



Hemotropic mycoplasmas and Bartonella spp. in dogs from Southern Italy: prevalence and risk factors

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Abstract

Canine vector-borne diseases (CVBDs) include several zoonoses and represent an important concern where veterinarians play a major role. Although usually responsible for asymptomatic infections in animals and humans, these agents may also have fatal consequences in immunocompromised patients. In this study, different categories of dogs from the Campania region (southern Italy) were investigated for three canine vector-borne bacteria (i.e., *Candidatus Mycoplasma haematoparvum*, *Mycoplasma haemocanis*, and *Bartonella* spp.) by using specific real-time PCR protocols. Out of 187 dogs, 45 (i.e., 24.1%) tested positive for at least one pathogen, with *Candidatus Mycoplasma haematoparvum* (14.4%) being the most prevalent, followed by *Mycoplasma haemocanis* (12.8%) and *Bartonella* spp. (1.6%). Coinfections with more than one agent were observed in 4.8% of the samples. The analysis of risk variables indicated province, activity, and lifestyle as risk factors associated with greater prevalence values. Non-hunting dogs (OR=0.11) showed a significantly lower likelihood of being positive for at least one infection. Living outdoors (OR=3.46), in Salerno province (OR=13), had a higher likelihood of testing positive for at least one hemotropic species. These findings revealed a wide distribution of CVBDs in dog populations of the study area, clearly underlining the necessity for effective efforts to minimize ectoparasite activity.

Keywords CVBDs · Mycoplasma · Candidatus · Bartonella · Vector-borne bacteria

Introduction

Canine vector-borne diseases (CVBDs) represent a challenge where the veterinarians play a major role, especially for zoonotic pathogens (Shapiro et al. 2017). Climate change, vector propagation, and increasing animal movements all contributed to the rapid spread of CVBDs (Ferrara

et al. 2023). The importance of these pathogens is primary considering the animal and human health concerns caused by their circulation and diffusion. While certain CVBDs receive continuing attention and have countrywide surveillance networks, the same is not true for some CVBDs that are deemed minor. Hemotropic mycoplasmas are bacteria that adhere to the surface of red blood cells (Huggins et al. 2022). Typical characteristics of mycoplasmas are that they cannot be cultivated, are small, have no cell wall, and have a relatively small genome (Huggins et al. 2022). The two most frequent species detected in dogs and wild animals (such as foxes, raccoons, lynxes, etc.) are *Mycoplasma haemocanis* (*M. haemocanis*) and *Candidatus Mycoplasma haematoparvum* (*Ca. M. haematoparvum*). Both agents can cause anorexia, lethargy, and hemolytic anemia, whose severity depends on the overall health and the immune system of the host (Sykes et al. 2005; Huggins et al. 2022). The transmission routes are poorly understood and depend primarily on the activity of blood-feeding arthropods, such as, for instance, the brown tick of the dog (*Rhipicephalus sanguineus sensu lato*). However, evidence supporting

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this transmission pathway remains limited, largely due to the inability to culture the microorganisms (Huggins et al. 2023). Given that transmission remains poorly understood, other possible routes of infection might involve the vertical route (dam-pup), fighting (pathogen DNA has been detected in the saliva), and blood transfusion (Huggins et al. 2023). Additionally, canine hemoplasmas have been shown to have a zoonotic potential with the occurrence of central nervous system disease (Dawood et al. 2022).

Similarly, *Bartonella* spp. is a worldwide-spread bacterium that affects companion cats and dogs. Fleas are the main vectors of numerous bacteria of the genus *Bartonella* (Chomel et al. 2006; Zarea et al. 2022). Several species of *Bartonella* are zoonotic, including *Bartonella vinsonii* subsp. *berkhoffi* that is typically found in dogs. Dogs can also harbor other *Bartonella* species, including *B. henselae*, *B. clarridgeiae*, and *B. rochalimae* (Chomel et al. 2006). Dogs can become infected through ingestion of flea feces, blood transfusions, or scratches/bites. *Bartonella* spp. has been described as responsible for splenomegaly, nasal discharge, and epistaxis. In severe cases diaz causes bacillary myocarditis, angiomatosis, endocarditis, granulomatous hepatitis, and lymphadenitis. Transmission of these *Bartonella* spp. to humans, mostly through cat scratches contaminated with infected flea stool, can cause a variety of clinical symptoms, whose severity is higher in immunocompromised individuals (Chomel et al. 2006).

The absence of specific clinical signs contributes to the difficulty of diagnosing these CVBDs without laboratory support. PCR protocols are the preferred diagnostic methods for canine hemoplasma and *Bartonella* spp. infection because they have higher analytical sensitivity and specificity, allowing for bacterial detection, particularly during chronic infection, as well as species identification (Roblejo-Arias et al. 2022).

Although hemotropic bacteria have been documented in hunting dogs, there is still little information about these pathogens in southern Italy (Cortese et al. 2020). The aim of this study was to evaluate the prevalence of hemoplasmas and *Bartonella* spp. in different categories of dogs, evaluating the potential risk factors correlated with the infection.

Materials and methods

Study area and sampling

This study was carried out in the Campania region, southern Italy, in an urban area. This area is located between the Tyrrhenian Sea in the southwest and the southern Apennines in the northeast and has a Mediterranean climate. Evidence of other CVBDs has been described in this area

in both wild and domestic canids (Minichino et al. 2025). This region is divided into five provinces (Naples, Salerno, Benevento, Avellino, and Caserta). In the study area there were approximately more than one million owned dogs (of which a small percentage were hunting dogs) and half a million stray dogs housed in public kennels. Several species of dog fleas (i.e., *Ctenocephalides canis* and *C. felis*) and ticks (i.e., *R. sanguineus* s. l., *Ixodes ricinus*, *Ixodes hexagonus*) have been reported in this area (Maurelli et al. 2018). The sample size was determined using Thrusfield's method for a theoretically "infinite" population and included the following information: estimated prevalence of hemoplasma based on previous research (10%), 95% confidence interval (CI), and required absolute precision (5%) (Cortese et al. 2020; Ravagnan et al. 2017). A random sampling was performed in three different animal groups (i.e., stray dogs, pet dogs, and hunting dogs) (Ferrara et al. 2024b). A whole blood sample (with K₃EDTA) of 5 mL was collected from a total of 77 stray dogs, 50 hunting dogs, and 60 pet dogs. Periodic and constant use of anti-ectoparasites was reported for all dogs through a questionnaire. All samples were collected from clinically healthy animals during the 2023 vector season (April-September). Recent data shows that vectors are most active in the months mentioned for the study region, although their presence has been documented throughout the year (SgROI et al. 2022). All samples were stored under cold chain conditions (± 4 °C) and delivered to the Department of Veterinary Medicine and Animal Production in Naples. Blood samples were kept at -80 °C before DNA extraction. A questionnaire survey was run to collect information about each sampled animal, including sex, breed, size, province, age, origin, and lifestyle (Ferrara et al. 2024a, c). Dogs weighing less than 15 kg are classified as small, 15 to 25 kg as medium, and 25 kg or more as large. The age of stray animals was estimated using a method described in the literature and the date of arrival at the shelter as a reference (Roccaro and Peli 2020). The animal study protocol was approved by the institutional ethics committee of the Department of Veterinary Medicine and Animal Production (Centro Servizi Veterinari), University of Naples, Federico II (PG/2022/0093420, 21 st July 2022).

Real-time PCR protocols

DNA was extracted from each sample using DNeasy[®] Blood & Tissue (Qiagen, Germany) following the manufacturer's instructions. After quantification with NanoDrop Microvolume Spectrophotometers (ThermoFisher Scientific, United States) the DNAs were used as templates for three different PCR protocols previously described in the literature for the detection of *M. haemocanis*, *Ca. M. haematoparvum*, and *Bartonella* spp. (Diaz et al. 2012; Willi et al. 2006).

Briefly, 50 ng of each sample or specific control (DNA extracted from samples that tested positive in previous studies or derived from laboratory strains, as in the case of *Bartonella*) was supplied to 1 µl of forward and reverse primers, respectively, 0.25 µL of probe and 2X iTaq Universal Probes Supermix (Biorad, Italy).

Supplementary file 1 summarizes primers, probes, and thermal conditions used in this study. Real-time positivity was further confirmed via end-point PCR by visualizing specific bands for each pathogen (Roblejo-Arias et al. 2022; Zarea et al. 2023a, b). The amplification products (amplicons) were resolved in agarose gels (1.5%) and visualized under a transilluminator (BioRad, Italy) after 15 min of running (the first 5 min at 40 V and the following 10 min at 70 V). DNA quality assessment was performed by amplifying a portion of the canine β-actin sequence using DNA extracted from Madin-Darby Canine Kidney cells (MDCK) as control (Ayers et al. 2007).

Statistical analysis

Chi-square statistics were used to investigate the association between the dependent variable (real-time PCR outcome) and independent factors (as extracted from the questionnaire); values of *p* (*p*-value) less than 0.05 were considered statistically significant. The independent factors included age (≤ 2 years considered young, > 2 and ≤ 6 years considered adults, > 6 years considered senior), sex (male or female), location (Avellino, Benevento, Salerno, Caserta, Napoli), activity (hunting or non-hunting), breed (mix or purebred), and origin (owned or stray) (Harvey 2021). When expected cell counts were below five, the chi-square was considered invalid and the exact Fisher's test was used. The variables found to be correlated with a higher risk of positivity in the univariate analysis were subjected to multivariate analysis using stepwise elimination in a logistic regression. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to determine the level of association between independent variables and molecular positivity to each pathogen. The Akaike Information Criterion (AIC) was used to evaluate fit models, and the ones that best fit the data were chosen. The Variance Inflation Factor (VIF) was utilized to determine collinearity. Statistical analysis was carried out using MedCalc Statistical Software version 16.4.3 (MedCalc Software, Ostend, Belgium).

Results

A total of 24.1% (45/187) of the dogs were positive for at least one bloodborne pathogen. *Ca. M. haematoparvum* was the most frequently detected pathogen (14.4%; 27/187),

followed by *M. haemocanis* (12.8%; 24/187) and *Bartonella* spp. (1.6%; 3/187). A total of 20 municipalities out of 62 (32.2%) resulted positive for at least one hemotropic pathogen (Fig. 1). Some coinfecting animals were also observed (9 out of 187 animals tested). More specifically, 8 animals tested positive for both *Ca. M. haematoparvum* and *M. haemocanis* (4.3%), and in one animal, the DNA of both *Bartonella* and *M. haemocanis* were identified (0.5%). Co-infections have been observed mainly in adult/senior hunting and kennel dogs. The analysis of risk factors identified province, activity, and lifestyle as risk factors correlated with higher prevalences, considering animals positive for at least one pathogen (Table 1). Dogs from the province of Salerno, particularly those used for hunting and living outdoors, showed the highest positivity rates. These correlations were confirmed by exclusively considering positives for *Ca. M. haematoparvum* (Supplementary file 2). As for *M. haemocanis*, however, age was found to be a statistically significant factor (although higher prevalences were observed in dogs coming from Salerno, hunting, and living outdoors) (Supplementary file 2). The risk analysis in the case of *Bartonella* was not possible given that few dogs tested positive for real-time PCR. The positive animals were a hunting dog and two stray dogs, all living outside. Multivariate analysis performed on variables statistically associated with higher prevalence confirmed lifestyle (outdoors) and province (Salerno) as the main risk factors related to the selected CVBDs (Table 2). Specifically, dogs living outdoors had an OR of 3.46, whereas dogs from the province of Salerno had an OR of 13. Similarly, non-hunting dogs had a significantly lower risk of testing positive than hunting dogs (OR=0.11).

The distribution of positive animals has been represented by a map (Fig. 1). All CVBDs investigated in this research were not widespread in the province of Naples.

Discussion

CVBDs represent a growing and alarming public and veterinary health concern, especially in the case of pathogens with zoonotic potential. The widespread presence of the causative agents in the canine population of southern Italy has been further supported in the present study, highlighting a high overall prevalence of 24.1% (Cortese et al. 2020). More specifically, the most frequently detected pathogen was *Ca. M. haematoparvum* (14.4%). The epidemiological picture varies significantly over the world. In Europe, evidence of hemoplasma infections is reported in Greece (4–6%), Albania (8.8%), and Bosnia (1%) (Colella et al. 2022; Hamel et al. 2016; Tennant et al. 2011). Overall, similar prevalences for *Ca. M. haematoparvum* have been described in Chile

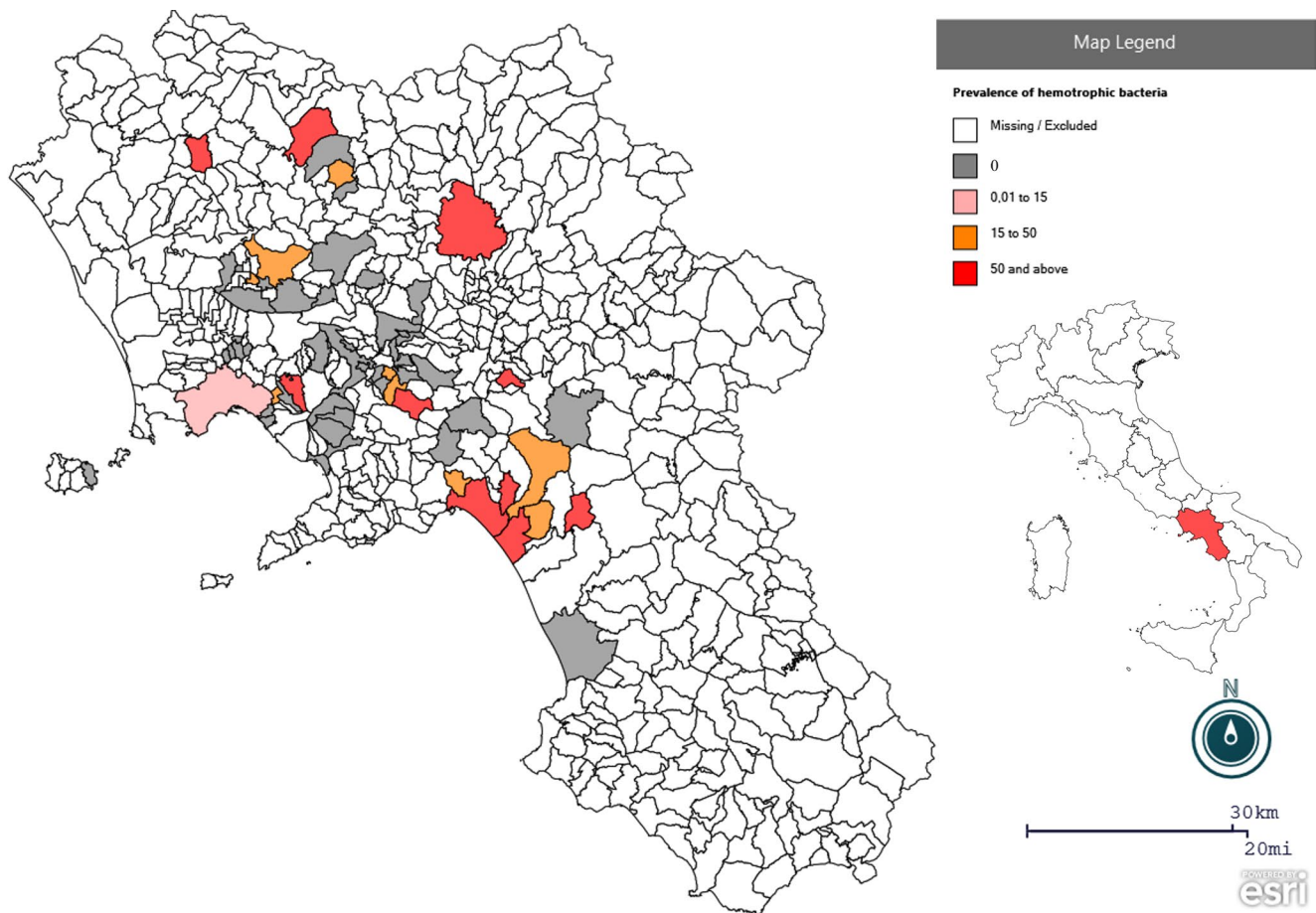


Fig. 1 Spatial distribution of districts tested positive for CVBDs in the Campania region, southern Italy. The distribution of positive animals is characterized by a high concentration in the province of Salerno for all pathogens, whereas *Ca. M. haematoparvum* was widespread in Benevento and *M. haemocanis* in Avellino. Positive districts were rep-

resented in red (overall prevalence $\geq 50\%$), in orange ($15 < 50\%$), or in pink ($0 < 15\%$). Negative districts were represented in grey, not sampled districts in white. Map was obtained using Epi Info (EPI Info™ software version 7.2.5.0, US).

(24.8%), Cuba (17.8%), and Turkey (15.3%) (Soto et al. 2017; Aktas and Ozubek 2018; Roblejo-Arias et al. 2022) although these countries have different environmental conditions from Italy. Higher molecular prevalences (47%), including a greater distribution of *Bartonella* spp. (17%), have been described in Egypt (Zarea et al. 2023a, b). Overall *Bartonella* spp. molecular prevalence of 56.7% and 33.8% have been recently described in Iran and Portugal (Beus et al. 2024). Some studies, however, have described lower prevalences using molecular methods. For example, a prevalence of 4.5% and 7.3% has been reported in Italy (northern areas) and Nigeria (Aquino et al. 2016; Ravagnan et al. 2017). These considerable discrepancies could be attributed to a variety of factors, including the PCR protocol employed, period of year when the surveys were done, epidemiological scenarios, and animal's population investigated. Generally, higher prevalences were observed in wild, free-roaming canids and dogfighters. For example, 30% of Darwin's foxes (wild canids) tested positive for *M. haemocanis* in Chile (Cataldo

et al. 2020). Evidence from Spain and Brazil highlighted the presence of hemotropic mycoplasmas (*Mycoplasma* spp.) in wolves, foxes, various mustelids, and wild rodents, supporting the hypothesis that the host range of these pathogens is broad and diversified (de Sousa et al. 2017). Another study conducted on dogfighters showed molecular prevalences of 32% and 30% for *Ca. M. haematoparvum* and *M. haemocanis*, respectively (Cannon et al. 2016). A study from Chile detected *Ca. M. haematoparvum* DNA in the blood of 31% of free-roaming dogs (Cevidanes et al. 2023).

A study conducted on only hunting dogs in the same study area as the present work identified an overall prevalence of 19.9%, with *M. haemocanis* and *Ca. M. haematoparvum* detected in similar percentages (13.1% and 11.4%, respectively) (Cortese et al. 2020).

The same previously listed technical and epidemiological factors could have an impact on the prevalence of one infection over the other. Overall, a predominance (also partially) of *M. haemocanis* over *Ca. M. haematoparvum* is reported

Table 1 Univariate analysis of potential risk factors for CVBDs (*Bartonella* spp., *Candidatus Mycoplasma haematoparvum*, *Mycoplasma hemocanis*) detection in the dog population in Campania region, southern Italy

Factor	At least one				χ^2	p
	n	Positive	%	95%CI		
Total	187	45	24.1	17.9–30.2		
Province						
Avellino/Benevento	46	11	23.9	11.6–36.2	14.95	<0.001
Salerno	45	20	44.4	29.9–59		
Caserta/Napoli	96	14	14.6	7.5–21.6		
Sex						
Male	111	25	22.5	14.8–30.3	0.35	0.55
Female	76	20	26.3	16.42–36.2		
Age						
Young/Adult	133	31	23.3	16.1–30.5	0.14	0.7
Senior	54	14	25.9	14.2–37.6		
Activity						
Hunting	50	18	36	22.7–49.3	5.3	0.02
Non Hunting	137	27	19.7	13–26.4		
Breed						
Mix	81	19	23.5	14.2–32.7	0.03	0.86
Specific bred	106	26	24.5	16.3–32.7		
Origin						
Stray	97	26	26.8	18–35.6	0.83	0.36
Owned	90	19	21.1	12.7–29.5		
Lifestyle						
In	56	5	8.9	1.5–16.4	10	0.02
Outside	131	40	30.5	22.6–38.4		
Size						
Small	71	18	25.3	5.2–35.5	0.87	0.65
Medium	81	17	21	12.1–29.9		
Large	35	10	28.5	13.6–43.5		

CI = Confidence intervals

χ^2 = Chi square

p = p-value

Table 2 Multivariate analysis of potential risk factors and odds ratio for CVBDs (*Bartonella* spp., *Candidatus Mycoplasma haematoparvum*, *Mycoplasma hemocanis*) detection in the dog population in Campania region, southern Italy

Factor	Coefficient (β)	OR	95% CI	p-value
Lifestyle (Outdoors)	1.2	3.46	1.2–10.2	0.02
Activity (No hunting)	-2.1	0.11	0.03–0.4	<0.001
Province (Caserta/Napoli)	-0.5	0.6	0.1–4.3	0.61
Province (Salerno)	2.56	13	1.92–88	0.009

CI = Confidence intervals, OR = Odds ratio, p = p-value

(Valle et al. 2014; Inpankaew et al. 2016; Ravagnan et al. 2017). Our results, however, coincide with those reported in two studies in Chile, where a slightly higher prevalence of *Ca. M. haetomaparvum* was observed (Soto et al. 2017; Cevidanes et al. 2023).

There are fewer reports of *Bartonella* spp. detection in dogs, as serological analyses have been preferred in this species. An example was described in the US, where 6.1% of dogs tested seropositive out of 5,957 examined (Lashnits et al. 2022). Recent global prevalence data in Europe indicated a molecular prevalence of 15.3% and 3.6%, respectively in cats and dogs (based on 20,133 cats and 9,824 dogs) (Zarea et al. 2023a, b). Moreover, *Bartonella* spp. infection was more common in rural or hunting dogs than in companion dogs in Mediterranean countries such as Italy (5%) and Greece (4%). The frequency was as low as 0.3% in Poland, and 0% in dogs from Portugal, Finland, and Spain (Zarea et al. 2023a, b). Other molecular investigations have identified no positive cases in dogs (in Australia, Chile, and Southeast Asia) (Zarea et al. 2022; Shapiro et al. 2017; Cevidanes et

al. 2023). *Bartonella* spp. prevalence in dogs was higher than in cats (42%) in Egypt (Zarea et al. 2023a, b).

Furthermore, in the literature described so far, a very low rate of coinfection always emerged, often between 1 and 7% among *M. haemocanis* and other CVBDs (Dordio et al. 2021). A higher co-infection rate between *Ca. Mycoplasma haematoparvum* and *M. haemocanis* has been described in Iran (31.9%) (Beus et al. 2024). This aspect could lead to suspicion of some type of competition that the various hemotropic bacteria may present in colonizing the red blood cells (although there is no evidence in vitro or in vivo).

Epidemiological evidence has identified numerous risk factors that are correlated with higher prevalences. The most frequently reported are tick infestation, origin from kennels, living in rural environments, and adult age (Aktas and Ozubek 2018; Barbosa et al. 2021; Roblejo-Arias et al. 2022). Additional variables that have been linked to the occurrence of specific CVBDs include activity and lifestyle. Hunting dogs and dogs that live outside showed clearly higher prevalence rates because they are more susceptible to continuous vector activity, particularly in the absence of a careful prophylaxis (Sgroi et al. 2022). Similarly, in some studies, certain areas exhibited greater prevalences, possibly due to climatic conditions that were more suitable to the pathogen's cycle throughout the period in question. This was seen in our research for the Salerno province. Recent research investigating the occurrence of CVBDs in the same study area consistently reported a greater frequency in the province of Salerno (Cortese et al. 2020; Sgroi et al. 2022). This suggests that this region has particularly favorable environmental circumstances for the vector's life cycle or, at the very least, for the transmission of these diseases. Older age is usually linked to predisposing factors including immunosuppression and other concurrent infectious and non-infectious disorders, and it also increases the likelihood that the animal will encounter the vector and, consequently, the pathogen. Despite this, in the present study no differences were observed based on the age of the animal. Although transmitted by the same vector, molecular positivity for *M. haemocanis* and *Ca. M. haematoparvum* correlated with different variables.

Finally, the present study highlighted how higher prevalences can occur in animals living outdoors, being more exposed to vectors and potential bites. Accordingly, control and surveillance strategies against CBVDs are important to guarantee animal health and also mitigate potential zoonotic transmission pathways. Even if the present study has demonstrated the presence and circulation of several CVBDs in the dog population of the Campania region and also evaluated the risk factors, it has not focused on clinical aspects and laboratory findings (such as blood smears or complete blood count tests).

Conclusions

This study reported the molecular prevalence of *Bartonella* spp., *M. haemocanis*, and *Ca. M. haematoparvum* in dog populations of southern Italy, indicating the main risk factors related to their presence. Although a small-scale sampling was performed, the findings clearly show a wide occurrence of canine vector-borne pathogens, thus the need for effective strategies for reducing ectoparasite infestations. More research is required to address the spread of pathogens in arthropod vectors and the risk of infection for humans, clarifying the epidemiological dynamics in the life cycle of CVBDs, as well as the clinical impact in the study area.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11259-025-11018-x>.

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Data availability Not applicable (no data sets were generated or analysed during the current study)

Declarations

Ethics approval The animal study protocol was approved by the Institutional Ethics Committee of Department of Veterinary Medicine and Animal production (Centro Servizi Veterinari), University of Naples, Federico II (PG/2022/0093420 20 July 2022).

Competing interests The authors declare no competing interests.

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