# The investigation of seroprevalence of *Borrelia burgdorferi* and *Rickettsia conorii* in people living in forest villages of Sinop

## Sinop'un orman köylerinde yaşayan insanlarda *Borrelia burgdorferi* ve Rickettsia conori seroprevalansının araştırılması

**Turabi Güneş**

Sivas Cumhuriyet University, Vocational School of Health Services, Sivas, Turkey
**Corresponding author:** Turabi Güneş, Sivas Cumhuriyet University, Vocational School of Health Services, Sivas, Turkey
**E-mail:** turabigunes@hotmail.com
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## SUMMARY

**Objective:** This study aimed to investigate the seroprevalence of *Borrelia burgdorferi*, the causative agent of Lyme disease, and *Rickettsia conorii*, the causative agent of Mediterranean spotted fever, in the Sinop region.
**Method:** In 2006 and 2007, the blood serum of 184 people, living in the villages of the central district of Sinop, was obtained and stored at 80ºC until the study. In these sera, IgG antibodies reactive to *B. burgdorferi* and *R. conorii* were investigated by ELISA method.
**Results:** Antibody positivity was found against *B. burgdorferi* in 36.41% and *R. conorii* in 45.10% of 184 people living in rural areas of Sinop. Reactive antibodies against *B. burgdorferi* were detected in 47.62% of 42 people with a history of redness and swelling at the contact site after the tick bite, and in 28.95% of 76 people without these symptoms **(p= 0.043, OR=2.23)**. The seropositivity to *R. conorii* was found in 57.14% and 46.05% of persons with and without these symptoms, respectively **(p= 0.363, OR= 1.56).** There was a statistically significant increase in seroprevalence of both *B. burgdorferi* and *R. conorii* with increasing age **(p<0.05).** *R. conorii* and *B. burgdorferi* co-seroprevalence were determined in 16.85% of serum samples. Since the ELISA test results in both pathogens were not repeated with confirmation tests, these findings were considered possible.
**Conclusions:** The findings obtained in this study revealed that Sinop and similar ecological features should be evaluated within the endemic regions for *B. burgdorferi* and *R. conorii*. It should be paying attention that infections from both pathogens may be likely to appear at these regions.
**Keywords**: *Borrelia burgdorferi*, *Rickettsia conorii*, lyme disease, mediterranean spotted fever, erythema migrans, tick bite

Turabi Güneş

ORCID IDs of the authors:
T.G. 0000-0002-8011-7844

## ÖZET

**Amaç:** Bu çalışmada, kene kaynaklı patojen arasındsa ön sıralarda olan Lyme hasta'lığının etkeni *Borrelia burgdorferi* ve Akdeniz benek ateşi etkeni olan *Rickettsia conorii*'nin Sinop yöresinde yaşayan insanlardaki seroprevalanslarının araştırılması amaçlanmıştır.

**Yöntem:** 2006-2007 yıllarında, Sinop'un merkez ilçesine bağlı köylerde yaşayan 184 kişinin kan serumu elde edilmiş ve çalışma anına kadar -80°C'de saklanmıştır. Bu serum örneklerinde ELISA yöntemiyle *B. burgdorferi* ve *R. conorii*'ye karşı reaktif IgG antikorları araştırılmıştır.

**Bulgular:** Sinop'ta kırsal kesimde yaşayan 184 kişinin %36.41'sında *B. burgdorferi*'ye ve %45,10'unda ise *R. conorii*'ye karşı antikor pozitifliği saptanmıştır. Kene tutması sonrası temas bölgesinde kızarıklığı ve şişlik öyküsü olan 42 kişinin %47,62'sinda, bu belirtileri olmayan 76 kişinin ise %28.95'inde *B. burgdorferi*'ye karşı reaktif antikorlar tespit edilmiştir **(p= 0,043, OR= 2,23)**. Bu belirtiler gösterenler ve göstermeyenlerdeki *R. conorii*'ye karşı antikor pozitifliği ise sırasıyla %57,14 ve %46,05 oranlarında elde edilmiştir **(p= 0,336, OR= 1,56).** Yaş artışıyla birlikte hem *B. burgdorferi*, hem de *R. conorii* seroprevalansında istatistik açıdan da önemli bir artış saptanmıştır **(p<0,05).** Serum örneklerinin %16,85'inde *R. conorii* ile *B. burgdorferi* ko-seropozitifliği saptanmıştır. Her iki patojen için de ELISA test sonuçları doğrulama testleri ile tekrarlanmadığından, bu bulgular olası olarak değerlendirilmiştir.

**Sonuç:** Bu çalışmada elde edilen bulgular, Sinop ve benzeri ekolojik özelliklere sahip yörelerin *B. burgdorferi* ve *R. conorii* için endemik yöreler içerisinde değerlendirilmesi gerektiğini ortaya koymuştur. Her iki patojenden kaynaklanan enfeksiyonların bu yerlerde görünmesinin muhtemel olabileceği dikkate alınmalıdır.

**Anahtar sözcükler**: *Borrelia burgdorferi*, *Rickettsia conorii*, Lyme hastalığı, akdeniz benekli ateşi, Eritema Migrans, kene ısırması

## INTRODUCTION

Blood-fed ticks; it serves as a vector for many tick-borne pathogens that are viral, bacterial and protozoan in animals and humans. *Borrelia burgdorferi*, the causative agent of Lyme disease, and *Rickettsia conorii*, the causative agent of Mediterranean spotted fever, are among the tick-borne pathogens [1,2].

The main vectors of *B. burgdorferi* are ticks of the genus *Ixodes*, and the reservoir is usually small rodents. The genus *Ixodes* show a wide geographic distribution on earth and the primary vector of *B. burgdorferi* in Europe is *I. ricinii* [1,2]. The most important tick vector of *R. conorii* is the *Rhipicephalus sanguineus*, and the essential mammal reservoirs are dogs, rabbits and hedgehogs [3].

Although there are many species in the genomic group *B. burgdorferi* sensu lato, in Europe, *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. spielmanii* and *B. bavariensi* cause Lyme borreliosis. The clinical picture of Lyme disease consists of 3 different stages: early local infection, early widespread infection and chronic infection. The most classic appearance of initial local infection is erythema migrans (EM), which macular or papular reddening lesions that develop between 3-30 days after tick bites. The most typical form of early diffuse infection is multiple erythema migrans (MEM), and the most characteristic clinical type of chronic disease is Acrodermatitis Chronica Atrophicans (ACA) [2,4,5]. Mediterranean spotted fever (MSF) caused by *R. conorii* is most common in Europe. There is a history of contact with the dog in 80% of patients with MSF. Therefore, most infections are likely to be transmitted by ticks found in dogs. Symptoms such as fever, headache and muscle pain occur 6-10 days after the tick bite. After the third week, maculopapular redness occurs in 95% of patients [3,6].

In epidemiological studies on Lyme disease and MSF, indirect serological methods are often used. Indirect Immunofluorescence (IFA), Enzyme Immunoassay (EIA, ELISA) and Western blot (WB) tests are one of the methods used to determine specific antibodies in serum. Although the sensitivity and specificity of ELISA have been increased in recent years, it is recommended to confirm positive samples in Lyme-ELISA with WB. Although the sensitivity of ELISA is low during early local infection, it is 97.5% (95% - 100%) at late stages [4,5].

The many regions of Turkey, including the Black Sea region, is suitable for *R. sanguineus* and *I. ricinus*. *I. ricinus* is known to be abundant, especially in our coastal areas [7]. This study aimed to investigate the seroprevalence of *B. burgdorferi* and *R. conorii* in humans with a history of tick bites in villages located in the forest of Sinop and to investigate this seroprevalence in terms of sociodemographic and medical parameters.

## MATERIAL AND METHODS

### Study area

In Sinop is located in the Central Black Sea region **(Figure 1),** rainfall is seen almost every season, the average annual rainfall is 679-1077 mm. The average temperature is 22°C in summer and 7°C in winter. Having a mild climate, Sinop is covered with rich forests and vegetation as it always receives rainfall. This forest and vegetation contain numerous species of rodents that serve as reservoirs for tick-borne microorganisms. Besides, *I. ricinus*, the vectors of many microorganisms like *A. phagocytophilum*, *B. burgdorferi* and *R. conorii*, are commonly found in Sinop [7]. The village settlement areas in the Sinop region are usually within the forested areas or on the edge of the forest.

### Collection of Blood Samples

Between May and June in 2006 and 2007, of the 42 villages in the central district of Sinop, 14 villages were identified by cluster sampling and 900 people were contacted, and blood samples were taken from 273 of them. During the blood sampling, information such as tick bite history in the last four years, occupation and gender were recorded. After obtaining the serum of blood samples were stored at -80ºC until the investigation. In our previous studies, seroreactive IgG antibodies against *A. phagocytophilum*, *Babesia microti* and Tick-borne encephalitis virus (TBEV) were also investigated in these sera [8–10]. In this study, according to the studies previously conducted in Turkey, it was assumed that the antibody prevalence of *B. burgdorferi* and *R. conorii* would be at most 10-14% [11–14]. According to 2006 data, the sample size was calculated as 182, for the population of approximately 10.000 people living in the central villages of Sinop, at the 95% confidence interval and the ± 5% error. In order to investigate the prevalence of reactive IgG against *B. burgdorferi* and *R. conorii*, 184 of from 273 serum samples that we used in previous studies were included in this study according to the random sampling method. Ethics committee approval was received from Cumhuriyet University Faculty of Medicine Ethics Committee for the research (2010-06 /26).

### Serological Tests (ELISA)

In the investigation of *B. burgdorferi*-IgG antibody in rural people, "Zeus Scientific, Inc. Netherland "commercial ELISA kit was used. Each 96-test ELISA test strip is in the format of 12 pieces (1x8 strip) and the microwells are coated with *B. burgdorferi* (strain B-131) antigen,

capable of binding to IgG antibodies against *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. Before starting the study, human serums and the contents of the ELISA kit were brought to room temperature, and the test procedure was performed. Finally, the absorbance (ABS) values of the serum samples and standard sera were determined by reading the microplates at 450 nm wavelength in ELISA microplate reader (Bio-Tek Instruments, Inc., Winooski, Vermont, USA). The cutoff value was found by multiplying the calibrator value by 0.25, as indicated in the kit insert. Serums with an absorbance value above cutoff value x 1.1 were considered positive. Serum samples positive for *B. burgdorferi* were subjected to RPR testing (Rapid Plasma Reagin, carbon slide) to eliminate the possibility of cross-reaction between *B. burgdorferi* and *Treponema pallidum*.

*R. conorii*-IgG antibody was investigated by using a commercial ELISA kit from "Vircell microbiologist, GRANADA, SPAIN". The micro-wells of each 96-test ELISA test strip were coated with *R. conorii* (Moroccan strain, ATCC VR-141) antigen. Each strip was set to be used 92 test sera, two cutoffs, one positive control and one negative control, and all procedures were performed according to the commercial test procedure. Finally, the test sera, control and cutoff sera were read on the spectrophotometer at a wavelength of 450 nm. The antibody index was calculated as indicated in the kit insert (optical density of test serum / optical density of cut of serum x 10). Serums with an antibody index of 11 or higher were considered positive.

### Statistics

Chi-square test and Binary logistic regression analysis tests were used to evaluate the differences in the seroprevalence of *R. conorii* and *B. burgdorferi* between tick-bite history, redness after tick bite history, gender, age and occupational groups. Spearman's rank correlation tests (Spearman's rho) were used to examine the relationship between age and ELISA-IgG absorbance values of subjects. Statistical analyses were performed using licensed SPSS-23 software (SPSS, Inc., Chicago, IL) and $p < 0.05$ was considered statistically significant.

## RESULTS

The distribution of *B. burgdorferi* and *R. conorii* seroprevalences according to different parameters in rural people in Sinop is given in **Table 1**. Since 3 of the 70 serum samples, which positive for *B. burgdorferi*, were also found positive in the RPR

test, these three samples were not evaluated. As a result, antibody positivity to *B. burgdorferi* was found in 67 (36.41%) and *R. conorii* in 83 (45.10%) of 184 people living in rural areas of Sinop **(Table 1).**

In the distribution of IgG seroprevalence of *B. burgdorferi* and *R. conorii*; there was no statistically significant difference between those with and without a tick-bite history in the last four years, and between occupation group, and between genders **(p>0.05).**

Of 118 people with a history of tick bites, 42 (35.59%) reported a significant redness and swelling at the contact site after the tick bite. Reactive antibodies against *B. burgdorferi* were detected in 20 (47.62%) of 42 subjects with redness and swelling at the contact area after tick bites, and 22 (28.95%) of 76 subjects without these symptoms after tick bites. The difference between the two groups was statistically significant **(p<0.05)**. The risk of *B. burgdorferi* infection (in terms of antibody positivity) was found to be approximately 2-fold higher in subjects with redness and swelling after a tick bite than those who did not **(p= 0.043, OR= 2.23 (CI; 1.02-4.88)**. Antibody positivity to *R. conorii* was observed in 57.14% and 46.05% of persons with and without redness and swelling after tick bites, respectively **(p= 0.356, OR= 1.56, CI; 0.73-3.34).**

In this study, both seroprevalences of *B. burgdorferi* and *R. conorii* were found to increase significantly with age **(p<0.05) (Table 1).** A significant correlation was found between the mean age and the prevalence of IgG in both *B. burgdorferi* and *R. conorii* **(p<0.05, r: 0.769 and 0.875, respectively).** The scatter plot curve between the ELISA absorbance values (*B. burgdorferi* or *R. conorii*) and the age of the subjects reveals the direction of this correlation **(Figure 2-3).** In this study, the mean age of 184 people was 44.07 (± 18.22), and the median was 44. Based on the median value, both the seroprevalences of *B. burgdorferi* and *R. conorii* were higher in persons older than 44 years, according to those younger than 44 years **(p<0.05).** The risk of *B. burgdorferi* seropositivity was approximately 2-fold **(OR= 1.92, CI= 1.05-3.55)** in individuals older than 44 years according to people under the age of 44, while the risk of *R. conorii* antibody positivity was approximately 3-fold **(OR= 3.03, CI= 1.66-5.55).**
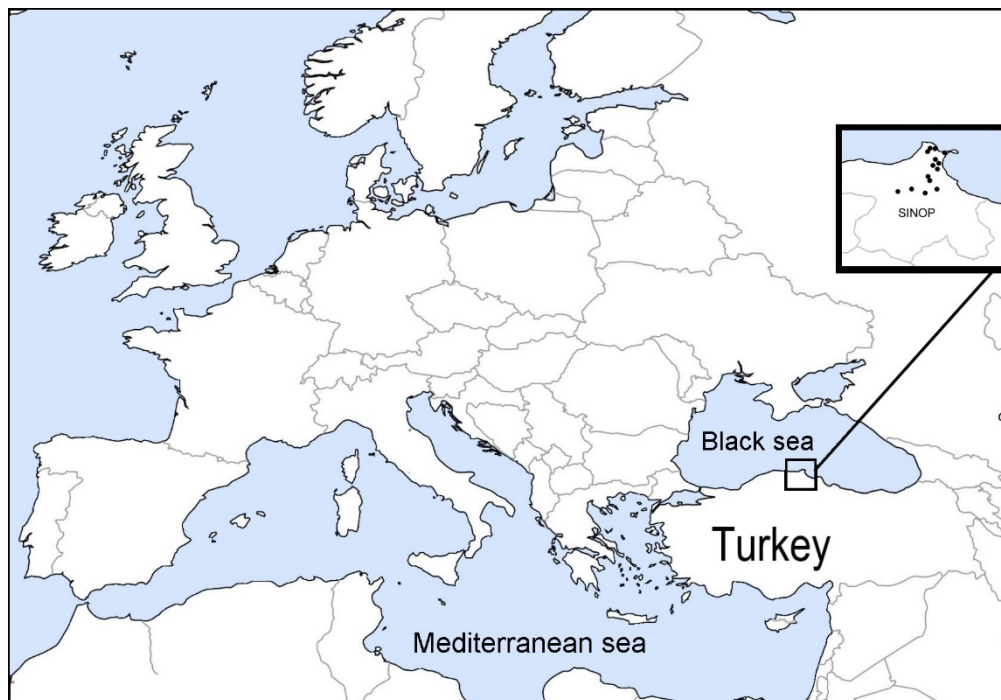


**Figure 1**: Forest villages of Sinop, Turkey, from which 184 persons were sampled.

**Table 1:** Seroprevalence and demographic characteristics of *B. burgdorferi* and *R. conorii* in humans, living in rural areas of the Sinop region

| Risk factors | n | *B. burgdorferi* Positive (%) | $X^2P$ | OR (CI%95) | *R. conorii* Positive (%) | $X^2P$ | OR (CI%95) |
|---|---|---|---|---|---|---|---|
| **Total** | 184 | 67 (36.41) | | | 83 (45.10) | | |
| **Gender** | | | | | | | |
| Female | 90 | 33 (36.67) | 0.944 | 1.02 (0.56-1.86) | 40 (44.44)) | 0.859 | 0.95 (0.53-1.69) |
| Male* | 94 | 34 (36.17) | | | 43 (45.74) | | |
| **Tick bite** | | | | | | | |
| No* | 66 | 25 (37.87) | 0.757 | 0.91 (0.49-1.69) | 24 (36.36) | 0.075 | 1.75 (0.94-3.25) |
| Yes | 118 | 42 (35.59) | | | 59 (50.00) | | |
| **Redness.Swelling** | | | | | | | |
| No* | 76 | 22 (28.95) | 0.043 | 2.23 (1.02-4.88) | 35 (46.05) | 0.336 | 1.56 (0.73-3.34) |
| Yes | 42 | 20 (47.62) | | | 24 (57.14) | | |
| **Occupation** | | | | | | | |
| Shepherd | 35 | 11 (31.43) | | 4.58 (0.52-40.38 | 19 (54.29) | | 990 (0.24-3.86) |
| farming | 138 | 55 (39.85) | 0.099 | 6.63 (0.83-53.24) | 58 (42.03) | 0.347 | 604 (0.18-2.01) |
| Forest Worker * | 11 | 1 (9.09) | | | 6 (54.55) | | |
| **Age. 44** | | | | | | | |
| ≤ 44 y* | 96 | 28 (29.16) | 0.033 | 1.92 (1.05-3.55) | 31 (32.29) | 0.001 | 3.03 (1.66-5.55) |
| > 44 y | 88 | 39 (44.32) | | | 52 (59.09) | | |
| **Age groups (M)** | | | | | | | |
| 11-20 (16.63)* | 24 | 8 (33.33) | | 0.44 (0.12-1.57) | 0 (0.00) | | ---- (-----------)** |
| 21-30 (26.14) | 28 | 5 (17.86) | | 0.70 (0.22-2.24) | 8 (28.57) | | 3.46 (1.17-10.26) |
| 31-40 (36.97) | 31 | 8 (25.81) | 0.012 | 1.27 (0.43-3.75) | 18 (58.06) | 0.001 | 2.24 (0.78-6.39) |
| 41-50 (46.08) | 36 | 14 (38.89) | | 0.67 (0.19-2.34) | 17 (47.22) | | 2.96 (0.94-9.31) |
| 51-60 (55.71) | 24 | 6 (25.00) | | 3.00 (0.93-9.63) | 13 (51.17) | | 4.44 (1.40-14.14) |
| 61-70 (64.60) | 25 | 15 (60.00) | | 4.40 (1.13-17.07) | 16 (64.00) | | 5.50 (1.44-20.96) |
| 71-80 (76.25) | 16 | 11 (68.75) | | | 11 (68.75) | | |

**OR:** Risk ratio, **CI:** Confidence Interval, **M:** Mean, **\*:** Reference category, **\*\*:** In terms of *R. conorii*, since the seroprevalence was "0" in the 11-20 age group, the 21-30 age group was taken as a reference category.
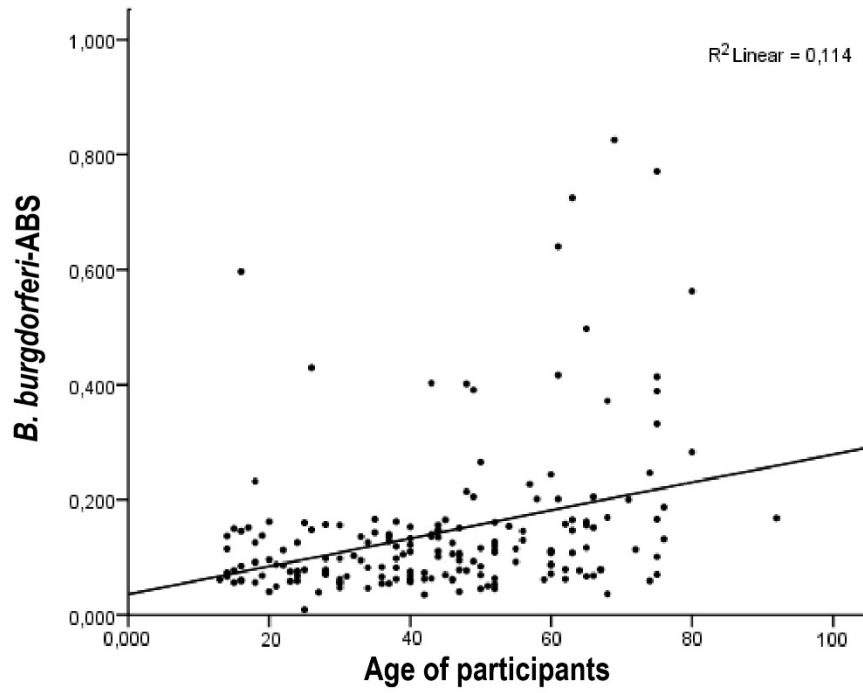
**Figure 2**: Scatter plot curve between ELISA absorbance values of *B. burgdorferi* and age of subjects
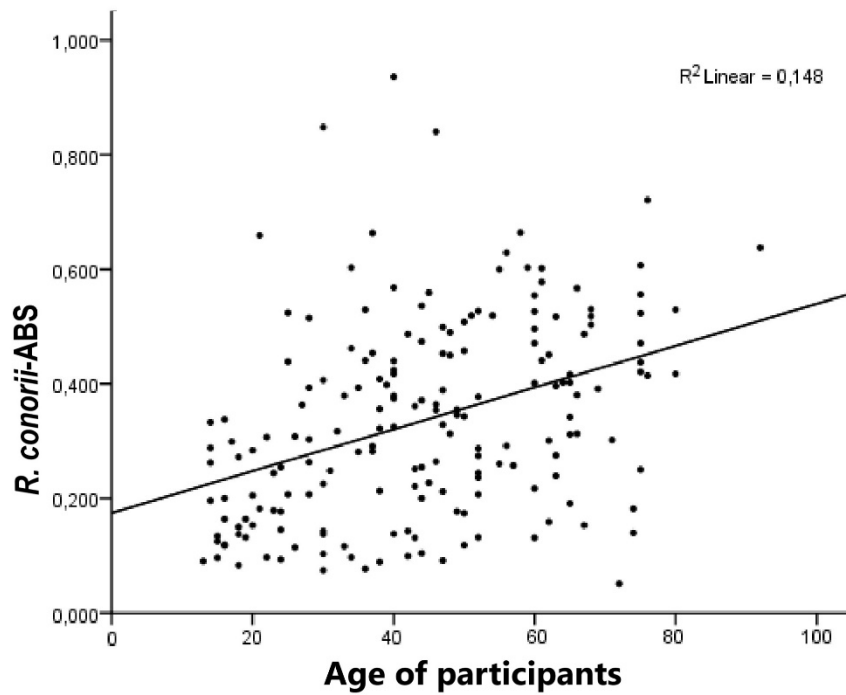


**Figure 3**: Scatter plot curve between ELISA absorbance values of *R. conorii* and age of subjects

In this study, co-seropositivity of *R. conorii* and *B. burgdorferi* was found in 31 (16.85%) of serum samples. A positive correlation was found between ELISA absorbance values of *B. burgdorferi* and *R. conorii*, and the result was statistically significant **(Spearman Rho, R= 0.198, p= 0.007)**. Regression was formulated as "*R. conorii* (ABS)= 0.303 + 0.174 x *B. burgdorferi* (ABS), (F= 5.71, p= 0.018).**

In the previous studies, reactive IgG antibodies against *A. phagocytophilum*, *B. microti* and TBEV were also investigated in serum samples of this study. In our earlier studies, we found that co-seroprevalence between *A. phagocytophilum* and *B. burgdorferi* was 3.30%, and that of TBEV and *B. burgdorferi* was 1.5% [9,10]. In the current study; in 184 serum samples, there were co-seropositivity 8 (4.35%) between *B. microti* and *B burgdorferi*, 11 (5.98%) in *A. phagocytophilum* and *R. conorii*, 8 (4.35%) in *R. conorii* and *B. microtii,* and 3 (1.63%) in TBEV and *R. conorii*.

Although the sensitivity and specificity of the *B. burgdorferi* IgG-ELISA test were very high in early generalised infection and chronic infection stages, *B. burgdorferi* seroprevalence detected in each parameter was evaluated as "possible results since the results were not confirmed by Western-Blot validation test.

## DISCUSSION

The most important factors that determine the prevalence of tick-borne pathogens in a region are the climate of that region, the reservoir of tick-borne pathogens, and the density of vector ticks. According to sociocultural and similar demographic parameters in people living in a district, the probability of contact with the tick and thus the prevalence of tick-borne pathogens may vary.

There may be differences in the incidence of MSF and Lyme disease among people living in urban and rural areas, as well as differences between different occupational groups in the same geographical area. The prevalence of antibodies to *R. conorii* in Europe and the Mediterranean coastal countries is generally between 2% and 30% in urban people and healthy blood donors [15,16]. However, the seroprevalence of *R. conorii* in persons in the countryside is between 10% and 60% [15,16]. Sero-epidemiological findings that have been made in Turkey is a similar direction [17]. Lyme seroprevalence in healthy blood donors and urban population ranged from 0.48% to 19.7% (18,19). Similar to *R. conorii*, the seroprevalence of *B. burgdorferi* in rural people is also high,

usually ranging from 2% to 60% [13,19–21]. The prevalence of *B. burgdorferi* (36.41%) and *R. conorii* (45.10%) obtained in this study reveals that Sinop and similar ecological district should be evaluated in endemic areas for both pathogens.

In this study, no difference was found between the occupational groups regard both the seroprevalence of *B. burgdorferi* and *R. conorii*. The reason for this may be attributed to the fact that 90% of the people living in this region are engaged in animal husbandry. In terms of gender, according to serological studies in Turkey and other countries, conflicting results related seroprevalence of Lyme disease and MSF was reported [14,17,20–22]. In this study, both *B. burgdorferi* and *R. conorii* seroprevalence were similar levels in men and women. This result may be because men and women living in rural areas of Sinop are engaged in similar agricultural and animal husbandry activities.

In terms of people living in rural areas, the seroprevalence of tick-borne infections is generally higher in people aged 40 and over [20,21]. Similarly, in the present study, *B. burgdorferi* and *R. conorii* seroprevalence were higher in individuals over 44 years of age, according to those under 44 years. Also, a linear increase in antibody prevalence against both *B. burgdorferi* and *R. conorii* was found in parallel with the increase in age **(Figure 2-3)**. IgG-type antibodies caused by *B. burgdorferi* and SFG-*Rickettsi*a infections can be detected in serum for years [23,24]. This result may lead to a higher prevalence of *B. burgdorferi* and *R. conorii* IgG in higher age groups. Generally, in rural areas, with the increase in age, the duration of intraday agriculture and animal husbandry and thus the possibility of tick bite exposure may increases.

In the study, there was no difference in antibody positivity to both *B. burgdorferi* and *R. conorii* between individuals with and without a history of tick bites. The fact that a significant proportion of tick bites occur unnoticed can be an essential reason. Of the 118 people with a history of tick bites, 42 reported considerable redness and swelling at the contact site after the event. It is doubtful to determine which of these lesions are definite EM lesions. It is conceivable to think that these people's statements about redness and swelling have to do with the blood-sucking time of ticks. These lesions after tick bites can be a sign of ticks sucking blood for a sufficient time. Studies have shown that a contact time of at least 12-24 hours is required for *I. ricinus* to transfer *B. burgdorferi* to the host [25]. Similar contact times

may also be necessary for *R. conorii* [26]. In this study, antibodies against *B. burgdorferi* were found in 47.6% of the subjects who showed signs of redness and swelling after ticks bite and 28.9% of those who did not, and the difference between the two groups was statistically significant **(p= 0,043, OR= 2,23, CI; 1,02-4,88).** Similarly, although there was no statistically significant difference, *R. conorii* seroprevalence was found to be higher in subjects with a history of swelling and redness after tick bites **(p= 0,336, OR= 1,56, CI; 0,73-3,34).**

The prevalence of Lyme disease in different countries of Europe varies between 0.8 and 315 per 100.000 **(2,18).** The annual incidence of MSF cases in the Mediterranean countries is around 50 per 100.000 [27]. In Turkey, although MSF and Lyme cases were seen, there is no comprehensive documentation on the annual incidence of these pathogens [28,29].

As a result, the high prevalence of antibodies against *B. burgdorferi* and *R. conorii* in humans in Sinop and similar climatic regions indicates that these people frequently encounter ticks and tick-borne pathogens. Accordingly, it should be taken into account by health institutions that symptomatic *R. conorii* and *B. burgdorferi* infections are likely to occur.

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