TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 727-732

doi:10.3906/sag-1504-100

Evaluation of *Borrelia burgdorferi* sensu lato seroprevalence in the province of Bolu, Turkey*

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Received: 21.04.2015 • **Accepted/Published Online:** 27.07.2015 • **Final Version:** 19.04.2016

Background/aim: The aim of the present study was to determine the seroprevalence of *Borrelia burgdorferi* sensu lato in the city center and the province of Bolu, Turkey.

Materials and methods: A stratified sampling method was used to determine the study population. A total of 196 blood samples were collected. A questionnaire was completed by each participant in the study. ELISA was performed and positive serologic results were confirmed using western blotting. Data were analyzed statistically using the chi-square test.

Results: Seropositivity rates of *B. burgdorferi* IgM and IgG were determined as 14.8% (29/196) and 13.7% (27/196) respectively by ELISA. A total of nine serum samples (4.6%) were found positive for IgG, and seven samples (3.8%) were positive for IgM according to western blotting. Seropositivity rates were found to be higher in people living in rural areas (11.1%), in women (8.3%), in people who were illiterate (10.0%), in people engaged in agriculture (10.8%), and in the age group of 40–60 years (10.0%). No statistically significant difference was found between seropositivity rates and survey data.

Conclusion: The seroprevalence of Lyme disease was determined in our region. Detection of endemic regions of Lyme disease with determination of seropositivity rates will increase the awareness among clinicians about this disease.

Key words: *Borrelia burgdorferi*, Lyme disease, western blotting

1. Introduction

Lyme disease is a multisystemic disease caused by *Borrelia* species, and it is transmitted to humans by ticks of the species *Ixodes*. There are three genotypes of the Lyme disease agent: *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii*. They are grouped together under the name *B. burgdorferi* sensu lato (1). The causative agent of Lyme disease, *B. burgdorferi*, was isolated in 1982 (2). Lyme disease is evident in three stages. The first stage includes erythema migrans (EM) and needs no serological testing. Diagnosis becomes more difficult in the second and third stages. In such cases, two-step diagnosis is necessary. The first step is based on an enzyme-linked immunosorbent assay (ELISA) test, and positive results should be confirmed by Western blot (WB) (3). Cross-reactions have been found between antibodies to syphilis, leptospirosis, relapsing fever, varicella, infectious mononucleosis, and some autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis), and ELISA tests may provide false positive results so the results should be confirmed by WB (4).

This disease is a common tick-borne zoonosis in European countries and the United States. In Turkey, the first cases were reported in 1990 in the Black Sea and Aegean regions (1). Because Lyme disease is not a compulsory notification disease in Turkey and due to confusion with clinical signs of other diseases, the exact prevalence is not known.

In the present study, the aim was to determine the seroprevalence of *Borrelia burgdorferi* sensu lato in the city center and the province of Bolu, Turkey.

2. Materials and methods

Permission for the study was obtained from the Abant İzzet Baysal University Clinical Research Ethics Committee, the Bolu Provincial Health Directorate, and the Association of Public Hospitals.

* This study was published as a poster at the 6th Congress of Infectious Diseases, 24–27 September 2014, Belgrade, Serbia (Poster No. 395).

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2.1. Sample size

Blood samples were collected between August and October 2013 from the Bolu city center and the Gerede, Mudurnu, Göynük, Yeniçağa, Seben, Mengen, Dörtdivan, and Kıbrıscık districts to investigate the seropositivity rates of Lyme disease. A stratified sampling method was used to determine the study population. The sample size was calculated using the following formula:

$$n = \frac{N \cdot t^2 \cdot p \cdot q}{d^2 \cdot (N-1) + t^2 \cdot p \cdot q}$$

In this formula, N represents the population size (the population in the province of Bolu is 276,500), t is a constant (1.96), p is the a priori assumption of the population parameter (0.15 according to the results of recent studies in Turkey), q is equal to 1 – p, and d is the margin of error (0.05%). The number of research samples (n) was defined as 196. The sample size was compared for each district according to population density.

2.2. Questionnaire

Each participant was asked about demographic information, place of residence, any clinical symptoms of tick exposure, where they spent the summer, and whether they dealt with agriculture.

2.3. Laboratory tests

Blood samples of at least 5 mL were taken from patients and were transferred in appropriate circumstances to the microbiology laboratory of Abant İzzet Baysal University, Faculty of Medicine. Blood samples were centrifuged at 4000 rpm for 10 min and the obtained sera were stored at –20 °C until analysis. First, a total of 196 blood samples were investigated for diagnosis of Lyme disease by the ELISA screening method. A commercial ELISA kit (Euroimmun, Germany) was used to investigate IgM against *B. burgdorferi*, *B. afzelii*, and *B. garinii* and IgG antibody levels against *B. burgdorferi*, *B. afzelii*, *B. garinii*, and *Borrelia* vlsE antigens. Results were evaluated semiquantitatively. At the end of the study, positive and borderline IgG and IgM results were included in the second round of study. ELISA serum samples with positive or borderline results were confirmed using the *Borrelia* EUROLINE RN-AT WB kit (Euroimmun). WB IgG and IgM results were evaluated in accordance with the criteria in the kit. ELISA and WB tests were performed according to the manufacturer's recommendations.

2.4. Statistical analysis

Data analysis was performed using SPSS 17.0 for Windows. Descriptive statistics were expressed as numbers and percentages. Differences between groups in terms of categorical variables were analyzed using the chi-square and Fisher exact tests. The results were evaluated at a 95% confidence interval and $P < 0.05$ was accepted as significant.

3. Results

The study included 196 individuals; 61.2% of them were female and 38.8% were male.

According to the ELISA test results 14.8% were positive (29/196) and 1.0% were borderline (2/196) for IgM, with 13.7% positive (27/196) and 5.1% borderline (10/196) for IgG. The borderline results from ELISA were negative in the WB confirmatory test. A total of nine serum samples (4.6%) were found to be positive for IgG, and seven samples (3.8%) were positive for IgM according to WB (Tables 1 and 2). One serum sample was positive in both IgG and IgM ELISA tests. This serum sample was confirmed as only IgM-positive by the WB test. Both IgM and IgG tests were not detected as positive in the WB test.

Seropositivity rates were found to be higher in women (8.3%), in the age group of 40–60 years (10.0%), for those living in rural areas (11.1%), for people who were illiterate (10.0%), those who had tick exposure (22.2%), those engaged in agriculture (10.8%), and those who spent the summer in rural areas (10.2%). Results of survey data and distribution of *B. burgdorferi* seropositivity according to the WB test are shown in Table 3. No statistically significant difference was found between seropositivity rates and survey data.

Demographic characteristics of *B. burgdorferi* serology-positive cases and serology results are listed in Table 4.

4. Discussion

Lyme disease is endemic to temperate regions of the northeastern United States and Central and East Europe. Since becoming a nationally notifiable disease in the United States in 1991, a total of 248,074 Lyme disease cases were reported to the Centers for Disease Control. During

Table 1. Comparison of the IgM ELISA and western blot IgM test results according to the number of patients.

ELISA IgM	Western blot IgM	
	Negative	Positive
Positive (n = 29)	22	7
Borderline (n = 2)	2	0

Table 2. Comparison of the IgG ELISA and western blot IgG test results according to the number of patients.

ELISA IgG	Western blot IgG	
	Negative	Positive
Positive (n = 27)	18	9
Borderline (n = 10)	10	0

Table 3. Results of survey data from people who gave blood samples and distribution of *B. burgdorferi* seropositivity according to the western blot test.

		N	%	*B. burgdorferi* seropositivity*		P
				n	%	
Gender	Female Male	120 76	61.2 38.8	10 6	8.3 7.9	0.913
Age group	0–20 21–40 41–60 >60	4 62 50 80	2.1 31.6 25.5 40.8	0 4 5 7	0 6.5 10.0 8.8	0.835
Residence	City Rural areas	97 99	49.4 50.5	5 11	5.2 11.1	0.128
Educational level	Illiterate Primary school Junior high school University	20 101 52 23	10.2 51.5 26.5 11.7	2 10 4 0	10.0 9.9 7.7 0	0.465
Tick bite history	Yes No	9 187	4.6 95.4	2 14	22.2 7.5	0.115
After tick contact	Clinical	0	0	0	0	0
Engaged in agriculture	Yes No	83 113	42.3 57.7	9 7	10.8 6.2	0.240
Location during summer vacation	Rural areas City	118 78	60.2 39.8	12 4	10.2 5.1	0.207

*According to IgM or IgG results of the western blot test.

a 15-year study period, the number of cases reported increased from 9908 in 1992 to 19,931 in 2006 (5). In the United States, approximately 30,000 cases of Lyme disease are reported annually. Estimates of the numbers of cases occurring in Europe are 80,000 per year (6). In Turkey, seroprevalences have been reported to vary between 7% and 36% (7–9).

B. burgdorferi infection can result in dermatologic, musculoskeletal, neurologic, or cardiac abnormalities. In approximately 70%–80% of cases, patients develop a characteristic rash, EM, within 30 days of infection. Lyme disease is diagnosed on the basis of clinical manifestations and a history of exposure to infected ticks. Laboratory tests are not required to confirm diagnosis for patients with characteristic EM. Positive results of the recommended two-tiered serologic testing can provide confirmation of infection in patients with musculoskeletal, neurologic, or cardiac symptoms (4,5).

Bennett et al. reported that in Switzerland 54.5% of the 3443 EM cases between 1997 and 2003 were women and especially in the age group of 50–74 years (60.1%) (10). It was reported in Turkey that Lyme positivity was higher in women in Samsun (11). In our study, the seropositivity rate was higher in women (8.3%) and 50% of them were in the age group of 40 to 60 years. As women are more involved in animal care and work in the fields more compared to men, their risk of contact with ticks is increased and we think this affects the emergence of high seropositivity among females.

Age-related attack rates of Lyme disease show bimodal distribution according to a study conducted in the United States. The first peak is from 5 to 9 years (8.9 cases per 100,000 people) and the second most common age range is 55–59 years (7.8 cases per 100,000 people) (5). Considering the age group in our study, the highest seropositivity rate was observed in the age group of 40–60 years (10%).

The Lyme disease agents, *Ixodes* spp. ticks, prefer forested areas especially and the edges of lakes and rivers (12). In rural areas, the risk of contact with ticks is greater. In a study conducted in Spain in 2004, Lyme seropositivity was 3.45%, with 4.19% reported in rural areas and 2.82% urban areas (13). In our study, higher seropositivity was determined among those living in rural areas (11.1% vs.

Table 4. Demographic characteristics of individuals with positive *B. burgdorferi* serology and results of serology.

	Age	F/M	Tick contact	Residence	Engaged in agriculture	ELISA IgM	ELISA IgG	WB IgM	WB IgG
1	>60	M	-	Rural area	+	+	-	+ (OspC Bg, OspC Ba)	-
2	20–40	M	+	Rural area	+	+	+	+ (OspC Bg, OspC Ba)	-
3	>60	F	-	Rural area	-	+	-	+ (OspC Bg, OspC Ba)	-
4	>60	M	-	Rural area	-	+	-	+ (OspC Bg, OspC Ba, OspC Bb)	-
5	40–60	F	-	City	-	+	-	+ (OspC Bg, OspC Ba)	-
6	20–40	F	-	Rural area	-	+	-	+ (OspC Bg, OspC Ba)	-
7	20–40	F	-	Rural area	+	+	-	+ (OspC Bg)	-
8	>60	F	-	Rural area	+	-	+	-	+ (vlsE Bb, Ba, Bg-p39-p19)
9	>60	F	-	Rural area	+	-	+	-	+ (vlsE Bb, Ba-p39-OspC)
10	40–60	F	-	City	+	-	+	-	+ (vlsE Bb, Ba)
11	>60	M	-	City	-	-	+	-	+ (vlsE Bb-p19-p21)
12	40–60	F	-	City	+	-	+	-	+ (vlsE Bb, Ba, Bg-p39-p83-LBb)
13	40–60	F	-	Rural area	-	-	+	-	+ (vlsE Bb)
14	20–40	M	-	City	-	-	+	-	+ (vlsE Bb-p18-p21)
15	>60	M	+	Rural area	+	-	+	-	+ (vlsE Bb-p83-OspC)
16	40–60	F	-	Rural area	+	-	+	-	+ (vlsE Bb-p39-p21-OspC)

5.2%, respectively). People who spent summers in rural areas were also found to have higher seropositivity (10.2%)

In our survey, the seropositivity rate was higher in people who were illiterate (10.0%). Increasing levels of education increase the level of knowledge in the fight against ticks and these people are less likely to live in rural areas or are less likely to be engaged in agriculture.

In North America and Europe, the nymphal stage of the tick especially is responsible for the emergence of Lyme disease. Tick exposure is less likely to be noticed as infected ticks in the nymph stage are small in size, and the bite does not cause pain and itching. A study in the United States showed that only 25% remembered a history of tick bite (14). In our study, tick exposure was stated only for two of 16 patients seropositive for Lyme disease. This suggests that tick exposure may not be remembered.

Development of Lyme disease after tick exposure and the appearance of symptoms varies between regions. In England, Lyme disease risk varies between 1.2% and 3.7% after tick exposure (6). Tick exposure was remembered by nine (4.6%) of 196 people who participated in the study and only two people (1.0%) had seropositivity. The reasons for not detecting seropositivity in people who have tick exposure are thought to be that ticks may not carry Lyme factors or recent tick exposure (<1 week) such that adequate antibody response has not yet occurred.

Due to direct contact with ticks, dealing with agriculture is a risk factor for *B. burgdorferi* infection. A study conducted in Samsun determined that the positive rate was high in those dealing with agriculture (11). In our study, the seropositivity rate was higher in people who dealt with agriculture.

The ELISA serologic method is recommended as a screening test in epidemiological studies (15). Reagent antigens used in the ELISA kit cannot be standardized so discrepancy may be found between the results obtained in the study. It is reported that vlsE surface protein has the highest sensitivity among recombinant antigens. The presence of antibodies against the vlsE immunodominant antigen is of great importance in the serological diagnosis of Lyme infection (16,17). In our study, both ELISA and WB tests included the vlsE recombinant antigen.

Güner et al. determined 98.8% to 100% similarity with genotypes of Europe (18). Thus, they proved that the antigen used in diagnostic kits imported from Europe may be used in the laboratory diagnosis of Lyme disease in Turkey. In our study, ELISA and WB kits used the antigen of the European strain.

Studies indicate that if the immunoblotting assay includes OspC antigens to detect IgM and vlsE antigen to detect IgG, the specificity of the diagnostic test is increased (15,17). In our study, in the WB test, the number of OspC antigen bands (other bands: p39, vlsE) in IgM-positive patients and the number of vlsE antigen bands in IgG-positive patients was seen to be more positive (other bands: OspC, p39, p83, p58, p21, p20, p19, and p18).

In a study conducted in Poland in 2008, Lyme seropositivity was 6% with the immunoblot method in healthy people living in urban areas and people working in rural areas were identified to have 33% seropositivity (19). In 2001, the seroprevalence of *B. burgdorferi* was 4.3% in healthy blood donors and 9.3% in forestry workers according to Hristea et al. (20). In a study conducted in Italy in 2005, 7.8% were positive for *B. burgdorferi* antibodies in the risk group and 4.9% in the control group according to ELISA, while with the immunoblot method these values were 7.03% and 3.56%, respectively (21). In a study conducted in healthy people in Poland using the ELISA method, seropositivity ranged from 11% to 13% (22).

Several studies have been conducted to determine Lyme seropositivity in Turkey. In 1990, the first reports of Lyme cases were reported from İzmir and Trabzon (1). In 1995, seropositivity was determined as 35.9% by ELISA in Antalya (7). Studies done in Ankara using ELISA found that seropositivity was 13% in patients and 7.8% in the general population in 1999 (9). In 2001 in studies done in Denizli, Trabzon, and Isparta, seropositivity was found by ELISA as 18.9%, 6.6%, and 17.07%, respectively (8,23,24). In 2008 in a study in Düzce, seropositivity was determined in the risk groups as 10.9% with ELISA and as 1.1% with WB (2). In the same study, although there was a history of tick exposure of 51.9%, *B. burgdorferi* sensu lato infection was reported to be rare in the area. A study conducted in the Van region in 2008 found that the seropositivity rate was 6.3% by ELISA (25). Aslan Başbulut et al. reported that the seropositivity rate was 4.1% by ELISA and 3.3% by WB (11). Correlation between seropositivity with a history of tick exposure was not detected in the same study.

In our study we investigated the Lyme seropositivity in Bolu Province and its districts. According to our WB test results, *B. burgdorferi* sensu lato seropositivity was detected as 4.6% for IgG and 3.8% for IgM. Our results are compatible with domestic and foreign data. *B. burgdorferi* sensu lato infection cannot be ignored in this province. Bolu is a province situated in the northwestern region of Turkey. Most of Bolu consists of forests and lakes, which may be a risk for tick exposure. Higher seropositivity rates seen in those living in rural areas, those engaged in agriculture, and people vacationing in rural areas found in this study support this hypothesis. Serological tests are of great importance in the diagnosis of Lyme disease because of people's inability to remember a history of tick exposure, EM lesions cannot always been seen and other clinical symptoms may be confused with other diseases. A limited number of studies have been completed about Lyme disease in Turkey, and the regions where the disease is endemic are not known yet. Detection of endemic regions of Lyme disease with determination of seropositivity rates will increase the awareness among clinicians about this disease. This research determined the seroprevalence of Lyme disease in our region. This study also shed light on other studies to be completed in the future and will contribute to the creation of seroprevalence maps in Turkey.

Acknowledgment

This study was funded by the Scientific Research Project Unit of Abant İzzet Baysal University (Project Number 2013.08.01.644).

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