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Pre-test probability and likelihood ratios for clinical findings in canine leishmaniasis

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Abstract

Canine leishmaniasis is a parasitic zoonosis mainly caused by *L. infantum*; an obligate intracellular protozoan transmitted by haematophagous insects of the genus *Phlebotomus*, which affects dogs and wild canids. The clinical implications of this disease are highly variable, since infected animals may remain asymptomatic (absence of observable clinical signs) or present a wide spectrum of clinical alterations and degrees of severity, including the death of the animal. Symptoms such as lymphadenomegaly, alopecia, weight loss, keratoconjunctivitis and onychogryphosis are usually the first diagnostic reference available. The objectives of this study are to evaluate the validity (sensitivity, specificity and likelihood ratios) and diagnostic utility (pre-test probability) of the clinical signs commonly associated with canine leishmaniasis based on the prevalence in the area and to explore the combination of symptoms that best predicts the diagnosis of canine leishmaniasis. It is a matched case-control study in the canine population of southern Spain based on the comparison of the findings collected in the clinical history and the results of the LeisSCAN quantitative ELISA. A total of 39 cases and 78 controls were analysed. Approximately 80% of the infected animals showed signs compatible with the disease. The most frequent alterations were cutaneous (64.1%), systemic (51.3%) and oculo-nasal (30.7%). The most useful signs to support this diagnosis were alopecia and epistaxis (LR+ 6.69 and 6.0, respectively) (pre-test leishmaniasis probability is ≥70% for prevalence ≥28% when alopecia or epistaxis is present), followed by lameness (LR+ 5.0). The combinations of signs that showed greater validity were alopecia with hyperkeratosis of the snout and alopecia with onychogryphosis (LR+ > 10). None of the observed signs or their combinations resulted useful to rule out the diagnosis (LR− 0.55 to 1.15). The results found show notable differences in the diagnostic value of the clinical signs, individually and in combination, so we believe that medical decisions should be based on their diagnostic validity (LR+) and the estimation of the pre-test and post-test probability.

KEYWORDS
canine leishmaniasis, clinical signs, likelihood ratios, predictive value

1 | INTRODUCTION

Leishmaniasis is a notifiable parasitic zoonosis caused by *Leishmania* spp., which can affect about 70 species of mammals, including humans. Although the natural infection in dogs and wild canids is more common (Ribeiro et al., 2018), causing a wide variety of clinical signs (cutaneous, oculo-nasal, systemic), many of them common to other pathologies transmitted by vectors (Cortes et al., 2012; Miró et al., 2013).

Canine visceral leishmaniasis (CVL) is mainly caused by *L. infantum*, an obligate intracellular protozoan transmitted by haematophagous insects of the genus *Phlebotomus*. This disease is endemic in America, the Mediterranean Basin, West Asia and the Middle East and is prevalent in at least 98 countries and 3 territories on 5 continents (Alvar et al., 2012; Ribeiro et al., 2018).

The assertive diagnosis of CVL is usually complex due to the lack of serological techniques with 100% sensitivity and specificity and the high cost of molecular and histopathological techniques (Travi et al., 2018; Laurenti et al., 2013). In the majority of pathologies, the physical examination does not allow the presence of a disease to be absolutely confirmed or ruled out. Moreover, it is important to mention that asymptomatic dogs are highly competent to transmit leishmania to the vector in endemic areas (Laurenti et al., 2013).

However, this information, together with the prevalence of the disease in the area and the epidemiological data (exposure to risk factors), allows us to make a first estimate of the probability that the animal suffers from a certain disease. In this way, it is possible, as for laboratory tests, to determine the validity and usefulness of the results of the examination by calculating the sensitivity, specificity and likelihood ratios (LR).

Numerous studies have detailed the frequency of presentation of signs associated with CVL in positive animals, attributing a diagnostic value to this frequency without considering that they may occur in other pathologies. These works divide the animals into asymptomatic, oligosymptomatic and polysymptomatic, depending on the number of clinical signs (0, 1–3 and +3, respectively), proposing different diagnostic and therapeutic guidelines for each group (Moreira et al., 2007; Romero and Sánchez, 2009; Travi et al., 2018). More recently Da Silva et al. (2017) proposed a clinical scoring scale (0–19), with a sensitivity of 60.71% and a specificity of 73.64%, based on the degree of association of clinical signs with the disease (Odds ratio); however, there are no previous references on the LR of the signs associated with CVL.

The LRs allow determining the potential usefulness of a diagnostic test and the probability that an animal has a certain disease based on its result and the prevalence in the area (post-test probability). They are basically a relationship between the probability that a certain sign is present (LR +) or not (LR–) in a sick animal with respect to the probability that it is present or not in an animal without the disease. However, LRs require one diagnostic item to be considered at a time and have never been validated for use in series or parallel. To assess combinations of signs, it is therefore necessary to compare the probability that they occur simultaneously in animals with and without the disease (Sackett et al., 2001).

This post-anamnesis probability may be used by the veterinarian to conduct the epidemiological data survey and readjust the probability of its first diagnosis, or to directly select the laboratory test and, once the result is known, estimate the final probability that the animal has leishmaniasis (Sackett et al., 2001; Santana and Esparza, 2014).

The objective of this work was to estimate the validity of different clinical signs associated with any kind of CVL and the pre-test probability that an animal has the disease based on symptoms and prevalence.

2 | MATERIAL AND METHODS

2.1 | Study design

Between 2017 and 2020, an age- and sex-matched case-control study was carried out in the dog population of the southern region of Spain (2 million animals, 29% of the country's census, MAGRAMA, 2015) to determine the diagnostic value of the main clinical signs associated with CVL, by comparing the frequency of presentation in animals with established infection (CVL-positive) and in 'healthy' animals (CVL-negative). Through the Andalusian Council of Official Veterinary Associations of Andalusia, veterinarians from hospitals, clinics, shelters, pack of hounds and kennels were contacted for the referral, with the informed consent of the owner, of a blood sample from all the seropositive animals treated for CVL in their establishments. The clinical history was also requested to determine the presence in the last month of cutaneous signs (alopecia desquamation, onychogryphosis, ulcerative dermatitis and hyperkeratosis of the snout), oculo-nasal signs (keratoconjunctivitis, epistaxis and glaucoma), systemic signs (weight loss, fever, adenopathy) and lameness (Manna et al., 2009; Solano-Gallego et al., 2011; Da Silva et al., 2017).

The samples were processed at the Department of Animal Health of the University of Córdoba using the commercial quantitative ELISA LeisSCAN (HYPRA Laboratories S.A., Girona, Spain), for the titration of specific antibodies against *Leishmania* spp. Due to its validity, this technique (Sensitivity 92.5%, Specificity 100%) is comparable to Indirect Immunofluorescence (IIF), considered a reference test (World Organization for Animal Health, OIE 2018). The results were read following the manufacturer's instruction using an ELISA reader at 450 nm.

After comparing the optical density of the samples individually and the positive control, it was considered as follows:

Sample/control ratio	ELISA	Correlation with IIF antibody titre
≤0.5	Negative	NEGATIVE
0.5–0.7	Negative	1/20–1/40
0.7–0.9	Negative	>1/40–1/80
0.9–1.1	Doubtful	1/80
1.1–1.5	Low positive	>1/80–1/160
1.5–2	Positive	>1/160–1/320
2–3	High positive	>1/320–1/640
> 3	Very high positive	> 1/640

Following the OIE guidelines (2018), any sample with a ratio equivalent to an antibody titre of IIF > 1/160 was considered a 'case' (animal with established infection).

For each confirmed case, the referring veterinarian was asked to randomly select 4 animals of the same sex, age and geographic location that were seronegative for CVL. After performing the quantitative test, 2 animals were randomly selected among those with a ratio < 1.1 to be used as negative controls. In total, 39 cases and 78 controls (1:2) were studied.

2.2 | Statistical analysis

The data were processed with the statistical program EPIDAT 3.1 (Epidemiology Service of the General Directorate of Public Health of Galicia, Spain) and the Microsoft Excel 2013 spreadsheet. Each clinical sign was considered as a diagnostic test to be included in the statistical program, positive if the clinical history collected its detection in the 30 days prior to the query, and negative otherwise. Taking the quantitative ELISA (imperfect gold standard test) as a reference, the following parameters were estimated: adjusted sensitivity (proportion of cases with a positive physical examination result) (True positives/True positives + False negatives); adjusted specificity (proportion of controls with a negative physical examination result) (True negatives/True negatives + False positives); and the likelihood ratios (probability of obtaining the result in an animal with the disease /probability of obtaining it in an animal without the disease) (LR result + = *S*/1–*E*) (LR result – = 1–*S*/*E*) (Sackett et al., 2001).

Subsequently, the components of the clinical history present with greater frequency in animals with CVL were chosen and the ability of various combinations to predict the disease was estimated. The interpretation of the results was performed based on the criteria of Sackett et al. (2001) and McGee (2002).

LR+	LR−	LR interpretation
>10	<0.1	High diagnostic value. They entail very important and usually decisive changes in probability (±45% minimum) They will normally allow discrimination between healthy and diseased animals
5–10	0.1–0.2	Moderate changes in probability (± 30%–45%) Its diagnostic utility will depend on the prevalence
2–5	0.2–0.5	Small but sometimes important changes (according to the prior probability) (± 15%–30%)
1.0–2	>0.5	Rarely noticeable changes

Finally, based on the LR+, the pre-test probability of suffering from CVL associated with the presence of one or more clinical signs based on the prevalence in the area was estimated using Bayesian methodology (Sackett et al., 2001).

3 | RESULTS

3.1 | Analysis of the clinical history

Of the 39 cases included in this study, 30 (76.9%) reflected clinical signs compatible with CVL in the anamnesis (Table 1): 23.1% showed 1–2 signs; 30.8%, 3 signs; 15.4%, 4 signs; and 7.7 %, 6 or more signs. Moreover, 47.4% (37/78) of the controls showed some of the signs described.

The most frequent alterations observed in infected animals were cutaneous (64.1% of animals with one or more lesions), followed by systemic signs (51.3% of animals with one or more signs) and oculonasal (30.7% of animals with one or more alterations). Individually, the most frequent signs were alopecia (48.7%), thinning (38.5%), onychogryphosis (35.9%) and lymphadenomegaly (35.9%), followed by keratoconjunctivitis (25.6%), muzzle hyperkeratosis (15.4%) and lameness (12.8%). In none of the cases was the presence of glaucoma recorded. The alterations that appeared together most frequently were alopecia thinning (30.7% of cases), alopecia lymphadenomegaly (28.2%) and alopecia onychogryphosis (20.5%).

In polysymptomatic cases (+3 signs), the most frequent findings were lymphadenomegaly (88.8%), alopecia (77.7%) and onychogryphosis (77.7%), presenting the three signs simultaneously in 55.5% of the animals.

TABLE 1 Description of clinical signs associated with canine leishmaniosis found in the infectious animals in this study

Clinical frame (% in infected animals)	Infected animal without clinical manifestation: 9 out 39 (23.1%) Infected animals with clinical manifestation: 30 out 39 (76.9%) infected animals Clinical signs observed in infected animals (% of presentation)									
	Cutaneous (64.1% with 1 or more sign)				Oculo-nasal (30.7%)		Systemic (51.3%)			Joint (12.8%)
	ALO	ULCER	NHYP	ONYC	CONJ	NOSEB	WLOOS	ADEN	FEV	LIMP
1 sign (15.4%)				*						
										*
				*						
					*					
					*					
						*				
2 signs (7.7%)	*						*			
	*							*		
		*		*						
3 signs (30.8%)	*	*		*						
	*						*	*		
	*				*		*			
	*				*		*			
	*			*			*			
	*		*		*					
	*		*				*			
	*						*	*		
	*		*					*		
	*						*	*		
				*	*	*				
				*			*	*		
4 signs (15.4%)	*			*			*	*		
	*		*		*			*		
	*			*				*		*
	*	*		*				*		
				*			*	*		*
					*		*	*		*
≥ *6 signs* (7.7%)	*	*	*	*	*		*			
	*		*	*		*	*	*	*	
	*			*	*		*	*		*
	48.7%	10.3%	15.4%	35.9%	25.6%	7.7%	38.5%	35.9%	2.5%	12.8%

Abbreviations: ALO, alopecia desquamation; ULCER, ulcer dermatitis; NHYP, nose hyperkeratosis; ONYC, onychogryphosis; CONJ, keratoconjunctivitis; NOSEB, epistaxis; WLOOS, weight loss; ADEN, lymphadenopathy; FEV, fever; LIMP, limp.

In controls, the most frequent clinical findings were lymphadenomegaly (33.3%), weight loss (15.4%), keratoconjunctivitis (14.1%) and onychogryphosis (11.5%). Less than 5% had persistent fever, lameness, ulcerative dermatitis, hyperkeratosis of the muzzle, or epistaxis. Glaucoma was not detected.

3.2 | Diagnostic validity and post-anamnesis probability

Table 2 details the sensitivity, specificity and likelihood ratios obtained for the different signs in isolation. As expected, most of the abnormalities were detected more frequently in cases than in controls.

TABLE 2 Adjusted sensibility, specificity and likelihood ratios are estimated for individually clinical signs

Clinical signs	Sensibility (95%CI)	Specificity (95%CI)	LR+ (95%CI)	LR− (95%CI)
Alopecia desquamation	48.8% (32.55%–65.21%)	92.7% (85.64%–99%)	6.69 (2.69–37.43)	0.55 (0.37–0.74)
Onychogryphosis	35.95% (21.45%–51.35%)	89.49% (81.60%–96.40%)	3.42 (1.55–10.99)	0.72 (0.53–0.91)
Ulcer dermatitis	10.26% (0%–21.6%)	94.94% (89.47%–100%)	2.03 (0.53–7.67)	0.95 (0.84–1.06)
Nose hyperkeratosis	12.82% (1.05%–24.59%)	94.87% (89.34%–100%)	2.50 (0.71–8.79)	0.92 (0.81–1.05)
Keratoconjunctivitis	23.09% (10.8%–37.2%)	86.28% (77.02%–93.94%)	1.68 (0.67–4.40)	0.84 (0.71–1.07)
Epistaxis	7.69% (0.00%–17.34%)	98.72% (95.58%–100%)	6.0 (0.64–55.82)	0.94 (0.85–1.03)
Weight loss	38.51% (23.09%–54.14%)	85.59% (76.66%–93.67%)	2.67 (1.31–6.52)	0.72 (0.53–0.93)
Fever	2.50% (0%–8.59%)	84.62% (75.97–93.26%)	0.16 (0.02–1.21)	1.15 (1.04–1.28)
Lymphadenopathy	35.90% (21.04%–51.39%)	66.78% (55.65%–77.36%)	1.08 (0.59–1.86)	0.96 (0.69–1.28)
Limp	12.82% (1.05%–24.59%)	97.44% (93.29%–100%)	5.0 (1.02–24.62)	0.89 (0.79–1.01)

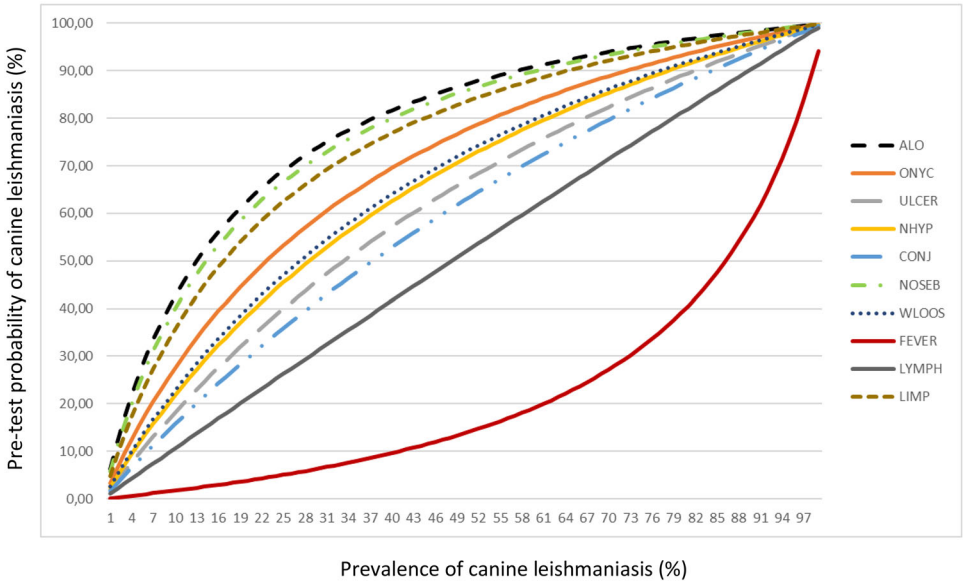

FIGURE 1 Estimated probability for the diagnosis of canine leishmaniasis based on the prevalence and the presence of a clinical sign. ALO, alopecia -desquamation; ULCER, ulcer dermatitis; NHYP, nose hyperkeratosis; ONYC, onychogryphosis; CONJ, keratoconjunctivitis; NOSEB, epistaxis; WLOOS, weight loss; LYMPH, lymphadenopathy; FEV, fever; LIMP, limp.

Based on the LR+, the sign with the highest value would be alopecia desquamation (LR+ of 6.69; 95%CI [2.69–37.43]), although its validity is moderate and its usefulness depends on prevalence. Thus, the probability pre-test that a dog with alopecia has CVL will be very high (≥70%) if the prevalence is greater than or equal to 26% (Figure 1). Similar results were obtained for the presence of epistaxis (LR+ 6.0) or lameness (LR+ 5). However, the lower limit of the estimated 95% CI for these signs was very close to 1, so we cannot rule out that its validity is actually null or very low. In the case of weight loss, hyperkeratosis of the snout and ulcerative dermatitis, the diagnostic relevance would be small (LR+ 2.03–2.67), so that only in areas with a prevalence ≥ 54% the probability of having a case of CVL would be elevated.

TABLE 3 Sensibility, specificity and likelihood ratios are estimated for combinations of clinical signs

	Sensibility (95%CI)	Specificity (95%CI)	LR+ (95%CI)	LR− (95%CI)
Alopecia + weight loss	30.77% (15.00%–46.54%)	94.62% (88.44%–99.77%)	5.73 (1.5–35.35)	0.73 (0.56–0.90)
Alopecia + lymphadenopathy	28.21% (12.8%–43.61%)	92.31% (85.75%–98.86%)	3.67 (1.46–9.18)	0.78 (0.63–0.96)
Alopecia + onychogryphosis	20.51% (6.56%–34.47%)	100% (99.36%–100%)	∞	0.79 (0.68–0.93)
Alopecia + keratoconjunctivitis	15.41% (5.13%–27.84%)	97.98% (93.83–100%)	7.62 (0–71.7)	0.86 (0.73–0.98)
Alopecia + nose hyperkeratosis	15.38% (2.78%–27.99%)	100% (99.36%–100%)	∞	0.85 (0.74–0.97)
Onychogryphosis + lymphadenopathy	17.97% (6.51%–30.99%)	91.40% (83.96%–97.9%)	2.09 (0.64–8.61)	0.90 (0.74–1.05)
Keratoconjunctivitis + lymphadenopathy	7.69% (0.00%–17.34%)	91.03% (84.04%–98.01%)	0.86 (0.23–3.13)	1.01 (0.90–1.14)
Keratoconjunctivitis + weight loss	12.82% (1.05%–24.59%)	96.15% (91.25%–100%)	3.33 (0.84–13.23)	0.91 (0.80–1.03)
Alopecia + weight loss + lymphadenopathy	15.41% (5.13%–27.84%)	97.98% (93.83%–100%)	7.62 (0–71.7)	0.86 (0.73–0.98)

When considering the joint presence of several signs (Table 3), we observed that most of the tested combinations increased the specificity with respect to the individual signs, but at the cost of reducing their sensitivity, since all the signs had to be present to lead to a positive result. As a consequence, only the presence of alopecia with hyperkeratosis of the muzzle or alopecia with onychogryphosis (LR+ ∞) would imply an important and generally decisive change in the initial probability (± 45% minimum). The combination of alopecia with keratoconjunctivitis (LR+ 7.62; 95%CI [0–71.7]) or with weight loss and lymphadenomegaly (LR+ 7.62; 95%CI [1.5–96.23]) represented in both cases an increase with respect to the validity of this sign isolated, although its usefulness remained modest. For the rest of the combinations, the LR+ was zero or very low (LR+ 0.86–3.67).

Regarding the validity of the signs observed to rule out the disease, both in isolation and in combination, it was very slight (LR− > 0.5) (Tables 2 and 3).

4 | DISCUSSION

Due to the increase in prevalence and spread of CVL in recent decades, as a consequence of climate change and the massive migration of human and canine populations, there are numerous studies that measure the frequency of presentation of clinical signs in affected animals with any kind of CVL and propose guides or scoring scales for the practical management of the disease (Manna et al., 2009; Solano-Gallego et al., 2011; Cortes et al., 2012; Da Silva et al., 2017). Medical decision-making in these cases is based solely on the number of signs observed or their degree of association with the disease. Therefore, they do not consider the initial probability that the animal has the infection (conditioned by clinical history and prevalence) and how this affects the final probability of diagnosis. This is especially relevant in CVL, a systemic disease that can manifest itself with a wide variety of non-specific clinical signs and whose transmission depends above all on the population density of the vector, which determines large variations in prevalence between contiguous territories (Solano-Gallego et al., 2011; Roura et al., 2013). The visceral form is of great importance in countries such as India, Ethiopia or Brazil (up to 85% depending on the bioclimatic zone), while the cutaneous form is endemic in America, West Asia, the Middle East and the Mediterranean Basin (6.4%–46.6% in central-southern Spain) (Alvar et al., 2012; Miró et al., 2013; Bouattour et al., 2021).

In most published studies, cutaneous and systemic signs constitute the most frequent manifestation in sick animals, with lymphadenomegaly being the most prevalent sign (84%–90% in infected animals) (Manna et al., 2009; Cortes et al., 2012; Da Silva et al., 2017). According to these authors, its presence in animals negative for serology or with low titres of antibodies is indicative of a state of mild disease (LeishVet Guide) (Solano-Gallego et al., 2011). Lymphadenomegaly is a normal response of the body to infectious agents and, consequently, its frequency in animals without CVL can be very high. According to data collected by Da Silva et al. (2017) (68% in negative animals and 83.9% in positive animals), we cannot establish a statistically significant association between this alteration and CVL (OR 2.2; 95%CI [0.94–5.15]. This result coincides with the low validity obtained for lymphadenomegaly in our work (LR+ 1.08), although the frequency of presentation of this sign in both groups was lower (35.9% in positive animals and 33.3% in negative animals).

Hair loss is one of the main manifestations of skin diseases in dogs. This disorder, very common in clinical practice, can be caused by multiple aetiologies (parasites, bacteria, fungi, allergies, endocrine disorders) (Greene, 2012). Alopecia desquamation was the most

frequent clinical sign in CVL-positive dogs (48.7%), being detected in only 8.8% of CVL-negative dogs. Although these values differ from those found by Da Silva et al. (2017) (39.29 and 22.48%, respectively), they attribute moderate validity to this sign (LR+ 6.69), coinciding with the degree of association described by this author (OR 2.25; 95%CI [1.14–4.48]) and the classification of the LeishVet guideline (Stage II, moderate disease) (Solano-Gallego et al., 2011; Da Silva et al., 2017). Based on our result, the probability of CVL if the animal presents alopecia desquamation will be high if the prevalence of the disease in the area is moderate. However, if the prevalence is low (<7%) the probability will be less than 30% and with prevalence between 13% and 19% the uncertainty of the diagnosis will be supreme (50%–60%). Similar results were found for lameness (LR+ 5.0), present in 12.8% of positive animals. However, taking into consideration that some authors describe up to 25% presentation in animals with CVL (Manna et al., 2009), this sign is not included in many clinical assessment scales (Solano-Gallego et al., 2011; Da Silva et al., 2017).

Within the variety of pathological alterations that can occur in CVL, several clinical signs (keratoconjunctivitis, onychogryphosis, epistaxis, nutritional status, alopecia desquamation and hyperkeratosis of the snout) seem to be more associated with the disease (Da Silva et al., 2017). According to Da Silva et al. (2017) the mere presence of keratoconjunctivitis (OR 5.4; 95%CI [2.54–11.44]), muzzle hyperkeratosis (OR 4.65; 95%CI [2.21–9.75]), bleeding (OR 4.52; 95%CI [1.26–16.14]), or onychogryphosis (OR 3.5; 95%CI [1.83–6.79]) would be highly correlated with CVL. In our study, the diagnostic validity of keratoconjunctivitis, hyperkeratosis and onychogryphosis was low (LR+ 1.68, 2.50 and 3.42, respectively) and moderate in the case of epistaxis (LR+ 6). This result, consistent with the presence of these signs in other pathologies (coagulation problems, foreign bodies, poisoning, cancer, parasites, infectious diseases, etc.) (Manna et al., 2009; Greene, 2012), differs notably from findings of Da Silva et al. (2017), although we must point out that our results could be underestimated based on the width of the confidence interval obtained. Regarding systemic signs (fever, weight loss and anorexia), we confirm the frequency of presentation described by other authors in infected animals (40%–60%) (Manna et al., 2009; Solano-Gallego et al., 2011), as well as its lack of specificity.

That an isolated symptom is of little use in diagnosing a disease is to be expected and coincides with the findings published by other authors on the diagnosis of CVL (Solano-Gallego et al., 2011; Da Silva et al., 2017). In fact, when the clinician evaluates a patient, he does not consider each sign in isolation, but all the data as a whole (prevalence, history, risk factors and clinical examination) and each positive response increases or adjusts the probability that the animal suffers from a certain disease. Thus, the combination of several positive signs can give such an important likelihood ratio that it will allow us to practically confirm or rule out a diagnosis (Lugo-Reyes et al., 2011). Most of the combinations evaluated in this study did not significantly improve the predictive capacity of the clinical examination. Only in case of observing alopecia with hyperkeratosis of the snout or onychogryphosis, the probability of being in a case of CVL would reach a high level of certainty, even in areas where the infection is rare, which corroborates the important role of these signs in the prediction of the CVL indicated by Da Silva et al. (2017).

The information provided in our work could help to identify the most useful clinical manifestations to establish the probability of finding a case of CVL and to select the best candidates for carrying out complementary tests. Ordering a diagnostic test is not always appropriate and can lead to confusion and poor patient management. If the pre-test probability is very high or very low, performing a test (almost always imperfect) can be useless, if it confirms what we already knew, or confusing, if it gives an erroneous result that contradicts our initial suspicion (Lugo-Reyes et al., 2011). However, it is not uncommon for owners and veterinarians to attribute 100% credibility to the test result of the test. As we have mentioned, the presence of one or more signs combined with an LR+ > 10 will generally support the diagnosis with sufficient certainty, and tests with moderate specificity can be used to confirm it, and therefore, they are more affordable economically (Sackett et al., 2001). Thus, in areas with limited economic resources (India, Sudan, Ethiopia, etc.), where the prevalence of the disease is unknown or diagnostic techniques are not applicable, clinical examination can be an important tool in making medical decisions (Soltani and Moayyeri, 2007).

5 | CONCLUSION

In this work, we verified that there are signs and combinations of signs with a notable diagnostic value that would allow us to confirm CVL with enough certainty, but which presence in areas of very low prevalence would not be conclusive. The results found also show notable differences in the validity of the clinical findings, which is why we consider that medical and therapeutic decisions should not be based so much on the number of signs that an animal presents, but on the probability ratios and the estimation of the pre-test and post-test probability. However, when using LRs it is important to understand their limitations. The validity of this parameter depends entirely on the quality of the studies that generated the data and may be subject to precision bias (size and representativeness of the sample), information bias (especially in retrospective studies) and diagnostic bias (homogeneity of criteria, quality of the clinical examination, validity of the diagnostic technique). Another limitation is the wide confidence intervals of the LRs due to the paucity of data at the extremes of the disease spectrum, where the LRs are likely to be most useful. Finally, since LRs are calculated from sensitivity and specificity, like these parameters, they can be affected by the severity of the disease (Parikh et al., 2009). Based on this, we consider it important to continue this work, including other geographical areas, in order to increase the sample and, in this way, improve the reliability of our estimates, include new symptoms associated with LC and assess other combinations.

AUTHOR CONTRIBUTIONS

LGG and AGR performed all experiments in this study. AM, CT, RJA, IL and BH designed the study. AM and BH carried out the statistical analysis. LGG and AGR wrote the manuscript down with the invaluable

insights of AM, CT, RJA, IL and BH. AM and BH directed and supervised the whole study.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required.

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REFERENCES

Alvar, J., Vélez, I. D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., & de Boer, M. (2012). Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*, *7*, https://doi.org/10.1371/journal.pone.0035671

Bouattour, A., Amri, A., Belkhiria, J. A., Rhim, A., Fezaa, O., Gantier, J. C., & M'ghirbi, Y. (2021). Canine leishmaniosis in Tunisia: Growing prevalence, larger zones of infection. *PLoS Neglected Tropical Diseases*, *15*, 1–17. https://doi.org/10.1371/journal.pntd.0009990

Cortes, S., Vaz, Y., Neves, R., Maia, C., Cardoso, L., & Campino, L. (2012). Risk factors for canine leishmaniasis in an endemic Mediterranean region. *Veterinary Parasitology*, *189*, 189–196. https://doi.org/10.1016/J.VETPAR.2012.04.028

Da Silva, K. R., Mendonça, V. R. R. D., Silva, K. M., Do Nascimento, L. F. M., Mendes-Sousa, A. F., De Pinho, F. A., Barral-Netto, M., Maria Prado Barral, A., & do S P E Cruz, M. (2017). Scoring clinical signs can help diagnose canine visceral leishmaniasis in a highly endemic area in Brazil. *Memórias do Instituto Oswaldo Cruz*, *112*, 53–62. https://doi.org/10.1590/0074-02760160305

Greene, C. E. (2012). *Infectious diseases of the dog and cat* (4th edn., pp. 495–520). Amsterdam: Elsevier Publisher.

Laurenti, M. D., Rossi, C. N., VL, R. M., Tomokanea, T. Y., Corbett, C. E. P., Secundinoc, N. F. C., Pimentac, P. F. P., & Marcondes, M. (2013). Asymptomatic dogs are highly competent to transmit Leishmania (Leishmania) *Infantum chagasi* to the natural vector. *Veterinary Parasitology*, *196*, 296–300.

Lugo, R. S. O., García, C. M. L., & Terán, J. L. M. (2011). Valor predictivo de signos y síntomas respiratorios en el diagnóstico de atopia. *Alergia, Asma e Inmunología Pediátricas*, *20*(1), 29–33.

Manna, L., Reale, S., Vitale, F., & Gravino, A. E. (2009). Evidence for a relationship between Leishmania load and clinical manifestations. *Research in Veterinary Science*, *87*, 76–78. https://doi.org/10.1016/j.rvsc.2008.12.009

McGee, S. (2002). Simplifying likelihood ratios. *Journal of General Internal Medicine*, *17*, 647–650. https://doi.org/10.1046/j.1525-1497.2002.10750.x

Miró, G., Montoya, A., Roura, X., Gálvez, R., & Sainz, A. (2013). Seropositivity rates for agents of canine vector-borne diseases in Spain: A multicentre study. *Parasites and Vectors*, *6*, 1–9. https://doi.org/10.1186/1756-3305-6-117

Moreira, M. A. B., Luvizotto, M. C. R., Garcia, J. F., Corbett, C. E. P., & Laurenti, M. D. (2007). Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different clinical signs. *Veterinary Parasitology*, *145*, 245–252. https://doi.org/10.1016/j.vetpar.2006.12.012

Parikh, R., Parikh, S., Arun, E., & Thomas, R. (2009). Likelihood ratios: Clinical application in day-to-day practice. *Indian Journal of Ophthalmology*, *57*, 217, https://doi.org/10.4103/0301-4738.49397

Ribeiro, R. R., Michalick, M. S. M, Eduardo da Silva, M., Peixoto dos Santos, C. C., Frézard, F. J. G., & Magno da Silva, S. (2018). Canine leishmaniasis: An overview of the current status and strategies for control. *BioMed Research International*, *2018*, 3296893. https://doi.org/10.1155/2018/3296893

Romero, M., & Sánchez, J. (2009). The diagnosis of canine visceral leishmaniasis: Dilemmas and challenges. *Biosalud*, *8*, 105–116.

Roura, X., Fondati, A., Lubas, G., Gradoni, L., Maroli, M., Oliva, G., Paltrinieri, S., Zatelli, A., & Zini, E. (2013). Prognosis and monitoring of leishmaniasis in dogs: A working group report. *Veterinary Journal*, *198*, 43–47. https://doi.org/10.1016/j.tvjl.2013.04.001

Sackett, D. L., Straus, S. E., Richardson, W. S., Rosenberg, W., & Haynes, R. B. (2001). Diagnóstico y cribado. In: *Medicina Basada en la Evidencia: cómo practicar y enseñar la MBE* (2nd edn., pp. 57–81). California: Harcourt Publisher.

Santana, L. A., & Esparza, R. I. (2014). Razonamiento con los signos y los síntomas. *Medicina Interna de México*, *30*, 442–450.

Solano-Gallego, L., Miró, G., Koutinas, A., Cardoso, L., Pennisi, M. G., Ferrer, L., Bourdeau, P., Oliva, G., & Baneth, G. (2011). LeishVet guidelines for the practical management of canine leishmaniosis. *Parasites & Vectors*, *4*, 86, https://doi.org/10.1186/1756-3305-4-86

Soltani, A., & Moayyeri, A. (2007). Deterministic versus evidence-based attitude towards clinical diagnosis. *Journal of Evaluation in Clinical Practice*, *13*, 533–537. https://doi.org/10.1111/j.1365-2753.2007.00716.x

Travi, B. L., Cordeiro-da-Silva, A., Dantas-Torres, F., & Miró, G. (2018). Canine visceral leishmaniasis: Diagnosis and management of the reservoir living among us. *PLoS Neglected Tropical Diseases*, *12*, 1–13. https://doi.org/10.1371/journal.pntd.0006082

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