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Serosurveillance of *Leishmania infantum* in Zoo-Kept Animals in Spain

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ABSTRACT

Introduction: Leishmaniosis is a sand fly-borne zoonosis mainly caused by *Leishmania infantum* in Europe. Exposure to this protozoan has been widely reported in many domestic and wild species. However, epidemiological surveys evaluating the circulation of *L. infantum* in zoo-kept animals remain limited.

This large-scale study aims to evaluate the seroprevalence of *L. infantum* in zoo-kept species in Spain as well as alterations in serum protein levels in *L. infantum*-seropositive individuals, to identify potential risk factors associated with *L. infantum* exposure, and to assess the dynamics of seropositivity in animals longitudinally sampled during the study period.

Methods: Between 2007 and 2023, serum samples from 429 zoo-kept animals belonging to 72 species were collected in nine zoos in Spain using convenience sampling. Additionally, 29 of these individuals from six of the tested zoos were also longitudinally sampled.

Results: Anti-*L. infantum* antibodies were detected in 22 (5.1%; 95% CI: 3.0–7.2) of the 429 animals using an in-house ELISA, as well as in 13.9% (10/72) and 66.7% (6/9) of the species and zoos tested, respectively. Serum protein electrophoresis analyses revealed that polyclonal gammopathy was the most common alteration in *L. infantum*-seropositive individuals. Three animals longitudinally surveyed seroconverted throughout the study period. The multivariate analysis identified the family Canidae as a risk factor for *L. infantum* exposure.

Conclusions: Our results indicate a moderate, widespread and endemic circulation of *L. infantum* in zoo-kept animals from Spain, which may be of animal health, conservation, and public health concern. Surveillance programs and control measures should be implemented in zoos to minimise the exposure of these species to *Leishmania* spp., particularly in hotspot areas.

Summary

- This study represents the largest serosurvey of *L. infantum* in zoo-kept species worldwide.
- Moderate, widespread, and endemic circulation of *L. infantum* in zoo-kept animals was identified.
- Surveillance and control strategies to prevent the spread of *L. infantum* in zoo-kept animals are recommended.

1 | Introduction

Leishmaniosis is a neglected vector-borne disease caused by different species of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) and transmitted by female sand flies (Diptera: Psychodidae) (Akhoundi et al. 2016). This disease constitutes a major public health concern worldwide, being endemic in at least 99 countries with more than a thousand million under infection risk (WHO 2024). Annually, 1 million new human cases and around 30,000–50,000 deaths are caused by this parasitic disease (Carvalho et al. 2024; WHO 2024).

In Europe, the Mediterranean basin constitutes the most important hotspot for leishmaniosis, being *Leishmania infantum* the main species circulating, and sand flies belonging to the genus *Phlebotomus* the competent vectors of this protozoan (Maia et al. 2023). The dog (*Canis lupus familiaris*) is considered the main domestic reservoir of *L. infantum* in Europe (Priolo et al. 2024). However, exposure to the parasite has also been documented in a wide range of domestic and wild species, which may act as reservoirs or spillover hosts (Arce et al. 2013; Cardoso et al. 2021). Previous epidemiological studies have also highlighted the relevance of captive wildlife as potential reservoirs of *L. infantum* in urban and periurban areas (Gonzálvez et al. 2017; Cantos-Barreda et al. 2020; Iatta et al. 2020). In this context, the role of zoos in the epidemiology of *L. infantum* is supported by the presence of a high variety of microenvironmental conditions that favour the presence and maintenance of sand fly populations in these complex multi-host systems (Muñoz et al. 2019). Additionally, clinical and/or fatal leishmaniosis cases have been documented in zoo-kept animals in Europe, which evidence the importance of *L. infantum* not only for animal health, but also for the conservation status of threatened species kept in zoos (Luppi et al. 2008; Montoya et al. 2016; Miró et al. 2018; Iatta et al. 2020).

To date, there is a limited number of epidemiological studies evaluating the circulation of *L. infantum* in zoo-kept animals, which are based on a scarce variety of animal species analysed, low sample size, and/or restricted geographical areas. The aims of the present study were: (1) to evaluate the seroprevalence of zoo-kept animals to *L. infantum* in Spain, (2) to identify serum protein alterations in *L. infantum*-seropositive animals, (3) to investigate the potential risk factors associated with *L. infantum* exposure in these individuals, and (4) to determine the dynamics of seropositivity in animals longitudinally sampled during the study period.

2 | Material and Methods

2.1 | Sampling

A cross-sectional study was conducted to evaluate the prevalence of antibodies against *L. infantum* in zoo-housed species from Spain. The minimum sample size was calculated based on an assumed seroprevalence of 50% (which yields the highest sample size in studies with unknown prevalence) with a 95% confidence interval (95% CI) and an accepted error of 5%, resulting in a required minimum sample size of 385 individuals (Thrusfield et al. 2018). Finally, a total of 429 animals belonging to 72 species were sampled at nine different zoological institutions (A–I) in Spain between 2007 and 2023 (Table S1; Figure 1). Serum samples were obtained from serum banks or animals subjected to health programs, surgical interventions, or routine medical check-ups during the study period. Samples were stored at −20°C until laboratory analysis. Epidemiological information, including family, species, sex, age, animal origin (zoo) and sampling date, was gathered from each animal, whenever possible. Additionally, 29 of the 429 (6.8%) analysed animals, belonging to 20 different species, were longitudinally sampled (two to four samplings per animal) in six of the analysed zoos (Table 1). The median period between consecutive samplings was 20.7 months (range: 1.2–79 months).

2.2 | Laboratory Analyses

The presence of antibodies against *L. infantum* was determined using an in-house ELISA (sensitivity of 99.4% and specificity of 97.5%), as previously described (Villanueva-Saz, Lebrero, et al. 2024) with some modifications. Briefly, each plate was coated with 100 μL/well of the *L. infantum* antigen solution in 0.1 M carbonate/bicarbonate buffer and incubated overnight at 4°C. A 100-μL aliquot of serum samples, diluted 1:100 in phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST) and 1% dry skimmed milk (PBST-M) as a blocking agent, was added to each well. The plates were incubated for 1 h at 37°C in a moist chamber. After washing the plates with PBST for 3 times during 3 min each one, followed by one wash with PBS for 1 min, 100 μL of Protein A/G conjugated to horseradish peroxidase diluted 1:10,000 in PBST-M was added per well. This reagent interacts with immunoglobulin G in different mammal species, allowing the use of positive and negative controls from different species in the absence of controls for the species being serologically tested. The standardisation of the ideal concentration of serum dilution and conjugated dilution was based on serological results obtained from previous studies in dogs (Basurco et al. 2020), cats (Villanueva-Saz, Giner, Tobajas, et al. 2022), sheep (Bauer et al. 2024; Villanueva-Saz, Lebrero, et al. 2024), goats (Ruiz et al. 2023), ferrets (Villanueva-Saz et al. 2021), European bison (Didkowska et al. 2024) and American and European mink (Giner et al. 2021). The plates were incubated for 1 h at 37°C in the moist chamber and were washed again with PBST and PBS as described above. The substrate solution (ortho-phenylene-diamine) and stable substrate buffer were added at 100 μL per well and incubated for 20 ± 5 min at room temperature in the dark. The reaction was stopped by adding 100 μL of 2.5 M H_2SO_4 to each well. Absorbance values

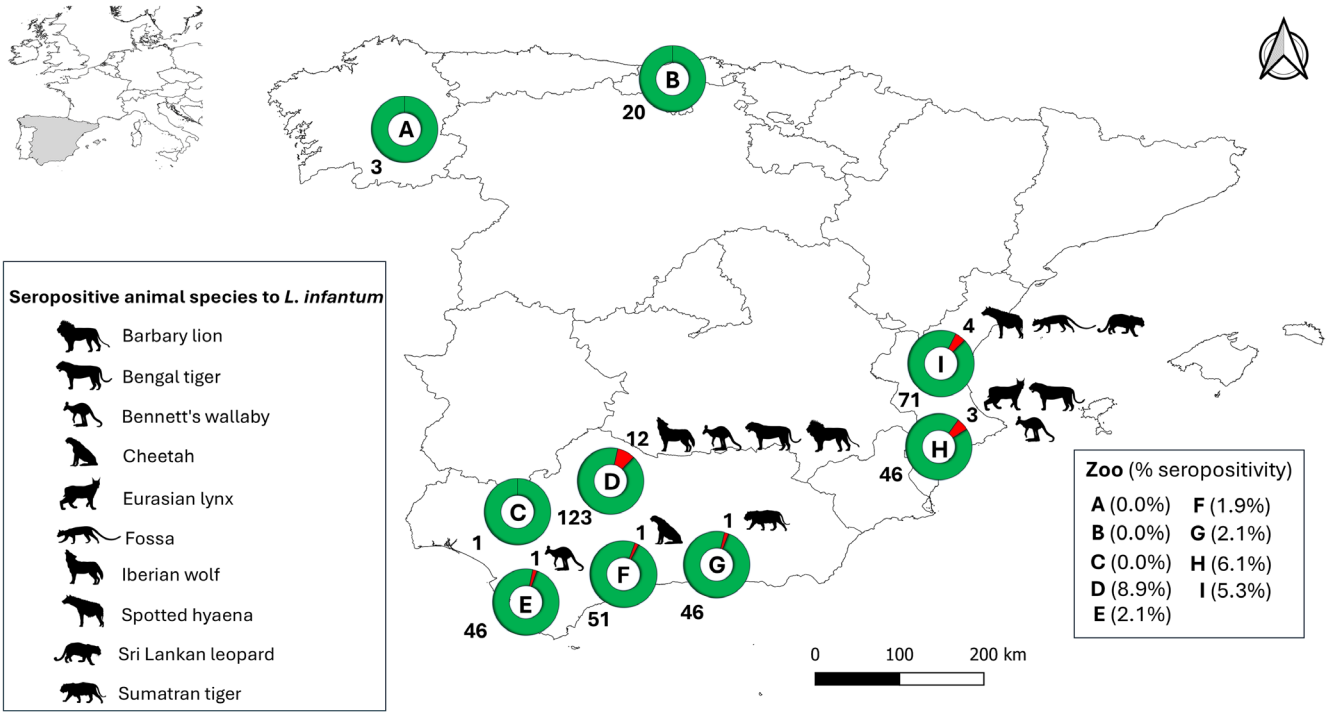

FIGURE 1 | Distribution of zoos (A–I) sampled in Spain. Doughnut charts show *L. infantum*-positive (red) and negative (green) animals, with the numbers outside the charts indicating the total of positive and negative animals in each zoo.

TABLE 1 | Seropositivity to *Leishmania infantum* in longitudinally sampled zoo-kept animals.

Species	Zoo	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Impala	I	▲					▲		▲ Apr; ▲ Jul						
African elephant	I						▲	▲	▲						
Thomson's gazelle	I						▲		▲						
Mouflon	D								▲ Jan; ▲ Dec						
Bennett's wallaby	D								▲ Jan; ▲ Nov						
Katanga lion	I						▲					▲			
African elephant	I											▲	▲		
Philippine spotted deer	G								▲	▲					
Philippine spotted deer	G				▲						▲				
Sri Lankan leopard	G						▲	▲							
Binturong	G						▲						▲		
South American coati	G				▲							▲			
Asian elephant	F									▲	▲				
Iberian lynx	F									▲	▲			▲	
Aoudad	F										▲			▲	
European bison	F										▲				▲
Aoudad	F										▲				▲
Dromedary	F										▲			▲	
Hartmann's mountain zebra	E												▲ Nov; ▲ Dec		
Aoudad	E												▲ Feb; ▲ Dec		
Aoudad	D										▲	▲			
Aoudad	D									▲	▲			▲	
											▲	▲			
Dama gazelle	H														▲ Jun; ▲ Oct
Eurasian lynx	D										▲	▲			
Spanish ibex	D									▲		▲			
Iberian wolf	D									▲		▲			
Iberian wolf	D									▲		▲			

Note: Coloured triangles indicate antibodies to *L. infantum* (red: positive; green: negative). When two samplings were carried out in the same year, the superscript indicates the sampled months.

were read at 492 nm in an automatic microELISA reader. All plates included the serum from a sick dog with a confirmed *L. infantum* infection as positive control (calibrator) and serum from a healthy dog tested as negative control. The same positive and negative controls were used in duplicate for all assays, and any plate with an inter-assay variation greater than 10% was discarded. The cut-off for carnivores was set to 0.22 based on the cut-off for dogs (Riera et al. 1999; Villanueva-Saz, Pérez, et al. 2024), while the cut-off for herbivores was set to 0.38 based on the cut-off for sheep (Villanueva-Saz, Lebrero,

et al. 2024). Sera were classified as high positive when they had an optical density equal to or higher than 1.5. Medium positive sera were classified as having an optical density equal to or higher than 0.7 but less than 1.5. Finally, low positive sera were defined as those with an optical density lower than 0.7 but higher than 0.22 for carnivores and 0.38 for herbivores.

Additionally, ELISA-seropositive animals were tested by serum protein electrophoresis (SPE) for serum protein fractions measured (α1-globulin, α2-globulin, β1-globulin, β2-globulin, and γ-globulin) using an agarose gel electrophoresis (AGE) system (Hydragel Kit 1-2, Sebia, Issy-les-Moulineaux, France) (Villanueva-Saz, Aranda, et al. 2024). Sera were electrophoresed for 21 min at 92V and stained with a diluted amidoschwarz dye at pH 2 (4 g/L amidoschwarz dye and 6.7% ethylene glycol). The AGE procedure was conducted according to the manufacturer's instructions. Dog, cat, sheep, cattle and horse sera were used as controls (Sebia, France). The electrophoretic curve for each sample was displayed and read with a GELSCAN TM densitometry system (Sebia, France). The electrophoretic curve for each sample was assessed using the Phoresis software.

2.3 | Statistical Analyses

The estimated seroprevalence of *L. infantum* was calculated by dividing the number of seropositive animals detected by the total number of individuals tested, based on binomial distribution data with a 95% CI (Thrusfield et al. 2018). Bivariate associations between the prevalence of anti-*L. infantum* antibodies and explanatory variables were evaluated using Pearson's chi-squared test or Fisher's exact test, as required. Variables with p-value ≤ 0.10 in bivariate analyses were selected for inclusion in the multivariate model. Collinearity between pairs of variables was tested using Cramér's *V*, selecting the variable with the highest biological plausibility associated with *L. infantum* exposure when the correlation coefficient between variables was >0.6 and $p \leq 0.05$. A Generalised Linear Model (GLM) was run using a binomial error distribution and a logit link function with the lme4 R-package (Bates et al. 2015). The significance of the fixed effect variables was determined using the car R-package (Fox and Weisberg 2018), and pairwise Tukey post hoc comparisons were calculated using the emmeans (Estimated Marginal Means) R-package to evaluate the differences among levels of the explanatory variables retained in the multivariate model. All statistical analyses were performed using R software version 4.1.3 (R Core Team 2023), and significant differences were considered with $p \leq 0.05$ for a two-sided test.

3 | Results

A total of 22 (5.1%; 95% CI: 3.0–7.2) of the 429 sampled animals showed antibodies against *L. infantum* (Table S1). Seropositivity to *L. infantum* was detected in 10 (13.9%) of the 72 species analysed, and in six (66.7%) of the nine zoos tested. Within each zoo, the average seroprevalence was 2.9% (range: 1.9%–8.9%) (Figure 1), defined as the proportion of animals testing positive among all animals sampled at that zoo. *Leishmania infantum*-positive animals were classified as low ($n=16$), medium ($n=2$) and high seropositivity levels ($n=4$) (Table 2). Seropositive individuals were detected in all sampling years from 2009 to 2023, except 2012 (Table 3).

The results obtained from the SPE are shown in Table 2. A total of 17 (77.3%) of the 22 seropositive individuals revealed alterations in the serum protein analysis, being polyclonal gammopathy the most common electrophoretic pattern detected (94.1%; 16/17). In addition, an alpha-2 globulins increased peak was evidenced in 29.5% (5/17) of seropositive specimens of which 80% (4/5) had also polyclonal gammopathy. No other alterations in SPE were evidenced in *L. infantum*-seropositive individuals.

Concerning the animals longitudinally sampled, three out of 29 specimens seroconverted during the sampled period. These animals were three Iberian wolves (*Canis lupus signatus*) from the same zoological centre (Zoo D); two of them were sampled in 2017 and 2019, and one in July 2018 and April 2019 (Figure 1; Table 1).

The GLM identified the "family" as a risk factor associated with *L. infantum* seropositivity in zoo-kept animals in Spain (Table 4). Specifically, the family Canidae showed significantly higher seroprevalence (42.1%, 8/19; OR: 7.4; 95% CI: 2.2–24.4; $p=0.012$) compared to the family Felidae (9.3%, 7/75). No significant differences regarding the seroprevalence to *L. infantum* were evidenced in the other families evaluated.

4 | Discussion

The present study constitutes the largest serosurvey evaluating the circulation of *L. infantum* in zoo-kept species worldwide. The overall seroprevalence (5.1%) detected here is in accordance with that found in a previous report in crab-eating foxes (*Cerdocyon thous*) (5.9%; 1/17) from two zoos in northeastern Brazil using commercial ELISA (Almeida et al. 2018). In contrast, a higher prevalence of anti-*L. infantum* antibodies was observed in various wild canid and felid species (42.9%; 9/21) housed at a zoo from southern Brazil, using commercial and in-house ELISAs (Tolentino et al. 2019). A similar seroprevalence (45.0%; 9/20) was reported in tigers (*Panthera tigris*) from one zoo in southern Italy using the indirect immunofluorescence antibody test (IFAT) (Iatta et al. 2020). In Spain, a similar seroprevalence to those obtained from the present survey was detected in captive wolves (*Canis lupus*) (6.1%; 2/33) by in-house ELISA (Sastre et al. 2008) and in Bennett's wallabies (*Macropus rufogriseus rufogriseus*) using IFAT (8.3%; 1/12) (Montoya et al. 2016). More recently, a seroprevalence of 4.0% (10/52) by IFAT was reported in captive non-human primates, with anti-*L. infantum* antibodies detected in white-crowned mangabey (*Cercocebus atys lunulatus*), vervet monkey (*Chlorocebus aethiops*), black mangabey (*Lophocebus aterrimus*), Barbary macaque (*Macaca sylvanus*), Tonkean macaque (*Macaca tonkeana*), orangutan (*Pongo pygmaeus pygmaeus*) and ring-tailed lemur (*Lemur catta*) (Barbero-Moyano et al. 2024). Nevertheless, comparisons between studies should be performed with caution due to differences in sample size, variety of species analysed, epidemiological scenarios, study designs, and diagnostic techniques employed.

Seropositive animals were detected in 66.7% (6/9) of the tested zoos and in all sampling years between 2009 and 2023, except in

TABLE 2 | Results of serum protein electrophoresis and seropositivity classification in seropositive zoo-kept animals to *Leishmania infantum*.

ID	Species	Origin zoo	Sampling year	Seropositivity classification (Optical density value, OD)	Serum protein electrophoresis results
15	Iberian wolf	D	2010	High (1.82)	Polyclonal gammopathy + alpha-2 globulins increased peak
70	Iberian wolf	D	2014	Low (0.36)	Polyclonal gammopathy
274	Eurasian lynx	H	2016	Medium (1.36)	Polyclonal gammopathy
907	Bennett's wallaby	H	2019	Low (0.39)	No alterations
829	Bennett's wallaby	E	2012	Medium (0.74)	Polyclonal gammopathy
1504	Bennett's wallaby	D	2019	High (1.77)	Polyclonal gammopathy
652	Iberian wolf	D	2019	Low (0.243)	Polyclonal gammopathy + alpha-2 globulins increased peak
1505	Bennett's wallaby	D	2019	High (2.03)	Polyclonal gammopathy
580	Bengal tiger	H	2019	High (1.64)	Polyclonal gammopathy
931	Sumatran tiger	G	2020	Low (0.28)	Polyclonal gammopathy
58	Bengal tiger	D	2012	Low (0.46)	Polyclonal gammopathy + alpha-2 globulins increased peak
415	Spotted hyaena	I	2009	Low (0.29)	Polyclonal gammopathy
416	Spotted hyaena	I	2011	Low (0.29)	Polyclonal gammopathy
426	Fossa	I	2015	Low (0.28)	No alterations
430	Sri Lankan leopard	I	2009	Low (0.42)	Alpha-2 peak globulins increased
650	Iberian wolf	D	2016	Low (0.44)	Polyclonal gammopathy
651	Iberian wolf	D	2017	Low (0.25)	Polyclonal gammopathy
653	Iberian wolf	D	2017	Low (0.30)	No alterations
1230	Cheetah	F	2021	Low (0.25)	No alterations
1506	Barbary lion	D	2021	Low (0.24)	Polyclonal gammopathy + alpha-2 globulins increased peak
647	Iberian wolf	D	2019	Low (038)	Polyclonal gammopathy
648	Iberian wolf	D	2019	Low (0.48)	Inconclusive

2012. These results indicate a widespread and endemic circulation of *L. infantum* in zoos from Spain, which is in accordance with those previously observed (Gálvez et al. 2020; Barbero-Moyano et al. 2024). Antibodies against *L. infantum* were found in 13.9% (10/72) of the tested species. This is the first report of *L. infantum* exposure in cheetah (*Acinonyx jubatus*), Eurasian lynx (*Lynx lynx*), fossa (*Cryptoprocta ferox*), spotted hyaena (*Crocuta crocuta*) and Sri Lankan leopard (*Panthera pardus kotiya*), increasing the host range of this protozoan. Of note, many of *L. infantum*-seropositive species (70%; 7/10) are threatened species, being listed as 'Vulnerable' or with higher conservation risk in the IUCN Red List of Threatened Species (IUCN 2024). In this context, it is relevant to consider that *L. infantum* has caused clinical cases and mortality in endangered mammal species housed in Spanish zoos, including Bennett's wallaby, Eurasian otter (*Lutra lutra*), Iberian wolf, and orangutan. These observations highlight the potential impact of *L. infantum* on conservation programs in zoos, particularly in zoo-housed species living in areas with a high risk of *Leishmania* circulation (Montoya et al. 2016; Miró et al. 2018; Cantos-Barreda et al. 2020; Merino-Goyenechea et al. 2024).

Regarding the SPE results, polyclonal gammopathy was the most common serum pattern detected in *L. infantum*-seropositive animals. This finding is consistent with previous studies conducted in domestic species such as dogs, cats, and

TABLE 3 | Frequency of seropositivity to *Leishmania infantum* in zoo-kept animals in Spain and results of the bivariate analyses.

Variables	Categories	% Seropositivity	No. of positive animals/no. of analysed animals[a]	*p*
Sex	Female	4.5	7/155	0.273
	Male	8.2	13/159	
Age	Adult	6.3	11/174	0.763
	Young	4.3	3/70	
Family	Bovidae	0.0	0/174	< 0.001
	Camelidae	0.0	0/24	
	Canidae	42.1	8/19	
	Caviidae	0.0	0/24	
	Cervidae	0.0	0/17	
	Elephantidae	0.0	0/6	
	Equidae	0.0	0/22	
	Eupleridae	50.0	1/2	
	Felidae	9.3	7/75	
	Herpestidae	0.0	0/8	
	Hyaenidae	22.2	2/9	
	Hystricidae	0.0	0/3	
	Macropodidae	40.0	4/10	
	Muridae	0.0	0/4	
	Mustelidae	0.0	0/12	
	Procyonidae	0.0	0/1	
	Suidae	0.0	0/8	
	Ursidae	0.0	0/8	
	Viverridae	0.0	0/3	
Sampling year	2007	0.0	0/6	0.894
	2008	0.0	0/14	
	2009	7.7	1/13	
	2010	4.0	1/25	
	2011	6.3	1/16	
	2012	0.0	0/8	
	2013	7.1	1/14	
	2014	8.3	1/12	
	2015	7.1	1/14	
	2016	7.7	2/26	
	2017	4.3	2/46	
	2018	2.4	1/41	
	2019	10.3	4/39	
	2020	3.7	1/27	
	2021	4.7	2/43	
	2022	2.4	1/42	
	2023	25.0	1/4	

[a]Missing values omitted.

TABLE 4 | Results of the multivariate model evaluating potential risk factors associated with *Leishmania infantum* seropositivity in zoo-kept animals. Pairwise Tukey post hoc comparisons among levels of the variables retained in the GLM are shown.

Variable	Categories	OR	95% CI	*p*
Family	Canidae–Felidae	7.4	2.2–24.4	0.012
	Canidae–Eupleridae	1.4	0.1–25.4	0.999
	Canidae–Hyaenidae	2.6	0.4–15.7	0.852
	Canidae–Macropodidae	1.1	0.2–5.2	1.000
	Eupleridae–Felidae	9.7	0.6–17.9	0.531
	Eupleridae–Hyaenidae	3.5	0.2–84.8	0.939
	Eupleridae–Macropodidae	1.5	0.1–31.6	0.999
	Hyaenidae–Felidae	2.8	0.5–16.0	0.785
	Macropodidae–Felidae	6.5	1.5–28.6	0.098
	Macropodidae–Hyaenidae	2.3	0.3–17.5	0.924

ferrets (Paltrinieri et al. 2016; Savioli et al. 2021; Villanueva-Saz, Giner, Fernández, et al. 2022; Giner et al. 2024). In these species, polyclonal gammopathy was associated with later stages of *Leishmania* spp. infection, suggesting disease progression (de Carvalho et al. 2024). Moreover, five animals presented an alpha-2 globulins increased peak, which could be related to active *L. infantum* infection (Villanueva-Saz, Giner, Fernández, et al. 2022). However, the results obtained in the SPE must be cautiously interpreted since infectious and/or inflammatory diseases other than leishmaniosis can also alter the serum protein profile (Fernandez-Gallego et al. 2020). In this regard, other complementary diagnostic techniques such as molecular methods or cytological examination should be contemplated to confirm *L. infantum* infection in zoo-kept animals and consequently evaluate its association with SPE results. Moreover, the absence of reference values for the species analysed should be considered when interpreting the results from SPE.

The result of the longitudinal study showed that three Iberian wolves from the same zoo seroconverted during the study period, two between 2017 and 2019 and one between 2018 and 2019. This finding suggests an active circulation of *L. infantum* in this zoo during those years. This hypothesis is supported by the confirmation of *L. infantum* infection in a Barbary macaque and a De Brazza's monkey (*Cercopithecus neglectus*) in 2018 and 2019 at the same zoo, respectively (Barbero-Moyano et al. 2024).

Risk factor analysis showed that *L. infantum* seroprevalence was 7.4 times higher in animals belonging to the family Canidae than in those of the family Felidae. This result aligns with previous studies describing the pivotal role of domestic and wild canids as reservoirs of *L. infantum* (Azami-Conesa et al. 2021; Vilas-Boas et al. 2024). Our findings are consistent with the particular immune response of species of the family Canidae to *L. infantum*, which is characterised by the predominance of a non-protective antibody response favouring disease progression (Solano-Gallego et al. 2001; García-Castro et al. 2022). In contrast, previous studies suggest that Felidae develops a lower production of antibodies against *L. infantum* infection compared to dogs, which would explain the lower seroprevalence obtained in the present survey (Priolo et al. 2019, 2022).

5 | Conclusion

The present large-scale serosurvey evidences a moderate, widespread and endemic circulation of *L. infantum* in zoo-kept animals in mainland Spain. Our results suggest the potential of SPE as a useful tool for monitoring and diagnosing leishmaniosis in *L. infantum*-susceptible zoo-kept species, complementing the results provided by serological methods. Surveillance programs and control measures against *Leishmania* spp., particularly focused on sand fly control, should be implemented in order to reduce the circulation of this protozoan in zoological institutions, and consequently its potential implications for animal and public health, particularly in leishmaniosis hotspot areas.

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Ethics Statement

All samples included in the present study came from serum banks or animals subjected to health programs, surgical interventions, or medical check-ups during the study period. Therefore, no ethical approval was necessary.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

Akhoundi, M., K. Kuhls, A. Cannet, et al. 2016. "A Historical Overview of the Classification, Evolution, and Dispersion of *Leishmania* Parasites and Sandflies." *PLoS Neglected Tropical Diseases* 10, no. 3: e0004349. https://doi.org/10.1371/journal.pntd.0004349.

Almeida, J. C., R. P. B. Melo, P. C. P. Kim, et al. 2018. "Molecular and Serological Investigation of Infectious Diseases in Captive and Free-Range Crab-Eating Fox (*Cerdocyon thous* – Linnaeus, 1776) From

Northeastern Brazil." *Acta Parasitologica* 63, no. 1: 184–189. https://doi.org/10.1515/ap-2018-0021.

Arce, A., A. Estirado, M. Ordobas, et al. 2013. "Re-Emergence of Leishmaniasis in Spain: Community Outbreak in Madrid, Spain, 2009 to 2012." *Eurosurveillance* 18, no. 30: 20546. https://doi.org/10.2807/1560-7917.ES2013.18.30.20546.

Azami-Conesa, I., M. T. Gómez-Muñoz, and R. A. Martínez-Díaz. 2021. "A Systematic Review (1990–2021) of Wild Animals Infected With Zoonotic *Leishmania*." *Microorganisms* 9, no. 5: 1101. https://doi.org/10.3390/microorganisms9051101.

Barbero-Moyano, J., R. Martínez, M. Gonzálvez, et al. 2024. "Monitoring of *Leishmania infantum* in Captive Non-Human Primates in Spain." *Research in Veterinary Science* 180: 105425. https://doi.org/10.1016/j.rvsc.2024.105425.

Basurco, A., A. Natale, K. Capello, et al. 2020. "Evaluation of the Performance of Three Serological Tests for Diagnosis of *Leishmania infantum* Infection in Dogs Using Latent Class Analysis." *Revista Brasileira de Parasitologia Veterinária* 29: e018020. https://doi.org/10.1590/S1984-29612020105.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67: 1–48. https://doi.org/10.18637/jss.v067.i01.

Bauer, B. U., M. E. Lebrero, M. Ganter, et al. 2024. "First Evidence of *Leishmania infantum* Antibodies in Sheep (*Ovis aries*) From Southern Germany." *Animals* 14, no. 13: 1860. https://doi.org/10.3390/ani14131860.

Cantos-Barreda, A., R. Navarro, L. Pardo-Marín, et al. 2020. "Clinical Leishmaniosis in a Captive Eurasian Otter (*Lutra lutra*) in Spain: A Case Report." *BMC Veterinary Research* 16, no. 1: 312. https://doi.org/10.1186/s12917-020-02509-x.

Cardoso, L., H. Schallig, M. F. Persichetti, and M. G. Pennisi. 2021. "New Epidemiological Aspects of Animal Leishmaniosis in Europe: The Role of Vertebrate Hosts Other Than Dogs." *Pathogens* 10, no. 3: 307. https://doi.org/10.3390/pathogens10030307.

Carvalho, B. M., C. Maia, O. Courtenay, et al. 2024. "A Climatic Suitability Indicator to Support *Leishmania infantum* Surveillance in Europe: A Modelling Study." *Lancet Regional Health* 43: 100971. https://doi.org/10.1016/j.lanepe.2024.100971.

de Carvalho, C. A., R. M. Hiramoto, L. R. Meireles, and H. F. de Andrade Jr. 2024. "Understanding Hypergammaglobulinemia in Experimental or Natural Visceral Leishmaniasis." *Parasite Immunology* 46, no. 1: e13021. https://doi.org/10.1111/pim.13021.

Didkowska, A., V. Martín-Santander, M. Wojciechowska, et al. 2024. "Serologic Evidence of Exposure to *Leishmania infantum* in Captive and Free-Ranging European Bison (*Bison bonasus*) in Poland, 2017–23." *Journal of Wildlife Diseases* 61, no. 1: 253–257. https://doi.org/10.7589/JWD-D-24-00032.

Fernandez-Gallego, A., L. Feo Bernabe, A. Dalmau, et al. 2020. "Feline Leishmaniosis: Diagnosis, Treatment and Outcome in 16 Cats." *Journal of Feline Medicine and Surgery* 22, no. 10: 993–1007. https://doi.org/10.1177/1098612X20902865.

Fox, J., and S. Weisberg. 2018. *An R Companion to Applied Regression*. SAGE Publications.

Gálvez, R., A. Montoya, I. Cruz, et al. 2020. "Latest Trends in *Leishmania infantum* Infection in Dogs in Spain, Part I: Mapped Seroprevalence and Sand Fly Distributions." *Parasites & Vectors* 13, no. 1: 204. https://doi.org/10.1186/s13071-020-04081-7.

García-Castro, A., A. Egui, M. C. Thomas, and M. C. López. 2022. "Humoral and Cellular Immune Response in Asymptomatic Dogs With Visceral Leishmaniasis: A Review." *Vaccine* 10, no. 6: 947. https://doi.org/10.3390/vaccines10060947.

Giner, J., M. E. Lebrero, D. López-Sahuquillo, et al. 2024. "Hyperbetaglobulinemia, Anaemia and Thrombocytopenia in a Domestic Ferret (*Mustela putorius furo*) Associated to *Leishmania infantum*." *Research in Veterinary Science* 166: 105096. https://doi.org/10.1016/j.rvsc.2023.105096.

Giner, J., S. Villanueva-Saz, A. Fernández, et al. 2021. "Detection of Anti–*Leishmania infantum* Antibodies in Wild European and American Mink (*Mustela lutreola* and *Neovison vison*) From Northern Spain, 2014–20." *Journal of Wildlife Diseases* 58, no. 1: 198–204. https://doi.org/10.7589/JWD-D-21-00027.

Gonzálvez, M., M. R. R. de Ybáñez, J. M. Rodríguez-Linde, E. Berriatua, J. Risueño, and J. Ortiz. 2017. "El Papel de los Centros Zoológicos Como Reservorios de Leishmaniosis en áreas Urbanas." *Anales de Veterinaria de Murcia* 33: 27–36.

Iatta, R., A. Zatelli, P. Laricchiuta, et al. 2020. "*Leishmania infantum* in Tigers and Sand Flies From a Leishmaniasis-Endemic Area, Southern Italy." *Emerging Infectious Diseases* 26, no. 6: 1311–1314. https://doi.org/10.3201/eid2606.191668.

IUCN. 2024. "The IUCN Red List of Threatened Species." https://www.iucnredlist.org/en.

Luppi, M. M., M. C. C. Malta, T. M. A. Silva, et al. 2008. "Visceral Leishmaniasis in Captive Wild Canids in Brazil." *Veterinary Parasitology* 155, no. 1: 146–151. https://doi.org/10.1016/j.vetpar.2008.04.024.

Maia, C., C. Conceição, A. Pereira, et al. 2023. "The Estimated Distribution of Autochthonous Leishmaniasis by *Leishmania infantum* in Europe in 2005–2020." *PLoS Neglected Tropical Diseases* 17, no. 7: e0011497. https://doi.org/10.1371/journal.pntd.0011497.

Merino-Goyenechea, J., J. Palacios-Alberti, T. Yanes-Martínez, M. Martínez-Valladares, and R. Balaña-Fouce. 2024. "Treatment and Healing of Leishmaniasis in a Wolf in Semi-Captivity Regime From an Educational Center of Zamora Province (Spain)." *Animals* 14, no. 10: 1436. https://doi.org/10.3390/ani14101436.

Miró, G., A. Troyano, A. Montoya, et al. 2018. "First Report of *Leishmania infantum* Infection in the Endangered Orangutan (*Pongo pygmaeus pygmaeus*) in Madrid, Spain." *Parasites & Vectors* 11, no. 1: 185. https://doi.org/10.1186/s13071-018-2772-1.

Montoya, A., L. P. de Quadros, M. Mateo, et al. 2016. "*Leishmania infantum* Infection in Bennett's Wallabies (*Macropus rufogriseus rufogriseus*) in a Spanish Wildlife Park." *Journal of Zoo and Wildlife Medicine* 47, no. 2: 586–593. https://doi.org/10.1638/2014-0216.1.

Muñoz, C., J. de la Martínez- Puente, J. Figuerola, et al. 2019. "Molecular Xenomonitoring and Host Identification of *Leishmania* Sand Fly Vectors in a Mediterranean Periurban Wildlife Park." *Transboundary and Emerging Diseases* 66, no. 6: 2546–2561. https://doi.org/10.1111/tbed.13319.

Paltrinieri, S., L. Gradoni, X. Roura, A. Zatelli, and E. Zini. 2016. "Laboratory Tests for Diagnosing and Monitoring Canine Leishmaniasis." *Veterinary Clinical Pathology* 45, no. 4: 552–578. https://doi.org/10.1111/vcp.12413.

Priolo, V., D. Ippolito, K. Rivas-Estanga, C. De Waure, and P. Martínez-Orellana. 2024. "Canine Leishmaniosis Global Prevalence Over the Last Three Decades: A Meta-Analysis and Systematic Review." *Comparative Immunology, Microbiology and Infectious Diseases* 112: 102211. https://doi.org/10.1016/j.cimid.2024.102211.

Priolo, V., P. Martínez-Orellana, M. G. Pennisi, et al. 2019. "*Leishmania infantum*-Specific IFN-γ Production in Stimulated Blood From Cats Living in Areas Where Canine Leishmaniosis Is Endemic." *Parasites & Vectors* 12, no. 1: 133. https://doi.org/10.1186/s13071-019-3386-y.

Priolo, V., P. Martínez-Orellana, M. G. Pennisi, et al. 2022. "*Leishmania infantum* Specific Humoral and Cellular Immune Responses in Cats and Dogs: A Comparative Cross-Sectional Study." *Veterinary Sciences* 9, no. 9: 482. https://doi.org/10.3390/vetsci9090482.

R Core Team. 2023. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.r-project.org.

Riera, C., J. E. Valladares, M. Gállego, et al. 1999. "Serological and Parasitological Follow-Up in Dogs Experimentally Infected With *Leishmania infantum* and Treated With Meglumine Antimoniate." *Veterinary Parasitology* 84, no. 1: 33–47. https://doi.org/10.1016/S0304-4017(99)00084-9.

Ruiz, H., J. Serra, A. Fernández, et al. 2023. "O-083 Clinical Leishmaniosis due to *Leishmania infantum* in a Goat: Clinical Findings and Treatment Response." *Animal—Science Proceedings* 14: 117. https://doi.org/10.1016/j.anscip.2023.01.159.

Sastre, N., O. Francino, O. Ramírez, C. Enseñat, A. Sánchez, and L. Altet. 2008. "Detection of *Leishmania infantum* in Captive Wolves From Southwestern Europe." *Veterinary Parasitology* 158, no. 1: 117–120. https://doi.org/10.1016/j.vetpar.2008.08.008.

Savioli, G., J. Archer, E. Brianti, et al. 2021. "Serum Amyloid A Levels and Alpha 2 and Gamma Globulins on Serum Protein Electrophoresis in Cats Exposed to and Infected With *Leishmania infantum*." *Parasites & Vectors* 14, no. 1: 217. https://doi.org/10.1186/s13071-021-04710-9.

Solano-Gallego, L., C. Riera, X. Roura, et al. 2001. "*Leishmania infantum*-Specific IgG, IgG1 and IgG2 Antibody Responses in Healthy and Ill Dogs From Endemic Areas." *Veterinary Parasitology* 96, no. 4: 265–276. https://doi.org/10.1016/S0304-4017(00)00446-5.

Thrusfield, M., R. Christley, H. Brown, et al. 2018. *Veterinary Epidemiology*. 4th ed. Wiley-Blackwell.

Tolentino, N., G. R. G. Pinheiro, J. Ottino, et al. 2019. "Serological Evidence of *Leishmania* Infection by Employing ELISA and Rapid Tests in Captive Felids and Canids in Brazil." *Veterinary Parasitology: Regional Studies and Reports* 17: 100308. https://doi.org/10.1016/j.vprsr.2019.100308.

Vilas-Boas, D. F., E. K. N. Nakasone, A. A. M. Gonçalves, et al. 2024. "Global Distribution of Canine Visceral Leishmaniasis and the Role of the Dog in the Epidemiology of the Disease." *Pathogens* 13, no. 6: 455. https://doi.org/10.3390/pathogens13060455.

Villanueva-Saz, S., M. d. C. Aranda, M. d. l. Á. Jiménez, et al. 2024. "Serum Protein Electrophoresis in European Mink (*Mustela lutreola*): Reference Intervals and Comparison of Agarose Gel Electrophoresis and Capillary Zone Electrophoresis." *Veterinary Quarterly* 44, no. 1: 1–11. https://doi.org/10.1080/01652176.2024.2318195.

Villanueva-Saz, S., J. Giner, A. Fernández, et al. 2022. "Serum Protein Electrophoretogram Profile Detected in Apparently Healthy Cats Infected With *Leishmania infantum* – Short Communication." *Acta Veterinaria Hungarica* 70, no. 1: 30–34. https://doi.org/10.1556/004.2021.00055.

Villanueva-Saz, S., J. Giner, A. P. Tobajas, et al. 2022. "Serological Evidence of SARS-CoV-2 and Co-Infections in Stray Cats in Spain." *Transboundary and Emerging Diseases* 69, no. 3: 1056–1064. https://doi.org/10.1111/tbed.14062.

Villanueva-Saz, S., J. Giner, M. Verde, et al. 2021. "Antibodies to *Leishmania* in Naturally Exposed Domestic Ferrets (*Mustela putorius furo*) in Spain." *Veterinary Parasitology* 296: 109492. https://doi.org/10.1016/j.vetpar.2021.109492.

Villanueva-Saz, S., M. E. Lebrero, A. Solsona, et al. 2024. "Presence of Anti-*Leishmania infantum* Antibodies in Sheep (*Ovis aries*) in Spain." *Veterinary Research Communications* 48, no. 1: 615–621. https://doi.org/10.1007/s11259-023-10221-y.

Villanueva-Saz, S., E. Pérez, A. Yzuel, A. Gomez, and M. T. Verde Arribas. 2024. "Proliferative Papulo-Nodular Glossitis due to *Leishmania infantum* in a Dog." *Veterinary Record Case Reports* 12, no. 1: e779. https://doi.org/10.1002/vrc2.779.

WHO. 2024. "Leishmaniasis." https://www.who.int/data/gho/data/themes/topics/gho-ntd-leishmaniasis.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Seropositivity to *Leishmania infantum* in zoo species sampled in zoo parks from Spain.