Seroprevalence of *Borrelia burgdorferi* infection among forestry workers and farmers in Duzce, north-western Turkey

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**SUMMARY**

*Borrelia burgdorferi* infection is the most frequent tick-transmitted disease worldwide. Our aim was to assess the seroprevalence of *B. burgdorferi* infection among forestry workers and farmers in Duzce, in the north-west region of Turkey. Blood samples from 349 forestry workers and farmers and 193 healthy blood donors were obtained to determine the presence of antibodies to *B. burgdorferi*. A two-step testing strategy was used; the sera were initially tested by ELISA and then by Western blot (WB) IgG. Demographic data regarding residence, age, gender, profession, tick bite history, contact with animals, and symptoms involving the skin, nervous system, and osteoarticular system were collected by questionnaire. All results were evaluated statistically using the χ² test. The seroprevalence of *B. burgdorferi* was 10.9% (n=38) in forestry workers and farmers and 2.6% (n=5) in blood donors by ELISA, with a statistically significant difference (p<0.001). Seropositivity rates were related to age, gender, and common risk factors for the disease. IgG seropositivity was confirmed in four (1.1%) sera by WB. In this first seroepidemiological report from the northwest region of Turkey, tick bite exposure was found to be high, whereas *B. burgdorferi* infection was not common. Preventive measures against tick exposure and further studies to determine the distribution of Lyme disease in Turkey are proposed.

**KEY WORDS**: *Borrelia burgdorferi*, Farmers, Forestry workers, Lyme disease, Seropositivity

**INTRODUCTION**

Lyme disease is caused by the spirochete *Borrelia burgdorferi sensu lato* complex, which is transmitted to humans by infected *Ixodes* ticks (Dennis, 1998). Lyme disease was first described as a new entity in the USA in the late 1970s, but many of its individual manifestations had been documented many decades earlier in Europe.

Infection with *B. burgdorferi sensu lato* can result in dermatological, neurological, cardiac, and musculoskeletal disorders. The basic clinical spectrum of the disease is similar worldwide, although there are differences in clinical manifestations, presumably because of differences in the rates of occurrence of infection caused by distinct *B. burgdorferi sensu lato* species (Aguero-Rosenfeld et al., 2005; Orloski et al., 2000). Lyme disease is the most commonly reported vector-borne disease in European countries and the USA (Orloski et al., 2000; Weber and Pfister, 1993). In Turkey, seroprevalences have been reported to vary between 1.5 and 4% (Birengel et al., 1999; Demirci et al., 2001; Hizel et al., 1997) in the healthy population and between 7.8 and 35.9% (Celik et al., 2001; Mutlu et al., 1995; Tuncer et al.,

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The risk of Lyme disease increases with increasing exposure to wooded, brushy, or overgrown grassy areas in endemic regions. Duzce is located in the north-west region of Turkey, and its rural areas have rich wooded areas, as well as large populations of animals. The forestry industry is the most important source of income, and most villagers are forestry workers. Also, there are close interactions between animals and people, especially farmers. Studies of the tick population and tick-borne diseases in Turkey have been limited. The results of one study conducted in Albania and Turkey showed that many tick-borne diseases were endemic in the Balkan area (Christova et al., 2003). In 2003, the first isolation and characterization of *Borrelia burgdorferi sensu lato* strains from *Ixodes ricinus* ticks was reported from the Black Sea coast of the European side of Turkey, close to the region in which our study was conducted (Guner et al., 2003).

The location, surroundings, and living conditions of the people of Duzce seemed indicative of a high risk for tick bites and related infections. This study was carried out to assess the seroprevalence of *B. burgdorferi* infection in the rural area of Duzce and to define associated risk factors in the high-risk group, comparing the results with those for a healthy population.

**MATERIALS AND METHODS**

The study group consisted of forestry workers and farmers in Duzce who are potentially exposed to tick bites. The forestry workers who participated were chosen from a regional forestry directory. The study was performed in nine villages located approximately 10-20 km from the city center and were surrounded by forests; the people living in these villages were either forestry workers or farmers, and they had lived in the area throughout their lives. Forestry workers and farmers were not evaluated in distinct groups, but instead were separated into three subgroups according to age (10-20, 21-40, and >40 years). The working team, consisting of a microbiologist, a dermatologist, a nurse, a laboratory technician, and doctors’ assistants, visited the forestry workers and farmers in Duzce. All participants completed a questionnaire administered by the doctors’ assistants that contained questions about residence, age, gender, profession, tick bite history, contact with animals, and symptoms involving the skin, nervous system, and osteoarticular system. Blood samples were then taken and were transported immediately to the laboratory of the Medical Faculty Hospital of Duzce University. Healthy blood donors (n=193) who were not forestry workers or farmers, who had no risk factors for the acquisition of the infection, and who were living in the city center located far from the risky areas were used as a control group. None of them reported neurological, psychiatric, dermatological, or rheumatological problems, and none had histories of tick bites or animal contact. Serum samples from both groups were preserved at -20°C until testing.

The recommended testing protocol for the serological diagnosis of Lyme disease consists of two steps. The first step is testing with a highly sensitive enzyme immunoassay (ELISA) or immunofluorescence assay (IFA). If the first test is either equivocal or positive, a Western blot test should be performed. If the first test is negative, no further testing is necessary (American College of Physicians, 2004; Brouqui et al., 2004; Centers for Disease Control, 1995).

Serum samples from patients and controls were examined using *Borrelia burgdorferi* ELISA (IBL, Germany) for IgM and IgG antibodies. The solid-phase enzyme-linked immunosorbent assay (ELISA) was based on the sandwich principle, and the wells were coated with antigen. In the *Borrelia* IgM ELISA, recombinant 14 kD protein and the native Outer surface protein C (OspC) protein, and in the *Borrelia* IgG ELISA, recombinant *Borrelia burgdorferi* VlsE antigen and a crude lysate antigen blend from *Borrelia burgdorferi sensu strictu*, *B. afzelii*, and *B. garinii* were used as coating antigens. The test procedure was performed according to the manufacturer’s instructions, and the optical density was measured with a photometer at 450 nm using an ELISA reader (EL×800 Biotek instrument). The sample results were evaluated directly using the standard curve or cutoff standard; absorbance greater than the cutoff standard was interpreted as positive. Serum samples with positive ELISA results were tested for Antistreptolysin-O (ASO-Dade Behring, Germany), Romatoid factor (RF-Dade Behring, Germany), TPHA (*Treponema pallidum* Hemag-
glutination, Omega Diagnostics, Scotland, UK), Monospot (Biokit, Spain) and Antinuclear antibody (ANA-Euroimmun, Germany) tests to exclude false positives (American College of Physicians, 2004).

The ELISA-positive samples were then analyzed by Anti-Borrelia Euroline-WB (Euroimmun AG, Germany) for IgG antibodies, following the manufacturer’s recommendations. Borrelia whole antigen extracts with recombinant VlsE were used as antigens. WB results were interpreted according to the European criteria for WB interpretation. At least a two-band criterion was used for the interpretation of a positive IgG immunoblotting result (Brouqui et al., 2004; Wilske and Schriefer, 2003).

Data were statistically evaluated using the $\chi^2$ (chi-square) test.

RESULTS

A total of 349 forestry workers (n = 153) and farmers (n = 196) in nine villages were included in the study group. The median age was 46.9 years (range: 14-92 years), with a gender distribution of 218 (62.5%) males and 131 (37.5%) females.

According to the questionnaire results, of these 349 people, 163 (46.7%) rheumatological, 102 (29.2%) neurological, 71 (20.3%) psychiatric, and 147 (42.1%) dermatological complaints or diseases were observed. One hundred and eighty-one (51.9%) had a history of tick bites and 303 (86.8%) had a history of animal contact.

Of the 349 sera obtained from forestry workers and farmers, 38 (10.9%) were determined to have IgG antibodies by ELISA. All samples were negative for IgM antibodies.

A total of 193 healthy people were used as controls: 115 (59.6%) males and 78 (40.4%) females. Of these 193 sera, 5 (2.6%) were determined to have IgG antibodies, and all samples were negative for IgM antibodies. When these results were compared with those for the forestry workers and farmers, the differences between the two groups were statistically significant ($p<0.001$) (Table 1).

When the 38 sera with positive ELISA results from the forestry workers and farmers were examined, ANA was positive in seven, and the monospot test was positive in three. ASO, RF, and VDRL tests were all negative. Five IgG-positive sera of controls yielded negative results in these tests.

The questionnaires were evaluated to investigate the effects of factors that might increase the risk for the acquisition of the infection, e.g., age, gender, profession, tick bite history, contact with animals, and symptoms related to Lyme disease. In the study group, the seropositivity rates in those aged 10-20 years ($p<0.05$) and in males ($p<0.01$) were significantly higher than in the other groups.

IgG antibody-positive subjects were generally determined to have histories of tick bite (50%) and/or animal contact (78.9%), in addition to non-specific clinical features such as arthralgia, myalgia, headache, malaise, tremor or skin lesions (89.5%). ELISA results in relation to gender, age, risk factors and clinical features of the study group are shown in Table 2.

### TABLE 1 - Determination of IgG and IgM antibodies against *B. burgdorferi* sensu lato in the study group (forestry workers and farmers) and controls (healthy blood donors) by ELISA.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Male</th>
<th>Female</th>
<th>IgG (+)</th>
<th>IgM (+) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>C</td>
<td>S</td>
<td>C</td>
</tr>
<tr>
<td>10-20</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>21-40</td>
<td>75</td>
<td>38</td>
<td>47</td>
<td>26</td>
</tr>
<tr>
<td>&gt;40</td>
<td>136</td>
<td>71</td>
<td>79</td>
<td>48</td>
</tr>
<tr>
<td>TOTAL</td>
<td>218</td>
<td>115</td>
<td>131</td>
<td>78</td>
</tr>
</tbody>
</table>

S: Study group. C: Control group
The seropositivity rate among the subjects with animal contact was significantly higher in both males and females >40 years of age (p=0.004), but seropositivity was not significantly related to tick-bite history (p>0.05) or clinical features (p>0.05).

Of the 38 IgG-positive sera from the study group, 4 (10.5%) reacted to *B. burgdorferi* antigens by WB. The IgG-positive sera of the control group did not react to *B. burgdorferi* antigens. According to the recommended testing protocol, the four positive sera by WB were considered positive results, and seropositivity was 1.1%.

Of the WB-confirmed results, one was in the 21-40 year age group (female) and three (two males, one female) were >40 years of age. The ASO, RF, TPHA, Monospot, and ANA tests were all negative. The p30, p31, p39, p83, and VlsE reacted with the antibodies in the patients’ sera. All of the patients had complaints such as arthralgia, malaise, myalgia, headache, tremor; as well as animal contact and histories of tick bites. Table 3 shows the characteristics and test results of the four WB-positive patients and the association with risk factors.

### DISCUSSION

Lyme disease is a chronic multisymptomatic zoonosis with dermatological, osteoarticular, neurological and organ symptoms. The etiological factor of this zoonosis is a spirochete, *Borrelia burgdorferi sensu lato*, which was isolated in 1982 from the digestive tracts of ticks and described by Willy Burgdorfer (Burgdorfer, 1991; Wilske and Schriefer, 2003). Epidemiological studies identified small rodents living in forests as the pathogen reservoirs, and the ticks *Ixodes ricinus* and *Ixodes persulcatus* in Europe and *Ixodes dammini (scapularis)* in North America as vectors (Aguero-Rosenfeld et al., 2005; Orloski et al., 2000; Wilske and Schriefer, 2003).

Lyme disease is common worldwide and is associated with areas inhabited by ticks (Gustafson, 1994; Wilske and Schriefer, 2003). The largest

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Gender</th>
<th>IgG (ELISA)</th>
<th>IgM</th>
<th>Positive bands (WB)</th>
<th>Clinical features</th>
<th>Tick bite</th>
<th>Animal contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>M</td>
<td>+</td>
<td>-</td>
<td>p39, p83</td>
<td>arthralgia, malaise</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>M</td>
<td>+</td>
<td>-</td>
<td>p39, VlsE</td>
<td>myalgia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>F</td>
<td>+</td>
<td>-</td>
<td>p30, p31, VlsE</td>
<td>headache, tremor</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>F</td>
<td>+</td>
<td>-</td>
<td>p31, p39, VlsE</td>
<td>arthralgia</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
number of cases of Lyme disease has been reported in the USA, where the disease is found in all states. In the Far East (Japan and China), Australia, South America, and Western Africa, the disease is diagnosed less frequently. In Europe, the disease is found in almost every country. According to a WHO report, all of Europe should be regarded as an endemic area of Lyme disease (Parola and Raoult, 2001; Wilske, 2005).

Routine diagnosis of borreliosis in humans is based on the determination of the levels of specific antibodies of the IgM and IgG classes, mainly in blood, cerebrospinal fluid, and synovial fluid. According to guidelines promulgated in the USA and Germany, serological diagnosis should follow the principle of a two-step procedure, with enzyme-linked immunosorbent assay (ELISA) or IFA as the first step; if reactive, this step is followed by immunoblotting (American College of Physicians, 2004; Brouqui et al., 2004; Centers for Disease Control, 1995). We screened the sera of forestry workers and farmers for B. burgdorferi antibodies by ELISA, and the ELISA-positive sera were retested by WB. Manelli et al. (1999) reported large numbers of seropositive results in groups at risk, including people occupationally exposed to ticks, which are mainly forestry workers and farmers.

Studies in healthy subjects in Europe showed that the frequency of specific antibodies for B. burgdorferi varied between 4.3 and 19.7% (Carlsson et al., 1998; Chmielewski and Tylewska-Wierzbanowska, 2002; Hristea et al., 2001). In some studies in European countries, the frequency of B. burgdorferi antibodies in forestry workers ranged between 4.7 and 40.7%. Seropositivities were 5.3% in Sweden (Werner et al., 2001), 4.7% in Croatia (Poljak et al., 2000), 13.7-31.4% in Germany (Burkert et al., 1996; Hulss and Von Stenglin, 1995; Munchhoff et al., 1987), 23.68-40.7% in Poland (Chmielewskab-Badora, 1998; Cisak et al., 2005; Pancewicz et al., 1996), 3-27.2% in Italy (Ciceroni and Ciarrocchi, 1998; Cristofolini et al., 1993), 35% in Spain (Arteaga Perez and Garcia-Monco Cara, 1999), 28% in the Netherlands (Kuiper et al., 1993), and 9.3% in Romania (Hristea et al., 2001). In Turkey, the seroprevalence of the healthy population was found to be between 1.5 and 4% (Birengel et al., 1999; Demirci et al., 2001; Hizel et al., 1997). Our seropositivity rate for healthy subjects as determined by ELISA was within the ranges obtained in Turkey, but was significantly lower than that for forestry workers and farmers (p<0.001).

In rural areas of Turkey, seroprevalences were reported as 18.9% in Denizli (Celik et al., 2001), 7.8% in Izmir (Tunger and Buke, 1995), and 22.1-35.9% in Antalya (Mutlu et al., 1995; Tuncer et al., 2000). These areas are located in the western or southern regions of Turkey. Duze is located in the northwest of Turkey, and our study group consisted of forestry workers and farmers living in the same environment, one that places them at risk for acquiring Lyme disease. In the rural area of Duze, which is surrounded by forests, the vector population is very high, and people have contact with animals. In such conditions, our seropositivity rates of 10.9% by ELISA and 1.1% by WB were lower than those in European studies, but the results obtained by ELISA were similar to those obtained in other Turkish study groups in rural areas. In this study group, 51.9% had a tick bite history, suggesting the heavy density of ticks in the region, and 86.8% had animal contact. The most affected groups according to age were 10-20, 21-40 and >40 years, respectively. Cumulation in the 10–20-year-old group was significantly high (p<0.05). In a study conducted in Bulgaria, the most affected age group was 5-9 years, followed by 45-49, 50-54, and 10-14 years (Christova and Komitova, 2004). There was also a correlation with gender. Males, who may be at greater risk for tick bites, were significantly more likely than females to have anti B. burgdorferi antibodies (p<0.01).

Testing for B. burgdorferi sensu lato antibodies should involve the two-tier approach, as currently recommended (Aguero-Rosenfeld et al., 2005). We retested the sera of people with B. burgdorferi antibodies detected by ELISA using WB. Some studies have shown the importance of the confirmation of ELISA results by WB (Gordillo et al., 1999; Gordillo-Perez et al., 2003), especially in evaluating patients with clinical symptoms. All four patients that were seropositive by both ELISA and WB had some potentially related health complaints, as well as histories of tick bite and animal contact.

In this first seroepidemiological report from the northwest region of Turkey, exposure to tick bites was frequently observed in forestry workers and farmers, but B. burgdorferi sensu lato infection
was rare. Additional measures to prevent tick exposure should be explored in this high-risk population, and further studies are needed to confirm and extend our seroprevalence findings in Turkey. One goal of future investigations will be to construct a map showing the distribution of the disease to allow the identification of high-risk areas.

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REFERENCES


Guneri E.S., Hashimoto N., Takada N., Kaneda K., Imai...


