cases (%)	Total No. Cases	Total No. Cases (%)
Sex	Female	38		27		29			43.3%
	Male	39		45		39			56.7%
Age	<10 years old							64	29.5%
	≥10 years old							41	18.9%
	Unknow Age							112	51.6%
Breed	Indetermined	32	41.56%	37	51.4%	25	37.89%	94	43.7%
	Unknown	10	12.9%	8	11.1%	11	16.7%	29	13.5%
	Pinscher	7	9.1%	3	4.2%	1	1.5%	11	0.9%
	Pitbull	1	1.3%	1	1.34%	0	0.0%	2	0.9%
	Cocker Spaniel	1	1.3%	0	0.0%	0	0.0%	1	0.5%
	Estrela Mountain	1	1.3%	0	0.0%	1	1.5%	2	0.5%
	Jack Russel Terrier	1	1.3%	0	0.0%	0	0.0%	1	0.5%
	Pointer	3	3.9%	1	1.34%	0	0.0%	4	1.9%
	German Spitz	1	1.3%	0	0.0%	0	0.0%	1	0.9%
	Yorkshire Terrier	1	1.3%	0	0.0%	1	1.5%	2	0.9%
	German Shepherd	3	3.9%	3	4.2%	5	7.6%	11	5.1%
	Boxer	2	2.6%	2	2.8%	0	0.0%	4	1.9%
	Labrador Retriever	2	2.6%	5	6.9%	6	9.1%	13	1.4%
	Rottweiler	2	2.6%	0	0.0%	1	1.5%	3	1.4%
	Podengo	3	3.9%	0	0.0%	0	0.0%	3	1.4%
	Poodle	1	1.3%	0	0.0%	0	0.0%	1	0.9%

	Teckel	1	1.3%	1	1.34%	0	0.0%	2	0.9%
	Chihuahua	1	1.3%	0	0.0%	0	0.0%	1	0.5%
	Transmontano Mastiff	2	2.6%	0	0.0%	0	0.0%	2	0.9%
	English Bulldog	1	1.3%	0	0.0%	0	0.0%	1	1.9%
	French Bulldog	0	0.0%	1	1.4%	3	4.6%	4	1.9%
	Setter	0	0.0%	2	2.8%	2	3.0%	4	1.9%
	Irish setter	1	1.3%	0	0.0%	1	1.5%	2	1.4%
	Beagle	0	0.0%	0	0.0%	3	4.6%	3	1.4%
	Portuguese Pointer	0	0.0%	0	0.0%	1	1.5%	1	0.5%
	St. Bernard	0	0.0%	1	1.4%	2	3.0%	3	1.4%
	Pug	0	0.0%	0	0.0%	1	1.5%	1	0.9%
	Chow-chow	0	0.0%	2	2.8%	0	0.0%	2	0.9%
	Brittany	0	0.0%	1	1.4%	0	0.0%	1	0.5%
	Siberian Husky	0	0.0%	1	1.4%	0	0.0%	1	0.5%
	Grand Danois	0	0.0%	1	1.4%	0	0.0%	1	0.5%
	Sharpei	0	0.0%	1	1.4%	0	0.0%	1	0.5%
	Golden Retriever	0	0.0%	1	1.4%	0	0.0%	1	0.5%
	Shitzu	0	0.0%	0	0.0%	1	1.5%	1	0.5%
	Cane Corso	0	0.0%	0	0.0%	1	1.5%	1	0.5%
Qualitative serological results	Negative	52	67.5%	48	66.7%	47	69.1%	147	67.7%
	Doubt	2	2.6%	2	2.8%	2	2.9%	6	2.7%
	Low Positive	5	5.2%	2	12.5%	6	2.9%	13	5.9%
	Positive	4	6.5%	9	2.8%	2	8.8%	15	6.9%
	High Positive	9	11.67%	9	12.5%	6	8.8%	24	11.0%
	Very High Positive	5	6.5%	2	2.8%	5	7.4%	12	5.5%

Table 2. Descriptive statistics analysis of the samples' Rz values between 2020 and 2022.

	2020	2021	2022
Minimum	0.07	0.06	0.06
1st quartile	0.19	0.15	0.18
Median	0.26	0.26	0.42
3rd quartile	1.27	1.55	1.49
Maximum	3.78	3.53	4.71
Mean	0.84	0.87	0.95
Standard error	0.13	0.13	0.15
Mode	0.26	0.18	0.23
Standard deviation	0.98	1.00	1.08

Table 3. Proportion (%) of female and male dogs among the sample between 2020 and 2022.

	2020	2021	2022
Female	43.5%	45.5%	30.0%
Male	56.5%	54.6%	70.0%

Table 4. Absolute and relative frequency (%) of seropositives according to the dogs' breed (2020-2022).

Breed	No. Seropositives	No. Seropositives (%)
Indeterminated	28	44.4%
Unknown	9	16.4%
Pointer	2	3.2%
Labrador Retriever	7	11.1%
Pinscher	1	1.6%
Serra da Estrela Dog	1	1.8%
Pitbull	1	1.8%
Epagneul Breton	1	1.8%
Siberian Husky	1	1.8%
Chow-chow	1	1.8%
German Shepherd	3	5.5%
English Setter	1	1.8%
Irish Setter	3	3.6%
St. Bernard	2	1.8%
Rottweiller	1	1.8%
French bulldog	1	1.8%

Most of the dogs that tested positive for antibodies had between 4-6 years (12.5% 4 years 12.5% 5 years, 10.9% 6 years) (Figure 1).

About 90.0% of the seropositive dogs were from the municipality of Chaves, 2.1% from that of Montalegre, 2.1% from Vila Pouca de Aguiar and 6.3% from Valpaços. The geographical distribution of seropositive animals is shown in Figure 2.

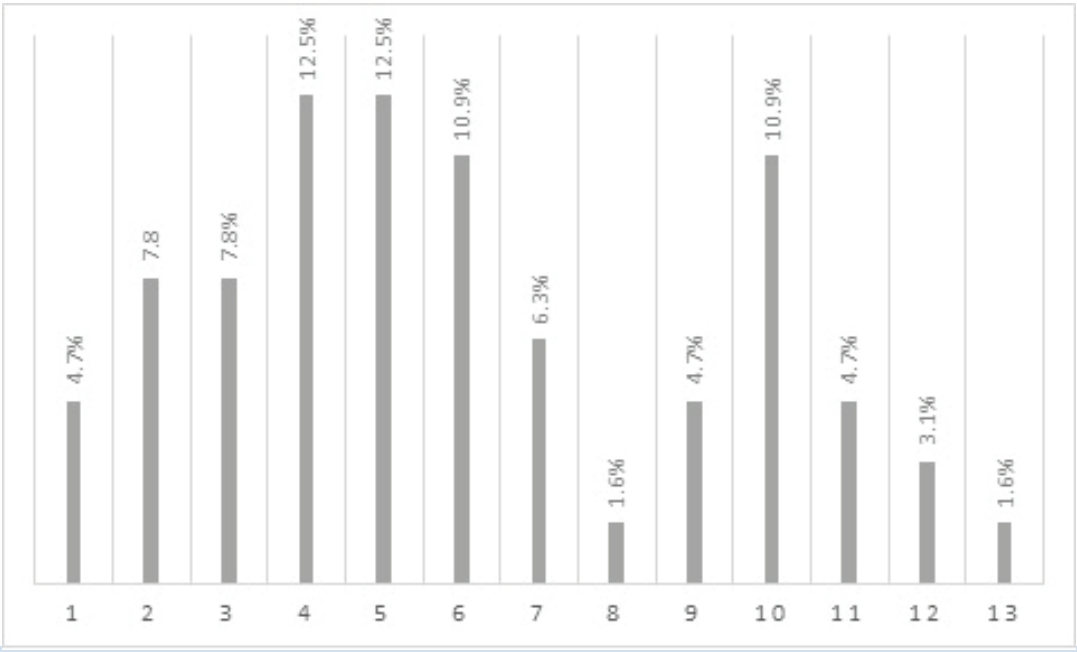

Figure 1. Percentage of seropositive dogs between 2020 and 2022according to age (years).

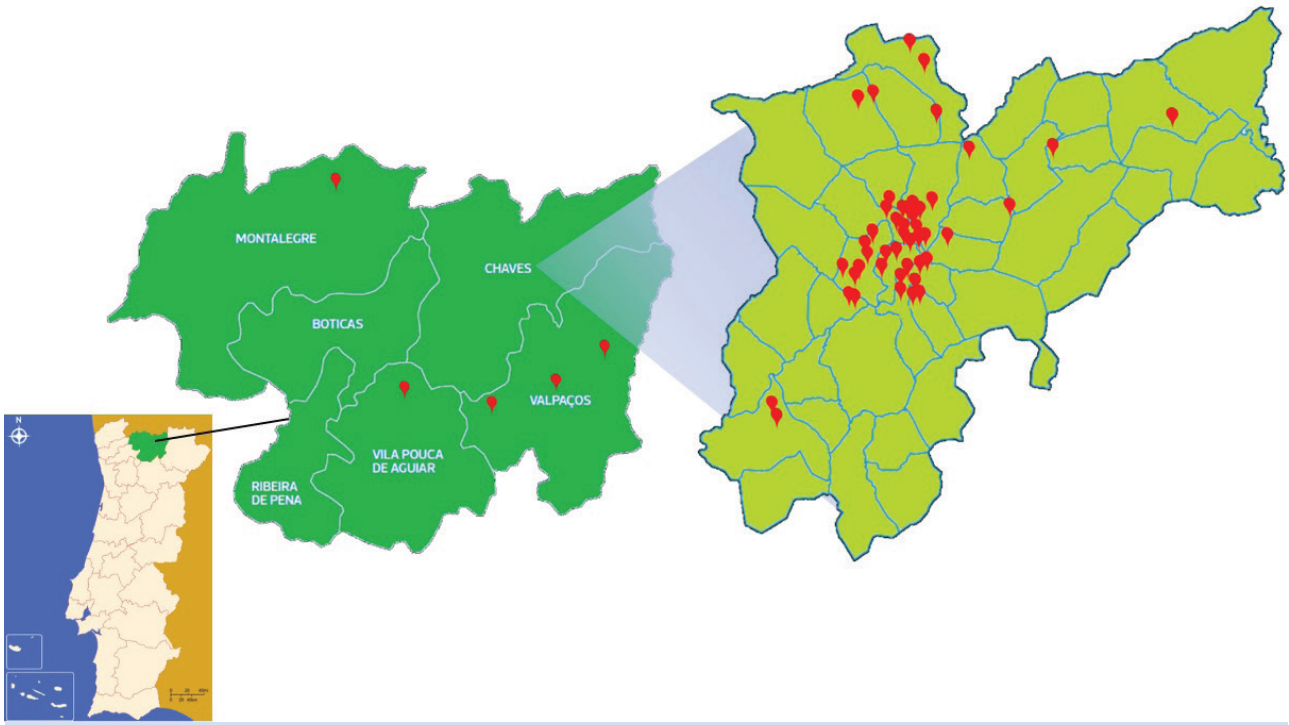

Figure 2. Geographical distribution of seropositive dogs between 2020 and 2022 in the Alto Tâmega region.

Human data, 2020-2022

According to the Public Health Unit of Alto Tâmegae Barroso, there were no human VL cases between 2020 and 2022. The last one recorded was in 2017. They were no known cutaneous leishmaniosis cases either, because the latter is not a notifiable disease.

Questionnaire

During the last fortnight of October 2022, a questionnaire was presented to the RVC. This fortnight, a low season, the number of clients rounded 130. One hundred clients were asked to answer the questionnaire and all of them were receptive. Table 5 summarizes the results of the questionnaire.

Table 5. Results obtained from the leishmaniosis questionnaire answered by 100 clients of the RVC presented in percentage.

Question/Answer	No. of clients (%)
1. Is leishmaniosis a disease that can affect humans?	
Yes	27,0%
No	39,0%
Don't Know	33,0%
2. Is leishmaniosis a disease that only affects dogs?	
Yes	32,0%
No	42,0%
Don't Know	24,0%
3. Leishmaniosis is caused by:	
Virus	20,0%
Bacteria	29,0%
Protozoa	12,0%
Fungi	2,0%
Nematode	1,0%
Other	24,0%
4. Is leishmaniosis transmitted directly from animal to animal?	
Yes	30,0%
No	33,0%
Don't Know	35,0%
5. The signs and symptoms of the disease in dogs are: apathy, skin lesions, hair loss, initially around the eyes and ears, weight loss, tearing and abnormal nail growth?	
Yes	56,0%
No	8,0%
Don't Know	34,0%
6. Have you ever had animals with leishmaniosis?	
Yes	17,0%
No	68,0%
Don't Know	11,0%
7. Is there a cure for leishmaniosis?	
Yes	32,0%
No	36,0%
Don't Know	31,0%
8.Do you consider leishmaniosis a public health problem?	
Yes	22,0%
No	33,0%
Don't Know	42,0%
9. Can leishmaniosis be prevented with a vaccine?	
Yes	53,0%
No	18,0%
Don't Know	26,0%
10. Can leishmaniosis be prevented with dewormers?	
Yes	47,0%
No	23,0%
Don't Know	27,0%

The majority of the clients (39.0%) believed that leishmaniosis does not affect humans. However, most of the clients (42.0%) answered that dogs are not the only victims. Furthermore, 29.0% marked that leishmaniosis is caused by bacteria, 20.0% by viruses, 12.0% by protozoa, 2.0% by fungi, 1.0% by nematodes, and 24.0% believed that it is caused by a different agent.

Thirty per cent of the answers indicated that leishmaniosis is directly transmitted from animal to ani-

mal. Fifty-six per cent were affirmative when asked if the signs of the disease in dogs were apathy, skin lesions, hair loss, initially around the eyes and ears, weight loss, tearing and abnormal nail growth. Seventeen per cent had had at least one animal with leishmaniosis.Thirty-six per cent responded that there is no cure for leishmaniosis.

The majority of the clients were unable to identify leishmaniosis as a public health problem. Thirty-three per cent considered that leishmaniosis is not a public health issue and 42% had no position about that.

DISCUSSION

Leishmaniosis is an endemic zoonosis in Trás-os-Montes and Alto Douro, where the Alto Tâmega region is located. (ECDC, 2022). The clinical signs of CanL may include progressive weight loss, generalised lymphademogelay, decreased appetite, epistaxis, hepatosplenomegaly, polyuria/polydipsia, skin lesions, onychogryphosis, among others (Solano-Gallego et al., 2011). Nevertheless, different studies have shown that more than half of the seropositive animals were apparently healthy (Solano-Gallego et al., 2001; Campino et al., 2022; Miró et al., 2007).

In the present study, the sample consists of dogs that presented clinical signs (specific and nonspecific) compatible with the disease. One limitation was the tutor's willingness to submit the animal to the test. Between January 2020 and the end of September 2022, there were 64 seropositive among dogs subjected to anti-Leishmania antibody detection for the first time, indicating that CanL had 29.5% of occurrence within the sample of the studied population. Around 67.7% of the animals with clinical signs compatible with the disease were seronegative. From these results, we can suggest that among these animals were dogs with clinical signs that were compatible with other diseases and conditions. Another explanation could be that in the early stages of the disease, the antibody titer is insufficient to be detected (Solano-Gallego et al. 2011).

According to some authors, the risk factors that may contribute to the clinical progression of the CanL are dog breed, body weight, living conditions, endoparasite control, CanL vaccination, age, and attitude (pet, watchdog, hunting dog) were confirmed in other studies (Gálvez et al., 2010; Miró and López-Vélez, 2018; Rombolà et al., 2021).

Some authors point to the predisposition of certain breeds, such as the Boxer, German Shepherd, and Rottweiler, to be more affected by the disease (Miró and López-Vélez, 2018). In addition, large breed dogs might be more likely to be kept outdoors and spend more time exposed to the vector than small dogs (Gálvez et al., 2010). Based on the results of the present study, we can hypothesize that people's traditions and mentality associated with animal ownership may influence the occurrence of the disease. Usually, animals sheltered in the backyard in endemic places belong to breeds that are less respected in the population's perception and with which there is less concern to take care of. Among seropositive animals, a large proportion were mongrels (44.4%). In Portugal, especially in the North, where a rural lifestyle is traditional, dogs are common backyard animals. Sadly, backyard animals receive limited veterinary care (Mihalca et al., 2019). Tutors tend to be more careless with mongrel animals and care more about pure breeds, giving them access to indoors and better veterinary treatments, among other benefits.

Most of the seropositive animals in this study were males (56.5%). Other studies showed that male dogs had a higher risk of infection (Miranda et al., 2008; Rombolà et al., 2021). One reason that can explain the higher prevalence of seropositive male dogs is that male dogs are preferred for guarding and hunting activities (Campinoand Maia, 2018).

Most of the seropositive animals in this study were between 4 and 6 years old, followed by the group of animals over 10 years old and the group between 1 and 3 years old. In other studies, the prevalence of *L. infantum* infection has been associated with the animal's age, maybe because older animals have been exposed for longer times to *Leishmania* (Rombolà et al., 2021). Some authors reported a bimodal age distribution of seroprevalence with one peak appearing in the young dogs (1-2 years) and a second more evident peak among the older ones (7-8 years) (Miranda et al., 2008; Gálvez et al., 2010).

One of the significant risk factors for CanL is the lack of knowledge among the population. This is a crucial area that needs immediate attention. As we observe from the sample of clients who responded to the questionnaire, there is a lack of information about the zoonotic potential of leishmaniosis. The majority of the respondents (clients with at least one animal) believed that the disease does not affect humans and that it is not a public health issue. This highlights the urgent need for education and awareness, especially

among veterinarians who play a key role in disseminating this information to their clients.

No declared human case was found between 2020 and 2022 in the studied geographical area. The last case was diagnosed in 2017, and, as explained by the health delegate, it was a complex process to determine whether the human patient was really affected by VL. One of the problems of this notifiable disease is a lack of information about leishmaniosis among the population and health professionals. There is also a high possibility of mistake in diagnosing this disease, as clinical signs can be the same as those of several other diseases. Furthermore, these couple of years were those of the COVID-19 pandemic period, which could have limited patients' search for healthcare.

CONCLUSION

We can consider the lack of knowledge of animal owners as another risk factor for CanL associated with other factors that have already been widely studied.Leishmaniosis is a public health issue, but it is poorly known even among pet tutors in endemic areas. It is necessary to improve the basic notions about this disease in order to mitigate cases in animals and humans by investing in education for prevention and prophylaxis. On the other hand, most people may not associate that the disease is a zoonosis and, therefore, there may be a risk of underdiagnosis and underreporting of human cases. It would be interesting to conduct a future study in an endemic region to assess how many of the animals diagnosed with leishmaniosis were previously subjected to prophylactic measures and how their owners were alert to the disease before knowing the diagnosis.

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