



Serological detection of *Anaplasma* spp., *Ehrlichia* spp., *Borrelia burgdorferi* s.l. antibodies, and *Dirofilaria immitis* antigen in stray dogs in Bosnia and Herzegovina

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Received: 19 February 2025 / Accepted: 3 June 2025
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Abstract

Stray dogs may be highly exposed to vector-borne pathogens (VBP), including zoonotic agents, and therefore may pose a high risk of spreading infection to other animals and humans. Our study is the first large-scale prevalence study of VBP in stray dogs in Canton Sarajevo, Bosnia and Herzegovina. During the period 2016–2018, a total of 3720 blood samples of stray dogs were screened by the SNAP 4Dx plus test for antibodies against *Anaplasma* spp., *Ehrlichia* spp., *B. burgdorferi* s.l., and *D. immitis*. The 910 dogs (910/3720, 24.46%) were seropositive for one or more CVBPs. The proportion of seropositive dogs against one, two, or three pathogens was 864 (23.23%), 43 (1.15%), and 3 (0.08%), respectively. The most prevalent canine VBP was *Anaplasma* spp. (21.59%), followed by *B. burgdorferi* s.l. (2.47%) and *Ehrlichia* spp. (1.13%). The lowest prevalence was recorded for *D. immitis* (0.5%). The number of seropositive dogs older than 1 year was 704 (704/2497, 28.19%), while the number of dogs younger than 1 year was 206 (206/1223, 16.84%). The most common dual coinfection detected was with *Anaplasma* spp. and *B. burgdorferi* s.l. (26/3720, 0.7%). The results show that stray dogs are exposed to at least one of these four CVBD pathogens. Since some of these pathogens cause zoonoses, controlling these infections is crucial for both veterinary and public health. These findings highlight the need for continuous serological monitoring of CVBD pathogens in stray dogs due to their risks to animal and human health.

Keywords Canine · Vector-borne diseases · Stray dogs · SNAP 4Dx

Introduction

Canine vector-borne diseases (CVBD) have the potential to greatly affect the health status of dogs. The etiology of these diseases is broad, including different pathogens (protozoans, helminths, bacteria, and viruses), all transmitted by hematophagous arthropods such as mosquitoes, ticks, fleas, lice, and phlebotomine sandflies (Otranto et al. 2009a; Beugnet et al. 2009). Dogs can serve as reservoirs for CVBD-causing pathogens, including zoonotic agents such as *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Dirofilaria immitis*, and *Leishmania infantum* (Otranto et al. 2009a). Human infection with *Ehrlichia canis* is rare, but has

been reported (Perez et al. 2006). Infected dogs can become asymptomatic carriers, posing a high risk of spreading infections to other animals and humans (Otranto et al. 2009a and 2009b). CVBDs are often present with nonspecific signs, and clinical symptoms vary based on the specific pathogen and host (Baneth et al. 2012).

Canine infection with *Anaplasma* spp., *Ehrlichia* spp., *B. burgdorferi* s.l., and *D. immitis* has been reported in many countries in Europe (Beugnet et al. 2009; Sainz et al. 2015).

The genus *Anaplasma*, including *A. phagocytophilum* and *A. platys*, is bacteria transmitted by ticks of the genus *Ixodes* and are the most commonly detected vector-borne pathogens (VBPs) in dogs throughout Europe (Maggi et al. 2014). *Anaplasma* spp. is responsible for cyclic thrombocytopenia, canine granulocytic anaplasmosis infection in dogs and zoonotic human granulocytic anaplasmosis (Sainz et al. 2015). Reports of canine anaplasmosis exist from various countries of southeastern Europe near Bosnia and Herzegovina, with seroprevalences of 4.5% (Croatia), 6.2% (Greece), 6.74% (Serbia), 24.1% (Albania), 24.8% (Kosovo), and

Section Editor: Van Lun Low

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46.1% (Bulgaria) (Savić et al. 2014; Pantchev et al. 2015; Hamel et al. 2016; Angelou et al. 2019; Jurković et al. 2019; Sinani et al. 2020). The first seroprevalence study was performed in Bosnia and Herzegovina recently and estimated *Anaplasma* spp. seroprevalence was 20.4% (Maksimović et al. 2022).

E. canis, primarily transmitted by the brown dog tick *Rhipicephalus sanguineus* sensu lato (s.l.) is the causative agent of acute and chronic canine monocytic ehrlichiosis (Sainz et al. 2015). In Europe, this bacterium has been the only *Ehrlichia* species detected (Aguirre et al. 2004). The prevalence of *E. canis* among dogs in southern Europe varies from 0.6 to 21% (Pantchev et al. 2015; Hamel et al. 2016; Jurković et al. 2019; Sinani et al. 2020). According to a recent study conducted in Bosnia and Herzegovina, the seroprevalence of *E. canis* in stray dogs was reported to be 0.22% (Maksimović et al. 2022).

B. burgdorferi sensu lato (s.l.) is a group of spirochetes transmitted by *Ixodes* ticks that can cause Lyme borreliosis in humans and dogs. About 65,000 human cases in Europe are reported annually (Hubálek 2009). The seroprevalence rates of *B. burgdorferi* s.l. in dogs vary across Balkan countries, ranging from 0.1% in Greece, 1.3% in Kosovo, to 25.8% in Serbia (Savić et al. 2014; Angelou et al. 2019; Sinani et al. 2020). Data from Bosnia and Herzegovina, as we know, is unavailable.

D. immitis is a zoonotic mosquito-borne nematode causing heartworm disease in dogs, and it rarely infects humans, leading to pulmonary dirofilariasis (Simón et al. 2012). Zahirović (2015) detected *D. immitis* in B&H with a prevalence of 3.11% in dogs. The prevalence of *D. immitis* in dogs from Southeast Europe ranges from 0.6% in Croatia (Jurković et al. 2019) to 27.6% in Serbia (Savić et al. 2014).

The aim of this research was to estimate the prevalence of *D. immitis* and the seroprevalence of *Ehrlichia* spp., *Anaplasma* spp., and *B. burgdorferi* s.l. in the stray dogs in Canton Sarajevo, Bosnia and Herzegovina.

Materials and methods

Study area

Canton Sarajevo is located in the central part of B&H, covering an area of approximately 1277 km². The region has a continental climate with an average annual temperature of 9.5 °C and 1000 mm of precipitation.

Dog population and sampling

The blood samples used in this study were obtained during routine monitoring of zoonotic infectious and parasitic diseases in the population of stray dogs in the canton Sarajevo,

B&H, over the period 2016–2018. The population of stray dogs in B&H is significant, especially in Sarajevo, although the absolute number of dogs is unknown. Officially in B&H in 2015–2017, there were about 11,000 stray dogs in the Sarajevo region (Maksimović et al. 2022).

The study included 3720 stray dogs from the state shelter located in the Sarajevo region. Prior to sheltering, all dogs lived in rural and urban areas, were exposed to ticks (no prophylactic measures), and had unknown clinical status. The dogs estimated ages ranged from 1 month to 9 years.

A total of 3720 ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood samples of all sheltered dogs were collected at the state veterinary station by the local veterinarians over the period 2016–2018. All blood samples were delivered on ice to the clinical laboratory of the Veterinary Faculty, University of Sarajevo, and stored at 4 °C (12–24 h) until further processing.

Serological testing

The samples were tested for antibodies against *A. phagocytophilum*/*A. platys*, *B. burgdorferi* s.l., *E. canis*/*E. ewingii*, and *D. immitis* antigen by use of the commercial point-of-care SNAP 4Dx plus assay (IDEXX Laboratories, Westbrook, Maine USA) according to the manufacturer's instruction.

Statistical analysis

The differences in the distribution of positive/negative results of the investigated pathogens between sexes and between young (< 1 year) and adult (> 1 year) dogs were tested using the chi-square test with Yates' correction for continuity. *p*-values less than 0.05 were considered statistically significant.

Results

A total of 910 dogs (910/3720, 24.46%) were positive for one or more CVBPs. The proportion of positive dogs against one, two, or three pathogens was 864 (23.23%), 43 (1.15%), and 3 (0.08%), respectively. The most prevalent CVBP was *Anaplasma* spp. (21.59%), followed by *B. burgdorferi* s.l. (2.47%) and *Ehrlichia* spp. (1.13%). The lowest prevalence was recorded for *D. immitis* (0.5%).

In relation to age, the number of positive dogs older than one year (704/2497, 28.19%) was significantly higher (*p* < 0.001) in comparison with the number of dogs younger than 1 year (206/1223, 16.84%). There were no statistically significant (*p* = 0.780) differences between positive male and female dogs.

Table 1 Number and percentage of positive dogs regarding age and sex of animals

			<i>Anaplasma</i> spp.	<i>Ehrlichia</i> spp.	<i>B. burgdorferi</i> s.l	<i>D. immitis</i>	SNAP 4Dx test
Sex of animals	Male (N= 1971)	n	427	19	51	8	478
		%	21.67	0.96	2.59	0.4	24.25
	Female (N= 1749)	n	376	23	41	11	432
		%	21.5	1.32	2.34	0.63	24.7
Animal ages	p=		0.934	0.392	0.514	0.47	0.78
	< 1 year (N= 1223)	n	178	13	21	7	206
		%	14.55	1.06	1.72	0.57	16.84
	> 1 year (N= 2497)	n	625	29	71	12	704
		%	25.03	1.61	2.84	0.98	28.19
	p=		< 0.001	0.92	0.049	0.902	< 0.001
Total			n	803	42	92	910
			%	21.59	1.13	2.47	24.46

N number of animals,

n - number of positive animals,

% - the percentage of the positive animals

Table 2 Number and percentage of dogs with one, two, or three CVBP

	N	% of positive samples (N= 910)	% of positive animals (N= 3720)
One pathogen	864	94.95	23.23
Two pathogens	43	4.72	1.15
Three pathogens	3	0.33	0.08
Total	910	100	24.46

N- number of animals

n- number of positive animals

% - the percentage of the positive animals

The most common dual coinfection detected was with *Anaplasma* spp. and *B. burgdorferi* s.l. (26/3720, 0.7%). Coinfection with *Ehrlichia* spp. and *D. immitis* was detected only in one sample (1/3720, 0.03%). Triple coinfection with *Anaplasma* spp., *Ehrlichia* spp., and *D. immitis* was detected in two samples (2/3720, 0.05%) and with *Anaplasma* spp., *B. burgdorferi* s.l., and *D. immitis* in one sample (1/3720, 0.03%).

Other results are shown in Tables 1, 2, and 3.

Discussion

To the best of our knowledge, this is the first report concerning the prevalence of four CVB pathogens detected with the SNAP 4Dx test in the stray dogs from B&H. Recently reported first data of *A. phagocytophilum*/*A. platys*, and *E. canis*/*E. ewingii* seroprevalence in dogs from B&H (Maksimović et al. 2022) have shown lower

Table 3 Coinfection of different CVB pathogens

Pathogens	Positive animals	
	n	%
<i>Anaplasma</i> spp. + <i>B. burgdorferi</i> s.l	26	0.7
<i>Anaplasma</i> spp. + <i>Ehrlichia</i> spp.	9	0.24
<i>Anaplasma</i> spp. + <i>D. immitis</i>	7	0.19
<i>Ehrlichia</i> spp. + <i>D. immitis</i>	1	0.03
<i>Anaplasma</i> spp. + <i>Ehrlichia</i> spp. + <i>D. immitis</i>	2	0.05
<i>Anaplasma</i> spp. + <i>B. burgdorferi</i> s.l. + <i>D. immitis</i>	1	0.03
Total	46	1.24

N number of animals, n - number of positive animals, % - the percentage of the positive animals

value (187/903, 20.7%) in comparison to our results. This difference could be due to the smaller number of serologically tested samples (903) compared to the number tested in the present study (3720). Additionally, the varying representation of older dogs may contribute to the difference in prevalence. It is important to note the difference in categorization between the two studies: our study categorized dogs as under or over 1 year old, while Maksimović et al. (2022) used a threshold of 2 years. Compared to other countries in the Balkan peninsula, detected prevalence is much higher than in Croatia (6.6%) and Romania (11.3%), at a similar level as in Greece (21.8%) and Albania (25.1%), but lower than in Kosovo (37.6%) (Mircean et al. 2012; Hamel et al. 2016; Jurković et al. 2019; Angelou et al. 2019; Sinani et al. 2020). The differences can be attributed to variations in sample sizes, sampling methods, the diverse population of dogs and ticks, the climatic and

geographic differences between the study areas, and variations in the assays used (Petrucelli et al. 2021).

Our study detected *Anaplasma* spp. as the dominant CVBP in stray dogs (21.59%). When comparing our results with previous studies in neighboring countries, the observed prevalence was higher than in Croatia (4.5%), Romania (5.5%), Greece (6.2%), and Serbia (6.74%), at a similar level as in Kosovo (24.8%) and Albania (24.1%) (Mircean et al. 2012; Hamel et al. 2016; Jurković et al. 2019; Angelou et al. 2019; Sinani et al. 2020). In Bulgaria, prevalence ranged between 13.4 and 46.1% depending on region and other factors related to tested animals and used tests (Panchev et al. 2015; Manev 2020; Iliev et al. 2020; Arnaudov 2021). As mentioned earlier, a smaller study of seroprevalence and molecular detection of *Anaplasma* spp. in Bosnia and Herzegovina was performed with a seroprevalence of 20.7%. Molecular detection shows *Anaplasma* spp. in only 26.09% of the seropositive samples, with the most detected species *A. phagocytophilum* (Maksimović et al. 2022). Omeragić et al. (2022) detected *Ixodes ricinus*, the primary vector for *A. phagocytophilum*, as the most dominant tick species in B&H. The results of these two recent investigations conducted in B&H (Omeragić et al. 2022; Maksimović et al. 2022) support the dominant seroprevalence of *Anaplasma* spp. observed in our study. Comparably, Mircean et al. (2012) in Romania detected a higher prevalence of *A. phagocytophilum* in an area where *I. ricinus* was the dominant tick species.

Panchev et al. (2015) reported a seroprevalence of *B. burgdorferi* s.l. of 2.4% in Bulgaria, which is similar to the seroprevalence detected in our study (2.47%). Curiously, most other countries of the Balkan peninsula reported prevalence of *B. burgdorferi* s.l. between 0 and 1.7% (Mircean et al. 2012; Hamel et al. 2016; Jurković et al. 2019; Angelou et al. 2019; Sinani et al. 2020; Manev 2020; Iliev et al. 2020). *B. burgdorferi* s.l. was detected in humans, and *I. ricinus* ticks were collected from humans in the Sarajevo region (Lasić et al. 2020). This data confirmed the potential significance of stray dogs as a reservoir of Lyme disease for humans. The results are particularly important as the test used in our study generally detects *B. burgdorferi* s.l. antibodies only in naturally infected dogs (Wagner et al. 2012), highlighting the zoonotic significance of stray dogs' health status.

In the present study, *E. canis*, the sole species of the *Ehrlichia* genus detected in Europe (Aguirre et al. 2004), was found in only 1.13% of stray dogs. This prevalence is similar to that observed in the Sofia region of Bulgaria (1.34%) among the tested population of stray dogs (Manev 2020). In comparison, notably lower values were found in Croatia 0.46–0.6% (Mrljak et al. 2017; Jurković et al. 2019) and Kosovo 0.7% (Sinani et al. 2020) and higher in Romania (2.1%) (Mircean et al. 2012), Serbia 11.06% (Bogičević

et al. 2017), Greece 12.5% (Angelou et al. 2019), Montenegro 19.3% (Laušević et al. 2020), Albania 20.8% (Hamel et al. 2016), and Bulgaria 13.4–30.4% (Panchev et al. 2015; Iliev et al. 2020; Arnaudov 2021). The main reason for this discrepancy can be explained by the distribution of the *Rhipicephalus sanguineus* s.l., the primary vector for *E. canis*. In regions with higher prevalence, vectors of this disease are present in high density compared with the areas with low vector density.

D. immitis was the less detected pathogen (0.5%) in our study. The prevalence of this nematode reported in other countries on the Balkan peninsula is as follows: Croatia (0.6%), Greece (9%), Kosovo (14%), Bulgaria (16.2%), and Serbia (27.6%) (Savić et al. 2014; Panchev et al. 2015; Jurković et al. 2019; Angelou et al. 2019; Sinani et al. 2020). One earlier study in Bosnia and Herzegovina detected *D. immitis* in 3.11% of tested dogs, but none from the Sarajevo region (Zahirović 2015). In our study, the detected presence of *D. immitis* infection in the Sarajevo region may be due to the tested animal population, stray dogs that potentially roamed across a larger area. Another possible reason could be the larger tested population, specifically from the Sarajevo region in comparison to the earlier study. Hence, further investigation is required to confirm the potential autochthonic presence of *D. immitis* infection in Canton Sarajevo.

Coinfection was detected in 46 stray dogs (1.24%), of which 43 were positive to two pathogens. Three dogs were positive to three pathogens. Studies from other countries have reported coinfection rates ranging from 0.5 to 4.5%. (Farkas et al. 2014; Mrljak et al. 2017; Jurković et al. 2019; Manev 2020; Petrucelli et al. 2021). *Anaplasma* spp. and *B. burgdorferi* s.l. coinfection was the most frequently detected (26 dogs) in our study. This result is not surprising considering that these infections were the most detected in our research and share the same vector, *I. ricinus*. Gunes et al. (2011) reported higher prevalence coinfection of *A. phagocytophilum* and *B. burgdorferi* s.l. in humans in an area with a higher prevalence of *I. ricinus*. *Anaplasma* spp. was detected as coinfection with all targeted CVB pathogens, while *B. burgdorferi* s.l. was detected as coinfection only with *Anaplasma* spp. The distribution of coinfection depends on the prevalence of CBV pathogens. Manev (2020) reported coinfection of *Anaplasma* spp. and *D. immitis* as the most prevalent. Other studies have similar results, where the occurrence of coinfections was correlated with the most detected CBV pathogens (Ural et al. 2014; Farkas et al. 2014; Panchev et al. 2015; Kovačević-Filipović et al. 2018; Angelou et al. 2019; Sinani et al. 2020; Petrucelli et al. 2021).

In the present study, the prevalence of detected CBV pathogens was significantly higher in dogs aged 1 year or older compared to younger dogs. Other authors, who got similar results, proposed the immunological status of the host or

the increased exposure to the vector with age as a possible explanation (Cardoso et al. 2012; Diaz-Reganon et al. 2020). In our research, no significant gender predisposition to infections was found, which corresponds to the results of numerous other investigations (Mrljak et al. 2017; Angelou et al. 2019; Petruccielli et al. 2021). In contrast, Manev (2020) found a higher prevalence in stray male dogs (57%). Curiously, Petruccielli et al. (2021) found that seropositivity to *Ehrlichia* spp. was associated with gender (male), but not with the age of the tested dogs.

Conclusion

This is the first large-scale prevalence study on stray dogs in Canton Sarajevo, Bosnia and Herzegovina. It is also the first to investigate the prevalence of *D. immitis* and *B. burgdorferi* s.l., and the second for *Anaplasma* spp. and *Ehrlichia* spp. in this region. The results show that stray dogs are exposed to at least one of these four CVBD pathogens. Given that some of these pathogens cause zoonoses, controlling these infections is crucial for both veterinary and public health, as evidenced by the presence of Lyme disease in humans in Canton Sarajevo. Continuous serological monitoring of CVBD pathogens in stray dogs is essential due to the risks they pose to both animal and human health.

Acknowledgements The authors gratefully acknowledge Prof. Ćazim Crnkić, University of Sarajevo-Veterinary Faculty, for helping with the statistical analysis of our study's results.

Author contributions Conceptualization- Denis Čamo, Alan Maksimović, and Amir Zahirović, Writing-original draft preparation -Denis Čamo Writing -review and editing - Alan Maksimović and Tarik Mutevelić Methodology - Denis Čamo, Amir Zahirović Formal analysis and investigation - Dajna Predžić and Jasminka Isović Funding acquisition- Amir Zahirović All authors read and approved the final manuscript.

Funding This work was supported by project (grant number 07–01–14–15410-1/16), “Monitoring of zoonotic infectious and parasitic diseases in the population of stray dogs in Sarajevo Canton” of the Ministry of Economy of the Sarajevo Canton, Bosnia and Herzegovina (2016–2018).

Data Availability No datasets were generated or analysed during the current study.

Competing interests

The authors declare no competing interests.

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