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

Ayse Demet Kaya[1], Ali Haydar Parlak[2], Cihadiye Elif Ozturk[1], Mustafa Behcet[1]

[1]Department of Microbiology and Clinical Microbiology, Duzce University, Medical Faculty, Konuralp, Duzce, Turkey;
[2]Department of Dermatology, Abant İzzet Baysal University, İzzet Baysal Medical Faculty, Bolu, Turkey

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 χ^2 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

Received July 27, 2007 *Accepted* October 12, 2007

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.,*

Corresponding author
Ayse Demet Kaya
Department of Microbiology and Clinical Microbiology
Duzce University, Medical Faculty
Konuralp, Duzce, Turkey
Zip code: 81 620
E-mail: ademetkaya@superonline.com

2000; Tunger and Buke, 1995) in rural areas. 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 aquisition 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 rhematological 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 χ^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%) rhematological, 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.*

Age (years)	*Male*		*Female*		*IgG (+)*			*IgM (+) (%)*	
	S	*C*	*S*	*C*	*S (%)*	*C (%)*		*S*	*C*
10-20	7	6	5	4	4 (33.3)	0 (0)	$p < 0.05$	0	0
21-40	75	38	47	26	14 (11.5)	2 (3.1)	$p < 0.05$	0	0
>40	136	71	79	48	20 (9.3)	3 (2.5)	$p < 0.05$	0	0
TOTAL	218	115	131	78	38 (10.9)	5 (2.6)	$p < 0.001$	0	0

S: Study group. C: Control group

TABLE 2 - *Correlation of risk factors (tick bite history, animal contact, clinical features) and seropositivity detected by ELISA in the study group.*

	IgG(+)	*IgM(+)*	*Tick bite history*	*Animal contact*	*Non-specific clinical features*
MALE (n = 25)					
10-20 years	3 (42.9%)	0	0	2 (66.7%)	2 (66.7%)
21-40 years	8 (10.7%)	0	5 (62.5%)	5 (62.5%)	7 (87.5%)
>40 years	14 (10.3%)	0	8 (57.1%)	14 (100%)	14 (100%)
FEMALE (n = 13)					
10-20 years	1 (20%)	0	1 (100%)	1 (100%)	1 (100%)
21-40 years	6 (12.8%)	0	4 (66.7%)	5 (83.3%)	4 (66.7%)
>40 years	6 (76%)	0	1 (16.7%)	3 (50%)	6 (100%)

The seroposivity 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 pateints 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 3 - *Characteristics and test results for four patients with antibodies against B. burgdorferi sensu lato detected by ELISA and Western Blot.*

Case	*Age*	*Gender*	*IgG (ELISA)*	*IgM*	*Positive bands (WB)*	*Clinical features*	*Tick bite*	*Animal contact*
1	70	M	+	-	p39, p83	arthralgia, malaise	+	+
2	47	M	+	-	p39, VlsE	myalgia	+	+
3	24	F	+	-	p30, p31, VlsE	headache, tremor	+	+
4	42	F	+	-	p31, p39, VlsE	arthralgia	+	+

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; Hulsse and Von Stenglin, 1995; Munchhoff *et al.,* 1987), 23.68-40.7% in Poland (Chmielewska-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 İzmir (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. Duzce 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 Duzce, which is sorrounded 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.

ACKNOWLEDGMENTS

This study was kindly supported by a grant from the University of Abant Izzet Baysal Research. Protocol number: 02.07.01.107. We thank the Directory of Regional Forestry, all of the participating forestry workers, farmers, and healthy blood donors, and D.V.M. Vildan Ozdemir.

REFERENCES

AGUERO-ROSENFELD M.E., WANG G., SCHWARTZ I., WORMSER G.P. (2005). Diagnosis of Lyme borreliosis. *Clin Microbiol Rev.* **18**, 484-509.

AMERICAN COLLEGE OF PHYSICIANS. (2004). Guidelines for laboratory evaluation in the diagnosis of Lyme disease. *Ann. Int. Med.* **127**, 1106-1108

ARTEAGA PEREZ F., GARCIA-MONCO CARA J.C. (1999). Risk factors associated with the presence of antibodies against *Borrelia burgdorferi*. *Rev Clin Esp.* **199**, 136-141.

BIRENGEL S., BOSCA A., KURT H., TEKELI E. (1999). Sağlıklı bireylerde ve bazı hasta gruplarında Lyme seropozitifliği. *Flora Derg.* **4**, 51-57 (In Turkish).

BROUQUI P., BACELLAR F., BARANTON G., BIRTLES R.J., BJOËRSDORFF A., BLANCO J.R., CARUSO G., CINCO M., FOURNIER P.E., FRANCAVILLA E., JENSENIUS M., KAZAR J., LAFERL H., LAKOS A., LOTRIC FURLAN S., MAURIN M., OTEO J.A., PAROLA P., PEREZ-EID C., PETER O., POSTIC D., RAOULT D., TELLEZ A., TSELENTIS Y., WILSKE B. (2004). ESCMID Study Group on Coxiella, Anaplasma, Rickettsia and Bartonella; European Network for Surveillance of Tick-Borne Diseases. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. *Clin Microbiol Infect.* **10**, 1108-1132.

BURGDORFER W. (19991). Lyme borreliosis: ten years after discovery of the etiologic agent, *Borrelia burgdorferi*. *Infection.* **19**, 257-262.

BURKERT S., ROSSLER D., MUNCHHOFF P., WILSKE B. (1996). Development of enzyme-linked immunosorbent assays using recombinant borrelial antigens for serodiagnosis of *Borrelia burgdorferi* infection. *Med Microbiol Immunol.* **185**, 49-57.

CARLSSON S.A., GRANLUND H., NYMAN D., WAHLBERG P. (1998). IgG seroprevalence of Lyme borreliosis in the population of the Aland Islands in Finland. *Scand J Infect Dis.* **30**, 501-503.

CELIK A.F., TURGUT H., CETIN C.B., YALCIN A.N., KALELI I. (2001). Denizli yöresinde *Borrelia burgdorferi* antikor sıklığının araştırılması. *Infeksiyon Derg.* **15**, 439-441 (In Turkish).

CENTERS FOR DISEASE CONTROL AND PREVENTION. (1995). Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *Morb Mortal Wkly Rep.* **44**, 590-591.

CHMIELEWSKA-BADORA J. (1998). Seroepidemiologic study on Lyme borreliosis in the Lublin region. *Ann Agric Environ Med.* **5**, 183-186.

CHMIELEWSKI T., TYLEWSKA-WIERZBANOWSKA S. (2002). Prevalence of *Borrelia burgdorferi* antibodies in healthy population in Poland. *Przegl Epidemiol.* **56**, 33-38.

Christova I., Komitova R. (2004). CLINICAL AND EPIdemiological features of Lyme borreliosis in Bulgaria. *Wien Klin Wochenschr.* **31**, 42-46.

CHRISTOVA I., VAN DE POL J., YAZAR S., VELO E., SCHOULS L. (2003). Identification of *Borrelia burgdorferi sensu lato*, *Anaplasma* and *Ehrlichia* species, and spotted fever group Rickettsiae in ticks from southeastern Europe. *Eur J Clin Microbiol Infect Dis.* **22**, 535-542.

CICERONI L., CIARROCCHI S. (1998). Lyme disease in Italy, 1983-1996. *New Microbiol.* **21**, 407-418

CISAK E., CHMIELEWSKA-BADORA J., ZWOLINSKI J., WOJCIK-FATLA A., POLAK J., DUTKIEWICZ J. (2005). Risk of tick-borne bacterial diseases among workers of Roztocze National Park (south-eastern Poland). *Ann Agric Environ Med.* **12**, 127-132.

CRISTOFOLINI A., BASSETTI D., SCHALLENBERG G. (1993). Zoonoses transmitted by ticks in forest workers (tick-borne encephalitis and Lyme borreliosis): preliminary results. *Med Lav.* **84**, 394-402.

DEMIRCI M, YORGANCIGIL B, TAHAN V, ARDA M. (2001). Isparta yöresinde kene ısırığı öyküsü olanlarda Lyme hastalığı seropozitifliği. *İnfeksiyon Derg.* **15**, 17-20 (In Turkish).

DENNIS D.T. (1998). Epidemiology, ecology, and prevention of Lyme disease. In: Rahn DW, J. Evans J (eds). Lyme Disease. *American College of Physicians.* Philadelphia, PA. 7-34.

GORDILLO G., TORRES J., SOLORZANO F., CEDILLO-RIVERA R., TAPIA-CONYER R., MUNOZ O. (1999). Serologic evidences suggesting the presence of *Borrelia burgdorferi* infection in Mexico. *Arch Med Res.* **30**, 64-68.

GORDILLO-PEREZ G., TORRES J., SOLORZANO-SANTOS F., GARDUNO-BAUTISTA V., TAPIA-CONYER R., MUNOZ O. (2003). Seroepidemiologic study of Lyme's borreliosis in Mexico City and the northeast of the Mexican Republic. *Salud Publica Mex.* **45**, 351-355.

GUNER E.S., HASHIMOTO N., TAKADA N., KANEDA K., IMAI

Y., MASUZAWA T. (2003). First isolation and characterization of *Borrelia burgdorferi sensu lato* strains from *Ixodes ricinus* ticks in Turkey. *J Med Microbiol.* **52**, 807-813.

GUSTAFSON R. (1994). Epidemiological studies of Lyme borreliosis and tick-borne encephalitis. *Scand J Infect Dis.* **92**, 1-63.

HIZEL K., ULUTAN F., AKTAS F. (1997). Lyme hastalığı ile uyumlu bulgusu olan hastalarda *Borrelia burgdorferi* antikorlarının araştırılması. *Infeksiyon Derg.* **11**, 87-91 (In Turkish).

HRISTEA A., HRISTESCU S., CIUFECU C., VASILE A. (2001). Seroprevalence of *Borrelia burgdorferi* in Romania. *Eur J Epidemiol.* **17**, 891-896.

HULSSE C. (1995). Von Stenglin M. Incidence of Lyme borreliosis in Mecklenburg-Vorpommern. *Gesundheitswesen.* **57**, 21-24

KUIPER H, VAN DAM AP, MOLL VAN CHARANTE AW, NAUTA NP, DANKERT J. (1993). One-year follow-up study to assess the prevalence and incidence of Lyme borreliosis among Dutch forestry workers. *Eur J Clin Microbiol Infect Dis.* **12**, 413-418.

MANNELLI A, CERRI D, BUFFRINI L, ROSSI S, ROSATI S, ARATA T, INNOCENTI M, GRIGNOLO MC, BIANCHI G, IORI A, TOLARI F. (1999). Low risk of Lyme borreliosis in a protected area on the Tyrrhenian coast, in central Italy. *Eur J Epidemiol.* **15**, 371-377.

MUNCHHOFF P, WILSKE B, PREAC-MURSIC V, SCHIERZ G. (1987). Antibodies against *Borrelia burgdorferi* in Bavarian forest workers. Zentralbl. *Bakteriol Mikrobiol Hyg [A].* **263**, 412-419.

MUTLU G, GULTEKIN M, ERGIN C, SAYIN F, KURSUN AE. (1995). Antalya yöresinde *Borrelia burgdorferi* antikorlarının ve vektörlerinin araştırılması. *Mikrobiyol Bült.* **29**, 1-6 (In Turkish).

ORLOSKI KA, HAYES EB, CAMPBELL GL, DENNIS DT. (2000). Surveillance for Lyme disease. United States, 1992-1998. *MMWR CDC Surveill Summ.* **28**, 1-11.

PANCEWICZ SA, JANUSZKIEWICZ A, HERMANOWSKA-SZPAKOWICZ T. (1996). Detection of antibodies of *Borrelia burgdorferi* among inhabitants of north-eastern Poland. *Przegl Epidemiol.* **50**, 375-381.

PAROLA P, RAOULT D. (2001). Tick-borne bacterial diseases emerging in Europe. *Clinical Microbiology & Infection.* **7**, 80-83.

POLJAK I, TROSELJ-VUKIC B, MILETIC B, MOROVIC M, RUZIC-SABLJIC E, VUCEMILOVIC A, MATERLJAN E. (2000). Low sero-prevalence of Lyme borreliosis in the forested mountainous area of Gorski Kotar, Croatia. *Croat Med J.* **41**, 433-436.

TUNCER D, OGUNC D, COLAK D, ONGUT G, SAYIN F, ERGIN C, TUNCER B, MUTLU G. (2000). Prevalence of *Borrelia burgdorferi* antibodies in urban and high-risk areas of Turkey. Information Circular - *WHO Mediterranean Zoonoses Control Centre.* **49**, 8-9.

TUNGER O, BUKE M. (1995). Lyme hastalığı: İzmir ve çevresindeki durum. *Infeksiyon Derg.* **9**, 345-9 (In Turkish).

WEBER K, PFISTER HW. (1993). History of Lyme borreliosis in Europe. In: Weber K, Burgdorfer W (eds). Aspects of Lyme borreliosis. Springer-Verlag, Berlin, Germany. 1-20.

WERNER M, NORDIN P, ARNHOLM B, ELGEFORS B, KRANTZ I. (2001). *Borrelia burgdorferi* antibodies in outdoor and indoor workers in south-west Sweden. *Scand J Infect Dis.* **33**, 128-131.

Wilske B. (2005). Epidemiology and diagnosis of Lyme borreliosis. *Ann Med.* **37**, 568-79.

WILSKE B, SCHRIEFER ME. (2003). *Borrelia*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (eds). Manual of Clinical Microbiology, 8th ed. American Society for Microbiology, Washington, D.C. 937-54.