

Original research-Orijinal araştırma

The seroprevalence of *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum* in clinically healthy dogs from Sinop region of Turkey

*Türkiye'nin Sinop yöresinde, klinik olarak sağlıklı görülen köpeklerde *Borrelia burgdorferi sensu lato* ve *Anaplasma phagocytophilum* seroprevalansı ve her iki enfeksiyon etkeninin epidemiyolojik benzerlikleri*

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Abstract

Aim. Ixodes ricinus ticks are frequently encountered in the Black Sea Region of Turkey, including the province of Sinop. The aim of this study was to investigate the seroprevalence of *Borrelia burgdorferi sensu lato* (s.l.) and *Anaplasma phagocytophilum* in dogs living in eight villages of Sinop and to clarify epidemiological similarities between both infectious agents. **Methods.** In August 2007, blood samples were collected from 93 dogs, which appeared clinically healthy. Serum samples were tested by the ELISA method for the presence of *B. burgdorferi* s.l. and *A. phagocytophilum* IgG antibodies. **Results.** *B. burgdorferi* s.l. IgG antibodies were detected in 26 of 93 dogs (28.0%) and *A. phagocytophilum* IgG antibodies in 28 of them (30.1%). Only *B. burgdorferi* s.l. antibodies were detected in 7 dogs (7.5%), only *A. phagocytophilum* antibodies in 9 dogs (9.7%) and co-seroprevalence was seen in 19 dogs (20.4%). Seroprevalence of *A. phagocytophilum* was higher in the seropositive dogs to *B. burgdorferi* s.l. than the group of seronegative dogs ($X^2= 28.90$, $p= 0.001$) and there was a positive correlation between both infectious agent (Phi coefficient= 0.584, $p= 0.001$). **Conclusion.** Dogs of Sinop in Turkey are under high risk to get infected with *B. burgdorferi* s.l. and *A. phagocytophilum* and most probably in similar climatic regions of the country. Also the seroprevalence rates in dogs are important to be an indicator of endemic status of a given region for humans, too.

Keywords: Canine borreliosis, canine anaplasmosis, *borrelia burgdorferi* s.l., *anaplasma phagocytophilum*, tick-borne infections, co-seroprevalence, Turkey

Özet

Amaç. Sinop'unda içinde olduğu Türkiye'nin Karadeniz bölgesinde *Ixodes ricinus* türü kenelere sıklıkla rastlanmaktadır. Bu çalışmanın amacı, Sinop'un sekiz köyünde yaşayan köpeklerde *Borrelia burgdorferi sensu lato* (s.l.) and *Anaplasma phagocytophilum*'un seroprevalansını araştırmak ve her iki enfeksiyon etkeni arasındaki epidemiyolojik benzerlikleri ortaya koymaktır. **Yöntem.** Sinop'ta, 2007 yılının Ağustos ayında klinik olarak sağlıklı görünen 93 köpekten kan örneği alınmıştır. Serum örneklerinde ELISA yöntemiyle *B. burgdorferi* s.l. ve *A. phagocytophilum* IgG antikorları araştırılmıştır. **Bulgular.** 93 köpeğin 26'sında (%28,0) *B. burgdorferi* s.l. ve 28'inde (%30,1) *A. phagocytophilum* IgG antikorları saptanmıştır. 7 köpekte (%7,5) sadece *B. burgdorferi* s.l., 9 köpekte (%9,7) sadece *A. phagocytophilum* antikorları ve 19 köpekte (%20,4) ise ko-seroprevalans tespit edilmiştir. *B. burgdorferi* s.l. seropozitifliği gösteren köpeklerde, seronegatif olanlarına göre *A. phagocytophilum* seropozitifliği daha yüksek bulunmuş olup ($X^2= 28,90$, $P= 0,001$), her iki enfeksiyon etkenleri arasında pozitif yönde bir korelasyon saptanmıştır (Phi korelasyon= 0,584, $p=0,001$). **Sonuç.** Sonuç olarak, Sinop ve benzer iklimsel bölgelerde, köpekler yüksek derecede *B. burgdorferi* s.l. ve *A. phagocytophilum* enfeksiyonu riski altındadırlar. Ayrıca köpeklerdeki seroprevalans oranları, belirli bir bölgenin endemik durumunun göstergesi olarak insanlar için de önem taşır.

Anahtar sözcükler: Köpek borreliosisi, köpek anaplasmosisi, *borrelia burgdorferi* s.l., *anaplasma phagocytophilum*, kene kökenli enfeksiyonlar, ko-seroprevalans, Türkiye

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Introduction

Anaplasma phagocytophila and *Borrelia burgdorferi* sensu lato (s.l.) are important tick-borne infections that are seen in dogs and humans [1]. Three genomic groups of Ehrlichia species have been described. *A. phagocytophilum* (formerly Ehrlichia phagocytophila), whose vector is Ixodes ticks, belong to the genomic group-2 and cause ehrlichiosis in dogs in Northern and Western USA, Northern and Central Europe [1, 2]. Borreliosis is caused by *B. burgdorferi* s.l. genomic group. *B. burgdorferi* sensu stricto in USA, *Borelia garinii* and *Borellia afzelii* in Europe and *Borellia japonica* in Japan are further species causing tick-borne borelliosis [1, 3]. Species of genus Ixodes, which are the principal vectors of both *B. burgdorferi* s.l. and *A. phagocytophilum*, exhibit a wide geographical distribution worldwide. *Ixodes ricinus* in Europe, *Ixodes persulcatus* in Asia, *Ixodes scapularis* and *Ixodes pacificus* in USA are known vectors of *B. burgdorferi* s.l. and *A. phagocytophilum* [4]. *Ixodes ricinus* ticks are known to be abundant, especially in the northern coastal regions of Turkey [5, 6]. In dogs, *A. phagocytophilum* infest monocytes, granulocytes and platelets. The most common clinical signs being lethargy, fever, anorexia and lameness, while neurologic manifestations have rarely been described [7]. *Borrelia* species cause various symptoms in dogs and specially damage the joints of dogs. In addition, they are responsible for neurological disorders in dogs, while cutaneous and cardiac symptoms are rare [1, 3]. Diagnosis of human granulocytic ehrlichiosis (HGE) and Lyme disease requires a combination of compatible clinical and laboratory findings, direct microscopic visualization or immunodetection of infective organisms in blood or infected tissue, microbial culture, serological testing, immunoblotting and polymerase chain reaction (PCR). In serology, indirect immunofluorescence (IFA) and enzyme-linked immunosorbent assay (ELISA) are commonly used techniques with specificity and sensitivity of approximately 93% [1, 7].

The aim of the present study was to investigate seroprevalence of *A. phagocytophilum* and *B. burgdorferi* in dogs living in Sinop area, which is located on the coastal region of northern Turkey, and to investigate the co-seroprevalance.

Materials and methods

Study area and blood collecting

Sinop is located between 41o-42o North-latitude and 34o-35o East-longitude on the coastal strip of the Black Sea Region. All year around, there are a lot of precipitations and accordingly the climatic conditions are suitable for ticks like *Ixodes ricinus*, which can be encountered extensively in the region [6]. In August 2007, with the approval of the ethics committee for animals at Cumhuriyet University in Sivas (Issue no: B.30.2.Cum.0.01.00.00-50/133), blood samples were collected from 93 dogs in 8 villages, which are located in the center district of Sinop. Serum samples were kept at -20oC until use.

ELISA

Serum samples collected from dogs were tested for antibodies of *B. burgdorferi* and *A. phagocytophilum* by using immunoglobulin G (IgG) ELISA kits (Helica Biosystema Inc; Fullerton, CA). Both ELISA tests were performed according to manufacturers' instructions and optical densities (OD) of samples were measured at 450 nm using an EL

312 Microplate Bio-kinetics Reader (Bio-Tek Instruments, Inc., Winooski, Vermont, USA).

Statistical analysis

The chi-square test and correlation tests were used to evaluate the differences between risk groups in dogs in terms of *B. burgdorferi* s.l. and *A. phagocytophilum*. SPSS (SPSS for Windows, version 17; SPSS Inc., Chicago, IL.) were used for the statistical analyses and $p < 0.05$ was regarded as statistically significant.

Results

Table 1 shows that 26 out of 93 dogs (28.0%) were seropositive for *B. burgdorferi* s.l. IgG while 28 dogs (30.1%) were seropositive for *A. phagocytophilum* IgG

Table 1. Risk factors related to seroprevalance of *B. burgdorferi* s.l. and *A. phagocytophilum* in dogs that live in Sinop region.

Risk factor category	n	No. seropositive dog (%)	
		<i>B. burgdorferi</i>	<i>A. phagocytophilum</i>
Total seroprevalence	93	26 (28.0)	28 (30.1)
Seroprevalence by gender			
Female	76	23 (30.3)	24 (31.6)
Male	17	3 (17.7)	4 (23.5)
Fisher's exact test (p value)		(0.380)	(0.575)
Seroprevalence by age (months)			
0-11	4	2 (50.0)	2 (50.0)
12-23	34	10 (29.4)	14 (41.2)
24-36	23	7 (30.4)	8 (30.4)
>37	32	7 (21.9)	4 (12.5)
Yates correction (p-value)		0.506 (0.918)	5.58 (0.134)
Single infection	93	7 (7.5)	9 (9.7)
Co-seroprevalance	93	19 (20.4)	19 (20.4)
<i>B. burgdorferi</i>			
Negative	67		9 (13.4)
Positive	26		19 (73.1)
Yates correction (p-value)			28.90 (0.001)
Phi coefficient (p)			0.584 (0.001)

IgG antibodies of *B. burgdorferi* s.l. and *A. phagocytophilum* were detected in 37.6% of the dogs. *B. burgdorferi* s.l. antibodies alone were found in 7 dogs (7.5%) and *A. phagocytophilum* antibodies alone in 9 dogs (9.7%). Co-seroprevalance was detected in 19 dogs (20.4%). Seroprevalance of *A. phagocytophilum* was higher in the seropositive dogs to *B. burgdorferi* s.l. than the group of seronegative dogs ($X^2 = 28.90$, $p = 0.001$) and there was a positive correlation between them (Phi coefficient = 0.584, $p = 0.001$). The scatter plot curve shows the correlation between *B. burgdorferi* s.l. and *A. phagocytophilum* according to ELISA absorbance values (Spearman's rho = 0.673, $p = 0.001$) (Figure 1). There were no significant differences between male and female dogs with regard to seroprevalance of *B. burgdorferi* s.l. and *A. phagocytophilum* ($p > 0.05$). In addition, there was no statistical difference between dog age groups for both *B. burgdorferi* s.l. and *A. phagocytophilum* ($p = 0.918$ and $p = 0.134$, respectively).

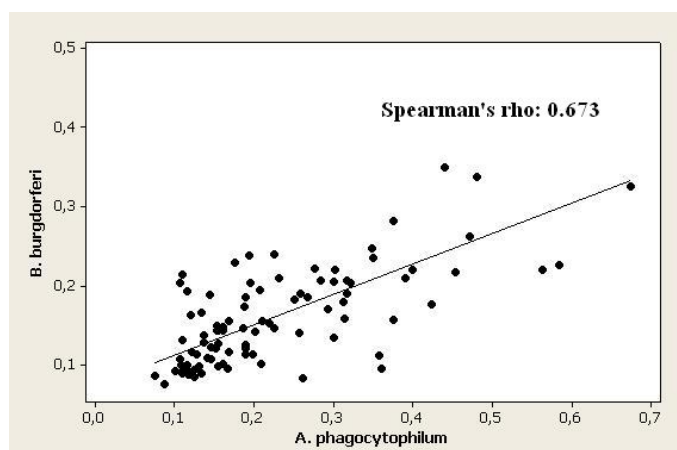


Figure 1. Relationship between *B. burgdorferi* and *A. phagocytophilum* with regard to their ELISA absorbance values.

Discussion

The high seroprevalence in dogs for *B. burgdorferi* s.l. and *A. phagocytophilum* in the region of Sinop and the high prevalence of *Ixodes* ticks [6], make the area highly endemic for these diseases [8]. Although the findings are different from country to country, seroprevalences of both *B. burgdorferi* s.l. and *A. phagocytophilum* in dogs can be as high as 30% [9-11]. The presence of *A. phagocytophilum* and *B. burgdorferi* s.l. infections in humans, domesticated animals and ticks in Turkey was demonstrated by molecular and serological techniques [6, 12, 13]. Epidemiological studies regarding dogs are however limited in Turkey [14]. In the present study, *B. burgdorferi* s.l. and *A. phagocytophilum* seropositivity was demonstrated in 28.0% and 30.1% of dogs examined, respectively. According to our findings, there are no differences with regard to seroprevalence of both *B. burgdorferi* and *A. phagocytophilum* between male and female dogs. Apparently, male and female dogs come equally in contact with infected ticks. Although it was expected that elderly dogs would show higher seropositivity than young dogs, no statistical differences between age groups were found. In dogs, antibodies to this infectious agents are short-lived and are significantly reduces within 8-12 months [7, 15, 16].

In the current study, *A. phagocytophilum* seropositivity was more higher in the *B. burgdorferi* seropositive dogs according to *B. burgdorferi* seronegative ones. The main reason for this situation may be due to the epidemiological similarities between both infection agents. A significant correlation ($\Phi = 0.584$) and the scatter plot curve between *B. burgdorferi* and *A. phagocytophilum* may be indicator of this epidemiological compliance. Observing the *Ixodes ricinus* having a role in the enzootic transmission of both infection agents widespread, may be the most important reason for the epidemiological similarity and high co-seroprevalance between both infectious agents. It is known that co-infection between *B. burgdorferi* s.l. and *A. phagocytophilum* in *Ixodes ricinus* ticks are between 2% and 5% [17, 18]. According to the present study, co-seroprevalance between *B. burgdorferi* s.l. and *A. phagocytophilum* were detected in the level of 20.4% in dogs. High level co-seroprevalance shows that dogs are repeatedly infested by ticks, especially by *Ixodes ricinus*. *B. burgdorferi* can show cross-reactivity with other *Borrelia* species and genus *Leptospira*. However, cross-reactivity between *B. burgdorferi* and *Ehrlichia* species is negligible in canine borreliosis [19, 20]. *A. phagocytophilum* frequently shows cross-reactivity with *Ehrlichia* species like *E. canis* and *E. platys* in canine anaplasmosis [18, 21]. It is recommended that dog sera, which are seropositive to *B. burgdorferi* and *A. phagocytophilum*, be also examined by the western immunoblot technique [1]. In the present study, this technique was not used.

In conclusion, dogs in Sinop area, where *Ixodes ricinus* ticks are endemic, are highly in risk to acquire *B. burgdorferi* s.l. and *A. phagocytophilum* infectious. Accordingly, canine borreliosis and canine anaplasmosis must be considered as health problems in dogs by veterinarians, in areas infested with vectors of tick-borne agents. Also the seroprevalence rates in dogs could be used as an indicator of endemic status of a given region for humans, too.

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