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Research Article

The Prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* Ticks in the Sinop Region of Turkey

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Abstract: This study was conducted in order to determine the prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks, which are the primary vectors of this spirochete, as well as to investigate the effect of *I. ricinus* population density on the prevalence of *B. burgdorferi* s.l. Ticks were collected by blanket dragging in 13 different forest regions of Sinop, Turkey, and its vicinity. Ticks were identified under a stereomicroscope. *I. ricinus* (n = 334), *I. hexagonus* (n = 5), *Rhipicephalus* spp. (n = 10), *Dermacentor* spp. (n = 7), and *Hyalomma* spp. (n = 5) were collected. Direct immunofluorescence was used to detect *B. burgdorferi* in collected ticks. *B. burgdorferi* was detected in 15.9% (53/334) of the *I. ricinus* specimens examined. This spirochete was not detected in *I. hexagonus*, *Rhipicephalus* spp., *Dermacentor* spp., or *Hyalomma* spp. The prevalence of *B. burgdorferi* infection in *I. ricinus* tended to be higher in regions where *I. ricinus* was most abundant.

Key Words: *Borrelia burgdorferi* sensu lato, *Ixodes ricinus*, tick vector, prevalence

Türkiye'nin Sinop Bölgesinde Bulunan *Ixodes ricinus* Kenelerindeki *Borrelia burgdorferi* sensu lato Prevalansı

Özet: Bu çalışmada temel vektörleri olan *Ixodes ricinus* kenelerinde *Borrelia burgdorferi* sensu lato prevalansının tespit edilmesi ve *I. ricinus* yoğunluğunun *B. burgdorferi* s.l. prevalansı üzerine etkilerinin araştırılması hedeflenmiştir. Sinop ve çevresindeki 13 farklı ormanlık alanda havlu çekme yöntemiyle keneler toplandı. Stereomikroskop altında sınıflandırılmaları sonucunda bu kenelerin 334'ü *I. ricinus*, 5'i *I. hexagonus*, 10'u *Rhipicephalus* spp., 7'si *Dermacentor* spp., 5'i *Hyalomma* spp. olarak tanımlandı. *I. ricinus*'ların % 15,9'unda *B. burgdorferi* s.l tespit edilirken, *I. hexagonus*'larda ve *Rhipicephalus* spp., *Dermacentor* spp. and *Hyalomma* spp. gibi diğer sert kenelerde ise tespit edilmemiştir. Bir bölgede *I. ricinus* yoğunluğu arttıkça, *I. ricinus*'lardaki *B. burgdorferi* s.l. prevalansı da artış göstermektedir.

Anahtar Sözcükler: *Borrelia burgdorferi* sensu lato, *Ixodes ricinus*, kene vektörlüğü, prevalens

Introduction

Borrelia burgdorferi sensu lato, the causative agent of Lyme disease, is an important tick-borne spirochete with an expansive range throughout the world (1). *B. burgdorferi* was defined as a new species in 1984 by Johnson et al. (2). Additional species having genotypic similarities to *B. burgdorferi* were described in the following years. In all, 11 species have been included in the *B. burgdorferi* s.l. species complex. *B. burgdorferi* sensu stricto *(B. burgdorferi)*, *B. garinii,* and *B. afzelii* cause disease in humans and animals. Studies designed to determine which other species of *B. burgdorferi* s.l. cause Lyme disease are in progress (3-5).

Primary vectors of *B. burgdorferi* s.l. belong to the genus *Ixodes*. Vectors of various species of *B. burgdorferi* include *Ixodes ricinus* in Europe and Asia, *I. persulcatus*, especially in northern and eastern Asia, *I. scapularis (I. dammini)*, *I. pacificus*, and *I. dentatus* in America, *I. ovatus* in Japan, and *I. granulatus* in China (3,4,6).

Rodents infested with *Ixodes* ticks are the most important reservoirs for *B. burgdorferi*. Birds are important as reservoirs and for transmitting ticks and spirochetes inter-continentally (6-8). We report the prevalence of *B. burgdorferi* in *I. ricinus* in the Sinop region of Turkey.

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Materials and Methods

Study area and tick collecting

The Sinop region is located in northern Turkey (lat 41-42°N, long 34-35°E). The main reason for selecting the Sinop region was the high prevalence of *I. ricinus* in its coastal regions. In order to investigate the presence and prevalence of *I. ricinus*, 13 different study areas were chosen in Sinop and its vicinity.

Tick collection was carried out between May and June 2004 by dragging a 1.2 m^2 (1.5 m × 80 cm) blanket over low-lying vegetation (Figure 1). Collection was performed in forest and bushy areas where *I. ricinus* and its hosts frequently appear. Since the fields were bushy and forested, ticks were counted according to number of ticks per hour (n/h) instead of number of ticks per square meter (n/m^2), and tick density was calculated according time spent dragging the blanket. Ticks that were collected at each site during each dragging session were placed in 1.5-ml labeled Eppendorf tubes containing wetted absorbent papers. Ticks were kept alive in a refrigerator at 4 °C until they were identified.

Figure 1. Collecting ticks in the field by blanket dragging.

Classification

In all, 361 live ticks were identified under a stereomicroscope (9). Ticks of the *Ixodes* genus were also classified according to stage of development (i.e. female, male, nymph, and larva) (Figure 2).

Figure 2. Appearance of *Ixodes ricinus.*

DFA technique

The surface of each tick was cleansed by plunging into 70% ethanol twice and then into distilled water. Adult ticks were dissected with sterilized microforceps and a small lancet under a stereomicroscope. Midguts were removed and placed on Teflon microscope slides with 8 wells, each containing PBS. Slides of nymphs and larvae were prepared by crushing each specimen in PBS solution with a sterile solid glass tube. In addition, slides of heat-killed *B. burgdorferi* s.l. were prepared as positive controls (Kirkegaard & Perry Laboratories, Inc.).

Control and test slides were air dried and fixed in cold acetone (4 °C) for 20 min. Diluted (1:15) fluorescein isothiocyanate (FITC)-labeled goat anti-*B. burgdorferi* IgG antibodies (Kirkegaard & Perry Laboratories, Inc.) were added to each well and the slides were incubated for 30 min in a humid incubator at 37 °C. Slides were washed with PBS and examined under a fluorescence microscope (50 visual fields at a magnification of 400×) for spirochetes (Figure 3).

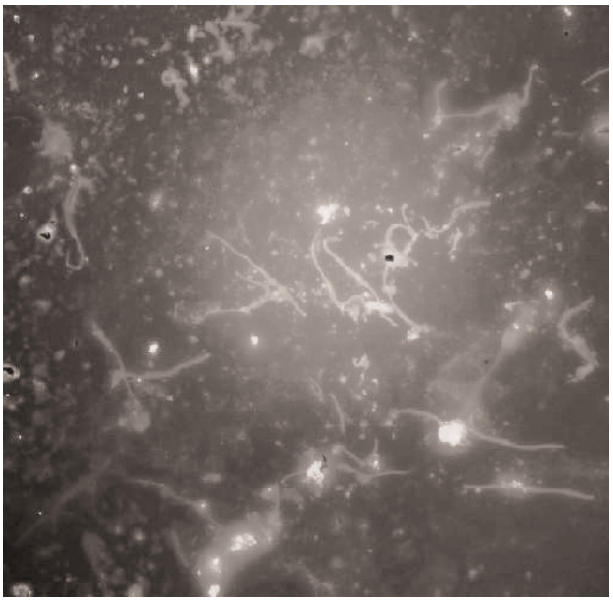

Figure 3. Appearance of *B. burgdorferi* s.l. under an immunofluorescence microscope.

Statistical Analysis

The chi-square test was used to evaluate the differences in the prevalence of *B. burgdorferi* between females, males, nymphs, and larvae. Similarly, differences between the prevalence of *B. burgdorferi* in the tick-dense areas and the tick-rare areas were analyzed by the chi-square test. Spearman's rank correlation test was used to examine the relationship between tick abundance and the prevalence of *B. burgdorferi* in ticks. SPSS v.10.0 was used for the statistical analyses and $P < 0.05$ was regarded as statistically significant.

Results

In all, 334 *I. ricinus* ticks were collected from 13 forest regions in Sinop and its vicinity, including 75 females, 33 males, 126 nymphs, and 100 larvae. Other ticks collected in this study were *I. hexagonus* (n = 5), *Rhipicephalus* spp. (n = 10), *Dermacentor* spp. (n = 7), and *Hyalomma* spp. (n = 5).

As seen in Table 1, 15.9% (53/334) of *I. ricinus* were infected. *B. burgdorferi* was detected in 28% (21/75) of the females, 42.4% (14/33) of the males, 11.1% (14/126) of the nymphs, and 4% (4/100) of the larvae. Spirochetes were not detected in *I. hexagonus* or other ticks.

The results showed that there were significant differences among infection rates between the 4 groups (females, males, nymphs, and larvae) (χ^2 correction: 38.387; P: 0.00001; $P < 0.05$). In addition, each group was individually compared to the other 3 groups. According to these results, infection rates were not significantly different between males and females (χ^2 correction: 2.18; P: 0.14; $P > 0.05$), but were significantly different between adults (males and females) and nymphs (χ^2 correction: 15.93; P: 0.0001; $P < 0.05$), and between nymphs and larvae (χ^2 correction: 3.846; P: 0.0499, $P = 0.05$).

Tick density at each collection site was calculated according to n/h. Thereafter, median tick density was computed as 1.111 (Table 2). Consequently, we categorized the study areas into low density (below 1.111) and high density (above 1.111) areas. The prevalence of *B. burgdorferi* s.l. in the high density areas

Table 1. Prevalence of *Borrelia burgdorferi* s.l. in *I. ricinus* collected in forest regions.

Tick	Number of specimens		Prevalence (%)
	Collected	Infected	
Female	75	21	28.0
Male	33	14	42.4
Nymph	126	14	11.1
Larvae	100	4	4.0
Total	334	53	15.9

Table 2. Distribution and density of *I. ricinus* collected in 13 areas of Sinop and the prevalence of *B burgdorferi* s.l. in *I. ricinus*.

No.	Study areas	Number of ticks collected (n)	Elapsed time for collection of ticks (h)	Tick density (n/h)	Number of ticks infected	Prevalence (%)
1	Uzungürgen/ Sinop	10	6	1.666	4	40.000
2	Bektaşağa/Sinop	11	7	1.570	8	72.727
3	Dibekli/Sinop	9	6	1.500	3	33.333
4	Osmanköy-Boyabat/Sinop	3	6	0.500	0	0.000
5	İmamlı-Boyabat/Sinop	4	7	0.571	1	25.000
6	Dağdalen-Durağan/Sinop	7	7	1.000	0	0.000
7	Hacıselli-Dikmen/Sinop	10	10	1.000	1	10.000
8	Abdaloğlu-Gerze/Sinop	11	8	1.375	6	54.545
9	Hıdırlı-Gerze/Sinop	4	7	0.571	1	25.000
10	Demirciköy/Sinop	6	8	0.750	1	16.666
11	Tepecik-Ayancık/Sinop	13	9	1.444	5	38.460
12	Hasandere-Erfelek/Sinop	10	9	1.111	3	30.000
13	Kabalı/Sinop	10	7	1.428	2	20.000
		108	97	14.486	35	

was 41.9% (31/74), and in the low density areas it was 11.8% (4/34) (Table 3). There was a statistically significant difference between the 2 groups (χ^2 correction: 9.7; P: 0.0019; $P < 0.05$). There was also a positive correlation between the density of *I. ricinus* and the prevalence of *B. burgdorferi* (Spearman's rho, r: 0,733; $P = 0.004$). According to Spearman's rho, the coefficient of determination (r^2) was 0.537. Both R-square and scatter plot (with regression line) showed that the prevalence of B. *burgdorferi* in ticks increased with tick density (Figure 4).

Table 3. The prevalence of *Borrelia burgdorferi* s.l. according to density of *I. ricinus.*

Density	Number of specimens		Prevalence (%)
	Collected	Infected	
Dense	74	31	41.89
Rare	34	4	11.76
Total	108	35	32.41

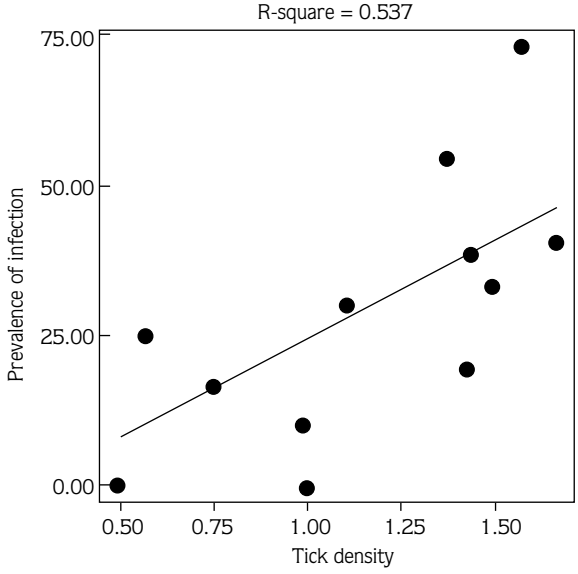

Figure 4. The relationship between tick density and the prevalence of ticks infected with *B. burgdorferi.*

Discussion

Various ecological components determine the incidence of Lyme disease in a region. The most important components are: the prevalence of *Ixodes* ticks in a region, the prevalence of *B. burgdorferi* in vector ticks, the abundance of vertebrate reservoirs for *B.*

burgdorferi, and the climate and geographic conditions of the region.

B. burgdorferi s.l. infection rates of unfed ticks collected from various European countries were reported as 0%-36% in adults, 0%-17% in nymphs, and 0%-6% in larvae (10-12). These findings are similar to our results (Table 1). The prevalence of infection in adult ticks was higher than in nymphs because the adults fed twice on host animals, whereas nymphs fed only once. Similarly, the prevalence of infection in nymphs was higher than in larvae because unfed larvae never fed on host animals. Hence, the fact that unfed larvae were infected suggests that *B. burgdorferi* was transmitted transovarially.

Others have reported differences in infection rates between male and female ticks. Some authors reported a predominance of female ticks over male ticks for carrying *B. burgdorferi* infection, while other authors reported the opposite (11,13). In this study, there was no significant difference in the infection rate of *B. burgdorferi* between male and female ticks.

There was an increase in the prevalence of *B. burgdorferi* where tick density was highest. Talleklint and Jaenson (14) observed that, in regions where nymphal density was ≥ 10 nymphs per 100 m^2, *Borrelia*-like spirochete infection was high as well. In our study, we found a higher prevalence of *B. burgdorferi* in high density regions than in low density regions, and this difference was statistically significant. Moreover, we detected a positive relationship between tick density and the infection rate of *B. burgdorferi*.

The climate and soil structure has a great effect on the density of *I. ricinus* in a given region (11,15). Our findings and those of others show that coastal regions of Turkey are suitable habitats for *I. ricinus* (10).

Domestic animals have greater exposure to vector ticks than humans. Thus, animals are at greater risk for acquiring Lyme disease than humans (16). In North America, *B. burgdorferi* is perpetuated by enzootic cycles involving rodents, such as white-footed mice (*Peromyscus leucopus*), and is transmitted to humans and other animals by Ixodid ticks (17). There is ongoing debate in Europe about the preferred animal hosts of *I. ricinus*. These ticks feed on more than 300 animal species, including large and small mammals, birds, and reptiles (18). In Asia, immature *I. persulcatus* were reported to commonly feed on voles, shrews, and birds, while adult ticks feed on larger animals, including hares, deer, and cattle (19).

It has been reported that 30 species of ticks in 6 genera have been detected in Turkey (20,21). Çalışır et al. (22) reported that ticks (n = 835) collected from domestic grazing animals were classified as *Ixodes ricinus, Rhipicephalus bursa*, *Hyalomma marginatum (plumbeum)*, *Boophilus calcaratus (annulatus)*, and *Dermacentor marginatus.* They did not detect *B. burgdorferi* in *I. ricinus* ticks. Yay et al. (23) reported that 6 tick genera and 11 species of ticks in Kayseri, Turkey, which feed on cattle and sheep, included *Rhipicephalus turanicus, Rhipicephalus sanguineus, Rhipicephalus bursa, Hyalomma anatolicum, Hyalomma excavatum, Hyalomma detritum, Haemaphysalis sulcata, Haemaphysalis parva, Dermacentor marginatus, Boophilus annulatus*, and *Ornithodoros lahorensis*. Guner et al. (24) reported that there was a low prevalence of *B. burgdorferi* in wild mice captured in northern Turkey; 2 out of 22 *Apodemus sylvaticus* specimens were seropositive for *B. afzelii*. Infection rates of species collected in our study were similar to the reports by others; *B. burgdorferi* was detected only in *I. ricinus.*

We conclude that tick control studies, as well as personal protection measures, repellents, and acaricides are essential tools for minimizing the incidence of Lyme disease. Further research is needed to assess the risk of exposure to Lyme disease in Turkey.

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