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Original Article

Vector-borne pathogens in Spanish greyhounds from Central Spain: Prevalence and hematobiochemical findings

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ABSTRACT

Vector-borne diseases are receiving increasing attention in public health, veterinary medicine, and epidemiological research. In this study, we investigated the seroprevalence of four major canine vector-borne pathogens in Spanish greyhounds, a breed reported to possess distinctive hematological and biochemical profiles. Serum samples from 160 dogs, originating from central Spain, were evaluated for antibodies against *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Leishmania infantum* using an indirect immunofluorescence assay (IFA), and for circulating *Dirofilaria immitis* antigens using ELISA. Comprehensive hematological and serum biochemical analyses were also performed. The overall seroprevalence rates were 10% for *A. phagocytophilum*, 3.1% for *E. canis*, 13.1% for *L. infantum* and 0% for *D. immitis*. A statistically significant association was identified between age and *E. canis* seropositivity, with higher rates observed in juvenile dogs, whereas *L. infantum* demonstrated a non-significant trend toward higher prevalence in adults. Seropositive dogs showed notable alterations in hematological and biochemical parameter, including thrombocytopenia, lymphocytosis/lymphopenia, anemia, and variations in platelet indices. These findings highlight the epidemiological relevance of serological screening and detailed clinical monitoring particularly given the breed's frequent involvement in hunting and prolonged exposure to outdoor environments conducive to vector transmission.

1. Introduction

Vector-borne diseases (VBDs) represent one of the most formidable challenges in both veterinary and human medicine owing to their global prevalence, pathogenic impact, and zoonotic potential (Beugnet and Marié, 2009; Mencke, 2013; Otranto et al., 2013). The increasing expansion of arthropod vectors such as ticks, mosquitoes, and sand flies, linked to climatic, ecological, and anthropogenic factors, as well as enhancer international transport and trade of animals and reservoir hosts, has resulted in sustained persistence, geographic expansion, and re-emergence of diseases that significantly affect animal and human health worldwide (Fooks and Johnson, 2014; Semenza and Suk, 2018). In dogs, several VBDs are particularly relevant, including *anaplasmosis, ehrlichiosis, leishmaniosis, and dirofilariosis* caused by pathogens capable of inducing wide spectrum clinical syndromes, ranging from subclinical infections to severe systemic illnesses, often characterized by marked hematological and biochemical disturbances (de Caprariis et al., 2011).

The Spanish greyhound is a native breed traditionally used for hunting hares in rural environments of the Iberian Peninsula and is regarded as physiologically distinct from other breeds (Zaldívar-López et al., 2011). Owing to their outdoor lifestyle and constant exposure to vectors, these dogs can serve as a sentinel population for monitoring VBDs in endemic regions. In addition to this breed's epidemiological relevance, several authors have reported hematological differences compared with other breeds. Spanish greyhounds present higher hematocrit, hemoglobin, and erythrocyte counts, along with lower platelet counts and larger red cell volumes (Mesa-Sánchez et al., 2012; Spada et al., 2015). These breed-specific peculiarities complicate clinical interpretation, as pathological changes may overlap with normal physiological ranges. Establishing breed-specific reference intervals is therefore crucial for an accurate diagnostic assessment (Kovarikova et al., 2024; Zaldívar-López et al., 2011).

Anaplasmosis in dogs is primarily caused by *A. phagocytophilum*, transmitted by *Ixodes* ticks (El Hamiani Khatat et al., 2021; Fourie et al., 2019). In Europe, the infection is considered underdiagnosed, although seroprevalence studies indicate notable level of exposure in

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Mediterranean regions (Dondi et al., 2014). Miró et al. (2022) reported rates ranging from 5.3% to 7.3%, while Montoya-Alonso et al. (2020) observed a seroprevalence of 4.26% in Spain.Ehrlichiosis, caused by *E. canis* and transmitted by the brown dog tick *Rhipicephalus sanguineus*, is considered one of the most clinically relevant vector-borne diseases in dogs (Groves et al., 1975; Sainz et al., 2015). In Europe, reported seroprevalence rates are relatively low, ranging from 3.4% to 4.3% (Miró et al., 2022), and in Spain from 3.4% (Montoya-Matute et al., 2025) to 4.26% (Montoya-Alonso et al., 2020).

Leishmaniosis, caused by *L. infantum* and transmitted by *Phlebotomus* sand flies, is one of the most prevalent zoonotic diseases in Mediterranean countries, where dogs are the main domestic reservoir for human visceral leishmaniosis (Pennisi, 2015; Ready, 2013).

In Europe, reported seroprevalence varies widely between countries and diagnostic techniques (Miró et al., 2022). In Spain, prevalence rates are highly heterogeneous from region to region, ranging from 2% to 57.1% (Vilas-Boas et al., 2024), with the most recent study reporting an average seroprevalence of *L. infantum* of 17.3% (Montoya-Matute et al., 2025).

The parasite *Dirofilaria inmitis* causes cardiopulmonary dirofilariasis and has a complex heteroxenous life cycle, requiring mosquito as intermediate host for its development. Species of *Culex*, *Aedes*, *Ochlerotatus*, and *Anopheles*, act as vectors, facilitating transmission to mammalian hosts (McCall et al., 2008; Simón et al., 2012). In Europe, reported seroprevalence rates in dogs range from 1.8% to 2.7% (Miró et al., 2022), while in Spain an overall seroprevalence of 6.25% has been estimated, with regional values generally below 10% except in the Basque Country (Montoya-Alonso et al., 2020); more recent data indicate a decline to a national seroprevalence of 3.2% (Montoya-Matute et al., 2025). Climate change, the increased movement of companion animals, and vector dispersal have all contributed to the widening of geographical spread of this parasite (Genchi et al., 2014).

The epidemiological situation of VBDs in Spain is associated with a wide range of risks, stemming from substantial ecological and climatic variability, directly influencing vector distribution. Central Spain, where most Spanish greyhounds are bred and used for hunting, constitutes an area of intense exposure to multiple vectors (Beugnet and Marié, 2009; Mencke, 2013). Considering the unique hematological profile of the Spanish greyhound and its high-risk outdoor lifestyle, this breed is an ideal population for evaluating the relationship between infection, clinical manifestations, and laboratory abnormalities.

In this context, the objectives of the present study were: (i) to determine the seroprevalence of *A. phagocytophilum*, *E. canis*, *L. infantum*, and *D. immitis* in Spanish greyhounds; (ii) to evaluate hematological and biochemical alterations associated with seropositivity; (iii) to investigate whether the distinctive hematological characteristics of Spanish greyhounds impinge upon the clinical or laboratory expression of these infections, and (iv) to assess age-related differences in infection patterns. This research aims to contribute to the understanding of VBDs in a particularly exposed canine breed, with implications for veterinary diagnostics, clinical strategies, and public health surveillance.

2. Materials and methods

2.1. Study population

The study population consisted of 160 Spanish greyhounds managed by a non-profit organisation from Madrid (central Spain), which is dedicated, among other activities, to the rescue and rehoming of greyhounds originating from central Spain. These dogs had been rescued, after being used for hunting and living outdoors. Samples and clinical data were collected between September 2016 and February 2018 during routine pre-adoption health evaluations of clinically healthy dogs performed in collaborating veterinary clinics, prior to national or international adoption. This is a convenience sample of dogs presented for routine pre-adoption health checks; within each clinic, dogs were enrolled consecutively whenever a serum sample was available. Inclusion criteria were restricted to the greyhound breed and the availability of a serum sample with basic signalment data. The dogs' age ranged from 10 months to 13 years (age data were missing for 12 individuals). Information on age was recorded when provided by the rescue organisation or adopters, whereas sex, previous treatments, housing conditions and intensity of work prior to rescue were not systematically registered and could not be analyzed.

Minimum sample size was estimated using the WinEpi 2.0 online tool (proportion estimation option) (http://www.winepi.net/winepi2, Vallejo et al., 2013). Due to absence of published prevalence data for greyhounds, expected prevalence values were based on studies from the general canine population in Spain: *E. canis* (3.4%), *D. immitis* (3.2%), *L. infantum* (17.3%) (Montoya-Matute et al., 2025), and *A. phagocytophilum* (3.1%) (Miró et al., 2013). With a 95% confidence level and 5% margin of error, the required minimum sample sizes were 51, 48, 220, and 47 animals, respectively.

2.2. Sample collection

Blood samples were obtained via cephalic or jugular venipuncture under standard aseptic conditions. Two aliquots were prepared: one with EDTA for hematological analysis and another without anticoagulant for serum separation. The latter was obtained by centrifugation of blood samples at 1500–2000 ×g for 10 min. Samples were stored at 4 °C and processed within 24 h of collection. All serological, biochemical, and hematological analyses were performed at Laboratorios Albéitar (Zaragoza, Spain).

2.3. Serological analysis

Antibodies against *A. phagocytophilum*, *E. canis*, and L. *infantum* were detected using indirect immunofluorescence assay (IFA) following the manufacturer's instructions included in the commercial kit (MEGACOR Diagnostik GmbH, Hörbranz, Austria). Briefly, antigen-coated slides were incubated with serum samples diluted according to the recommended working concentrations. After washing, fluorescein isothiocyanate (FITC)-conjugated anti-dog IgG was added as the secondary antibody. Slides were examined under a fluorescence microscope, and samples displaying specific cytoplasmic or membrane associated fluorescence patterns were considered positive.

Detection of circulating *D. immitis* antigens was performed using a commercial enzyme-linked immunosorbent assay (ELISA; VetLine Dirofilaria, NovaTec, Germany). Serum samples were added to wells coated with monoclonal antibodies against *D. immitis* antigens. Following incubation and washing, enzyme conjugate and chromogenic substrate solutions were added sequentially. Optical density was measured at 450 nm using a microplate reader, and results were interpreted according to the manufacturer's guidelines.

2.4. Hematological analysis

Complete blood counts (CBCs) were performed on EDTA samples using an automated hematology analyzer (SYSMEX XN-1000 V, Sysmex Corporation, Japan). We evaluated the following parameters: red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), white blood cell count (WBC), and differential leukocyte counts (lymphocytes, monocytes, neutrophils, eosinophils, basophils). Greyhounds are known to have higher RBC and Hct values and lower PLT counts compared to other breeds (Mesa-Sánchez et al., 2012; Zaldívar-López et al., 2011). These physiological characteristics were considered when interpreting deviations from reference limits.

2.5. Biochemical analysis

Serum biochemical parameters were measured using an automated biochemistry analyzer (BA 400 Byosistem, Spain). The following analytes were quantified: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, creatinine, urea, and total protein concentration. Hyperproteinemia and azotemia were interpreted as markers of potential renal involvement associated with leishmaniosis, whereas elevated liver enzymes were considered indicative of possible hepatocellular disturbance.

2.6. Statistical analysis

Prevalence values were calculated with 95% confidence intervals (CIs) using the VassarStats web-based statistical package (http://vassarstats.net/). Associations between age, hematological/biochemical indices, and pathogen positivity were evaluated using categorized variables. Age was grouped into two categories: puppies (0–3 years) and adults (>3 years). Infection status was classified as positive or negative, and hematological/biochemical indices were categorized as low/normal/high relative to reference ranges. Depending on distribution characteristics, either the chi-square test of independence or Fisher's exact test was applied. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS software (version 29, IBM Corp., Armonk, NY, USA).

3. Results

3.1. Overall seroprevalence

Among the 160 Spanish greyhounds included in this study, 38 (23.8%) tested seropositive for at least one of the pathogens analyzed. Four animals (2.5%) showed evidence of coinfection with more than one agent: two were seropositive for *A. phagocytophilum*, *Ehrlichia canis,* and *L. infantum*, and two for *A. phagocytophilum* and *L. infantum*.

Table 1 summarizes the seroprevalence rates of the four pathogens. *Leishmania infantum* was the most frequently detected agent (13.1%), followed by *A. phagocytophilum* (10%). *Ehrlichia canis* was detected in 3.1% individuals, and no cases of *D. immitis* were identified.

3.2. Age-related differences

In our age-related analysis, we excluded twelve animals due to missing age data; therefore, the analyses were based on remaining 148 individuals. A higher proportion of seropositivity was observed in puppies compared to adults for both *A. phagocytophilum* and *E. canis*, with the difference reaching statistical significance for *E. canis* ($p = 0.008$). In contrast, for *L. infantum*, we observed a reverse trend, with a higher proportion of positives among adults, although this difference was not statistically significant ($p = 0.27$) (Table 2).

Table 1
Seroprevalence of vector-borne pathogens in Spanish greyhounds ($n = 160$).

Pathogen	Prevalence (%)	95% CI
Anaplasma phagocytophilum	10.0	5.8–15.7
Ehrlichia canis	3.1	0.4–5.8
Leishmania infantum	13.1	8.3–19.4
Dirofilaria immitis	0.0	–
Co-infection: *A. phagocytophilum* and *L. infantum*	1.2	0.2–4.9
Co-infection: *A. phagocytophilum, E. canis* and *L. infantum*	1.2	0.2–4.9

3.3. Hematological and biochemical alterations

Table S1 summarizes the principal hematological abnormalities detected in terms of seropositivity, and Table S2 details their associations with hematological/biochemical parameters as determined by chi-square or Fisher's exact tests, as appropriate.

Lymphocyte and platelet counts were significantly associated with positivity for *A. phagocytophilum* ($p < 0.05$). Positive animals exhibited a higher frequency of altered lymphocyte counts (both increased and decreased) compared to negatives, and thrombocytopenia was more common among positive animals. We found non-significant trends for mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). No other hematological or biochemical parameters differed significantly between groups, in relation to *A. phagocytophilum*.

Platelet distribution width (PDW) was significantly associated with positivity for *E. canis*, and one of the five seropositive animals showed altered platelet volume distribution. We observed a non-significant trend for MCV; however, no other hematological, biochemical, or cellular parameters differed significantly between groups in relation to *E. canis*.

Regarding L. *infantum*, positive animals showed significant reductions in red blood cell (RBC) counts, as well as significant associations with mean platelet volume (MPV), platelet distribution width (PDW), serum protein concentrations, and alterations in lymphocyte counts. We did not detect any statistically significant differences for hemoglobin (Hb), mean corpuscular hemoglobin (MCH/MCHC), transaminases (ALT, AST), or urea levels.

4. Discussion

This study provides novel insights into VBDs in the Spanish greyhound, a native breed with distinctive physiological traits and an outdoor lifestyle that increases its risk of exposure to arthropod vectors. Our results reveal a substantial degree of occurrence of L. *infantum* and *A. phagocytophilum*, a lower prevalence of *E. canis*, and no cases of *D. immitis*. These findings highlight the epidemiological sentinel role of the Spanish greyhound in endemic regions of Spain, particularly in central areas where hunting activities and rural exposure are frequent.

The prevalence of *A. phagocytophilum* in our study (10%) is higher than that reported in two other Spanish studies; however, these comparisons warrant caution, as the cited estimates target different species and case definitions. Specifically, the 5.06% prevalence reported by Montoya-Alonso et al. (2020) referred to *A. platys*, while the 3.1% reported by Miró et al. (2013) combined seropositivity to *A. platys* and *A. phagocytophilum*. This difference may also be partially explained by our sample's deliberate limitation to the Spanish greyhound breed, as its traditional use in hunting may entail particularly prolonged exposure to arthropod vectors. The reported prevalence of *A. phagocytophilum* in Europe ranges widely, from 3% to 57% (Sainz et al., 2015). In Portugal, Santos et al. (2009) found a notably high value of 54.5% by IFA, a discrepancy likely driven by study design, as their cohort consisted of dogs already suspected of vector-borne diseases.

Regarding *E. canis*, our results (3.1%) are consistent with those previously reported in Spain by Montoya-Alonso et al. (2020) and Miró et al. (2013), who reported 4.26% and 5%, respectively. On the international level, *E. canis* prevalence varies widely, from 0% in South Korea to 86.9% in India (Kottadamane et al., 2017; Truong et al., 2021). Within Europe, values range from 0.2% in Hungary to 50% in Portugal (Montoya-Matute et al., 2025). Interestingly, Afonso et al. (2024), using ELISA, reported a seroprevalence of 0.9% in Portuguese shelter dogs, contrasting with the 3.1% obtained in our study. Such discrepancies are likely due to both ecological and methodological factors. The very low seroprevalence reported may reflect lower infection pressure in northern Portugal and shelter practices (ectoparasiticides on admission, short or variable length of stay) that limit or obscure tick exposure. In

Table 2
Distribution of Spanish greyhounds by age group (puppy vs. adult) according to serological status for vector-borne pathogens.

Age group	*A. phagocytophilum*		*E. canis*		*L. infantum*		*D. immitis*	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Adult ($N = 91$)	83 (91.2%)	8 (8.8%)	91 (100%)	0	76 (83.5%)	15 (16.5%)	91 (100%)	0
Puppy ($N = 57$)	50 (87.7%)	7 (12.3%)	52 (91.2%)	5 (8.8%)	52 (91.2%)	5 (8.8%)	57 (100%)	0
***P* value**	0.68*		0.008**		0.27*		1**	

*Fisher's exact test; *Chi-square test.

addition, the specific ELISA kit and conservative cut-off, with a further 3.5% of dogs classified as "borderline", may have led to underestimation of true seropositivity. Our results further suggest a possible association between seropositivity and age, in agreement with Kottadamane et al. (2017), who observed a higher prevalence in dogs aged 1–3 years (41.1%) and 3–6 years (32.8%), compared with lower values in older dogs (>6 years; 12.3%) and younger ones (<1 year; 13.7%). Afonso et al. (2024) similarly reported lower seropositivity in dogs less than one year old, although that difference was not statistically significant. In the context of Spanish greyhounds, that finding may be explained by variability in tick control practices during the first months after birth, together with the greater vulnerability of puppies' immature immune system to new infections.

Although both *A. phagocytophilum* and *E. canis* are tick-borne pathogens, their main vectors and transmission cycles differ. *Anaplasma phagocytophilum* is primarily transmitted by Ixodes spp. associated with wildlife reservoirs and rural habitats, whereas *E. canis* relies mainly on *Rhipicephalus sanguineus* and infected dogs (Groves et al., 1975; Sainz et al., 2015; Fourie et al., 2019; El Hamiani Khatat et al., 2021). In our hunting greyhound population, these ecological differences, together with tick-control practices in peridomestic environments, may have resulted in higher exposure to *A. phagocytophilum* than to *E. canis*.

For *L. infantum*, our prevalence estimate (13.1%) falls within the range previously reported in Spain: Montoya-Alonso et al. (2020) found 10.36% and Miró et al. (2013) reported 15.7%. Priolo et al. (2024) conducted a meta-analysis of 150 cross-sectional studies spanning 1990 to 2020 and reported a global pooled prevalence of 15.2% for *L. infantum* in dogs, with higher rates observed in rural settings (19.5%) and among owned animals (16.5%). Age-related differences in infection risk have been reported in previous studies, with higher prevalence rates in animals younger than 3 years and older than 8 years (Solano-Gallego et al., 2009). In the latter group, this trend may reflect the cumulative effect of prolonged exposure to sandfly vectors, resulting in an increased likelihood of infection over time. In our sample, there was an apparent increase in prevalence with age, but the association was not statistically significant.

Finally, for *D. immitis*, our findings diverge from those of Montoya-Alonso et al. (2020) (6.25%) and Miró et al. (2013) (1.25%), as we did not detect any seropositive cases in our sample of 160 Spanish greyhounds. This result also contrasts with the global prevalence of 10.91% reported by Anvari et al. (2020), suggesting substantial geographical variability and possible methodological influences on detection rates. A plausible explanation is that our sample originated in central Spain, where heartworm transmission tends to be lower than in coastal or more humid areas with higher mosquito densities and longer transmission seasons (Montoya-Alonso et al., 2020). In addition, antigen-based serology can fail to detect low-burden, male-only, or immune-complexed infections, and the absence of concurrent microfilaria testing may lead researchers to further underestimate prevalence by missing amicrofilaremic cases (Nelson et al., 2025).

Overall, our results confirm considerable variability in the prevalence of canine vector-borne pathogens, influenced by host-related factors (such as breed and age), ecological conditions, and diagnostic methodologies. These findings emphasize the importance of applying standardized approaches in prevalence studies and the need to account for both biological and methodological sources of variation when interpreting seroepidemiological data.

The hematological abnormalities we observed in seropositive dogs were consistent with disease profiles described elsewhere. Thrombocytopenia was the most frequent alteration, particularly associated with *E. canis* infection, aligning with the pathogenesis of canine ehrlichiosis, in which platelet destruction and bone marrow suppression are common (Skotarczak, 2003). Alterations in platelet indices (MPV, PDW) further support the involvement of bone marrow and platelet kinetics in such infections.

Lymphocytic imbalances, including both lymphopenia and lymphocytosis, were observed in dogs positive for *A. phagocytophilum*, consistent with previous reports of immunological dysregulation in canine anaplasmosis (El Hamiani Khatat et al., 2021). Anemia, frequently non-regenerative, was primarily associated with *L. infantum* positivity, reflecting chronic inflammation and bone marrow suppression associated with canine leishmaniosis (Molina et al., 2023). Hyperproteinemia, observed in several *L. infantum*-positive animals, likely reflects polyclonal gammopathy, a hallmark of chronic leishmaniosis (Baxarias et al., 2023). Increased urea levels in some positive dogs suggest early renal involvement, a common finding in advanced disease stages (Dias et al., 2020).

It is important to interpret these alterations in the context of the greyhound's unique physiology. Compared to other breeds, the Spanish greyhound is known to have higher hematocrit and hemoglobin values, as well as lower platelet counts (Mesa-Sánchez et al., 2012; Spada et al., 2015). Without breed-specific reference intervals, pathological changes might be misinterpreted as normal variations. This reinforces the need for veterinary practitioners to consider breed-specific baselines when evaluating laboratory results in greyhounds.

The epidemiological role of the Spanish greyhound extends beyond veterinary concerns. This breed's high mobility, both within Spain and across Europe through adoption programs, may facilitate the spread of VBDs to non-endemic regions (Fooks and Johnson, 2014). In particular, the international adoption of Spanish greyhounds rescued from rural environments increases the risk of introducing pathogens such as *L. infantum* into countries where competent vectors may be present (Wright et al., 2020).

The detection of coinfections in four dogs underscores the complexity of clinical profiles in endemic areas. Simultaneous exposure to multiple pathogens can exacerbate clinical signs, complicate diagnosis, and affect treatment outcomes (Beasley et al., 2021; De Tommasi et al., 2013). This highlights the importance of comprehensive screening protocols in Spanish greyhounds, particularly those intended for adoption or relocation.

Preventive strategies should focus on vector control, prophylactic treatments, and routine screening in high-risk breeds such as this one. The use of repellents and insecticides, vaccination where available (e.g., leishmaniosis vaccines), and regular serological testing are key in reducing disease burden (Baxarias et al., 2022). From a public health perspective, surveillance of the Spanish greyhound breed provides valuable insights into zoonotic risks, as infections such as leishmaniosis and anaplasmosis are transmissible to humans (Montoya-Matute et al., 2025).

Future studies should adopt a longitudinal approach to monitor infection dynamics and clinical outcomes over time. Molecular diagnostic techniques such as PCR could complement serology, providing more accurate information on active infections. Additionally, a larger sample size and the recording of clinical signs would allow for more robust correlations between serological status, laboratory alterations, and clinical disease.

5. Conclusions

In conclusion, our study confirmed that Spanish greyhounds serve as high-risk sentinels' animals for canine vector-borne pathogens in central Spain, with a pathogen profile featuring a relatively high seroprevalence of *L. infantum* and *A. phagocytophilum*, lower for *E. canis*, and no evidence of *D. immitis*. Collectively, these results reveal age-associated patterns of infection and emphasize the need for targeted preventive and surveillance program. Moreover, these findings confirm that hematobiochemical alterations consistent with disease pathophysiology should be interpreted using breed-specific reference intervals to avoid diagnostic misclassification. Finally, the observed coinfections underscore the need for comprehensive screening, particularly prior to adoption or relocation. Overall, this evidence supports the implementation of integrated vector control strategies, vaccination where available, and standardized serological panels to safeguard both animal and public health. Key limitations of this study, including geographically restricted sampling and reliance on serology alone highlight the need for longitudinal investigations incorporating molecular diagnostics, detailed clinical monitoring and expanded population coverage with the goal of refining risk assessment and guiding veterinary decision making and policy development.

CRediT authorship contribution statement

David Martínez-Durán: Writing – review & editing, Writing – original draft, Conceptualization. **Maider Mujika:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Mariano Morales:** Methodology, Investigation. **Juan Antonio Castillo:** Supervision, Conceptualization. **Bernardino Moreno:** Supervision, Conceptualization. **Sarah Delacour-Estrella:** Writing – review & editing. **Ignacio Ruiz-Arrondo:** Writing – review & editing. **María Paz Peris:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation.

Ethics declaration

This study's design complies with the ethical principles and animal protection standards for the use of animals in research and other scientific purposes, including teaching, as established by the University of Zaragoza. Accordingly, it received approval from the University of Zaragoza Advisory Ethics Committee for Animal Experimentation (Comisión Ética Asesora para la Experimentación Animal), reference PD38/24, dated 16 December 2024.

Declaration of competing interest

The authors have no conflicts of interest to declare

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2026.101437.

Data availability

Data will be made available on request.

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