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Seroepidemiologic Study On Lyme Borreliosis In Isparta Region In Turkey

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Abstract

The frequency of *anti-Borrelia burgdorferi* antibodies in human sera of people from Isparta region was studied. In this study, seroprevalance of IgG and IgM antibodies in 365 blood samples collected between June and September 2005, regardless of age and gender of the people who live within the borders of Province of Isparta in Turkey and who are engaged in farming through ELISA method was examined. The sera determined as IgG positive through ELISA were confirmed by Western Blot. Anti *Borrelia burgdorferi* antibodies were detected as an IgG positivity 12 (3.28%) in 365 blood samples was found in 143 men, 5 of 12 (3. 42%) and 219 women, 7 of 12 (3.19%). In the current study, positive IgM was not observed. Related studies conducted have shown that different results were obtained in different regions, because factors such as living conditions and climatic conditions contributed much to sero positivity of Lyme disease, and this study supported these findings.

Key Words: Lyme borreliosis, multiple sclerosis, seroepidemiology, ELISA, Western Blot

Introduction

Lyme Borreliosis or Lyme Disease (LD), represents a new global public health problem [1,2]. Lyme disease is the most common vector-borne disease in North America and Europe [3,4] and is an emerging problem in Northern Asia [5,6].

L.D. is a common world wide disease and it is associated with areas inhibited by ticks. Since the first isolation of Borrelia burgdorferi from Ixodes scapularis (*I. dammini*), numerous bacteriological examinations of ticks have been done to evaluate the frequency of infected ticks in various areas of the world [7, 8]. For example; it is seen that Ixodes scapularis plays a role as a vector in northeastern and western America [9], whereas Ixodes ricinus is the germ carrier ticks in Europe and Ixodes persulcatus in Asia [10]. With 17,027 cases reported in 2001 (Centers for Disease Control and Prevention 2002), Lyme disease has become a serious public health concern in the United States. It is transmitted to humans by the deer tick, Ixodes scapularis [11]. Vectors bearing the disease and clinical findings differ geographically

Symptoms of the disease caused by the factor and immune reaction vary in different areas. For example; whereas it is characterized by the symptoms of arthritis primarily in America, neurological disorders are the first to be considered in Europe. However, general characteristics of the disease are similar in the world [12]. The serological studies performed in Europe showed that populations exposed to contact with ticks more often have specific antibodies against B.burgdorferi than the rest of the population [13].

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Turkey, which has similar conditions in the regions where Lyme disease is endemically seen, does not have enough information on the distribution of this infection. Therefore, the distribution of the disease in Turkey is not exactly known. In 1994, the Second National Conference on Serological Diagnosis of Lyme Disease recommended a two-step testing system directed towards standardizing laboratory serological testing for B.burgdorferi. Since Enzyme-Linked Immunoassay (EIA) and Indirect fluorescent antibody (IFA) methods were not sufficiently specific to support clinical diagnosis ,it was recommended that positive or equivocal results from a sensitive EIA or IFA (first step) should be further tested, or supplemented, by using a standardized Western Blot (WB) method (second step) for detecting antibodies to B. burgdorferi. Two-step positive results provide supportive evidence of exposure to B. burgdorferi [14,15].

The goal of our study was an evaluation of the frequency of antibodies anti-Borrelis burgdorferi in human sera collected from farmers in Isparta province in Turkey.

Materials and Methods

Turkey is a country located in Southwest Asia with a small part in southeastern Europe. It extends from Southeastern Europe and Southwestern Asia, bordering the Black Sea, between Bulgaria and Georgia, and bordering the Aegean Sea and the Mediterranean Sea, between Greece and Syria. Province of Isparta is 400 km away from the capital of Turkey-Ankara and is located in the Lake District in northern Mediterranean (Longtidude/Latitude: E 320 20' and 310 33'-N 370 18; and 380 30'). Altitude is about 1050 metres (Figure 1) [16].

Isparta has 13 districts in total within its borders (Figure 2) [17].

In order to determine anti B.burgdorferi antibody sero-

prevalance in the districts of Isparta, 365 blood samples in total were gathered from 219 female farmers and 146 male farmers aged between 30 and 89 between June and September 2005. Before serological examinations, the epidemiological anamnesis was performed. This included questions about profession, place, tick bites and symptoms concerning skin.

Figure 1. Location of Isparta province in Turkey.

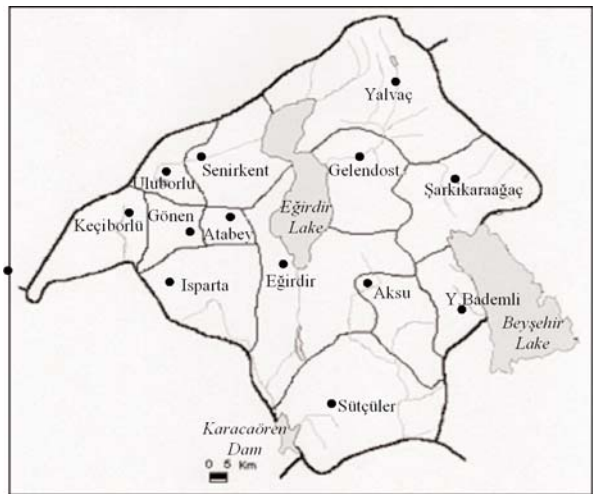

Figure 2. Districts of Isparta.

Collected sera were examined with quantitative indirect ELISA IgM and ELISA IgG. In order to examine anti-B. burgdorferi antibodies, the ELISA kit (Euroimmun, GmbH Germany) covered with OsP39 antigen from B. burgdorferi root was used. Assay was read in ELISA reader with filter 450 nm. A result was considered as positive if absorbance was greater than cut-off evaluated according to instruction. The rheumatic factor was absorbed in the course of reaction in the entire test ELISA kits. All samples of blood sera were examined twice, according to the manufacturer's instruction. We thawed the serum directly before processing and testing with separate ELISA kits. Repeat examinations were always conducted another day.

All the positive IgG results with ELISA were confirmed by Western Blot (WB; Euroimmun GmbH Germany) method. Chi-square test (x^2) was used in evaluation of the data statistically and in distribution of gender, whereas the slope chi-square test in distribution of age groups.

Results and Discussion

IgM seropositivity was not observed in the blood samples from 146 men and 219 women included in the study (365 in total), whereas IgG positivity was observed in 19 individuals through ELISA. All the positive results from ELISA were tested through WB. According to this, the positive rate was determined as (3. 28%). When gender of IgG seropositive cases was taken into consideration, it was found as 3. 19% in women (n=7) and 3.42% in men (n=5) (Table 1). Of 365 people included in the study, those aged between 40 and 49 (42.10%) had the highest seropositivity rate after both ELISA and WB tests. Distribution of seropositive cases according to age groups is presented (Table 2).

Table 1. Distribution of seropositive cases according to gender.

Gender	Number	ELISA (IgG)		WB (IgG Positive)	
		n	%	n	%
Female	219	11	5.02	7	3.19
Male	146	8	5.47	5	3.42
Total	365	19	5.20	12	3.28

Table 2. Distribution of seropositive cases according to age groups.

Ages	ELISA		WB	
	n	%	n	%
30-39	4	21.05	1	8.33
40-49	8	42.10	8	66.66
50-59	3	15.78	0	0
60-69	2	10.52	2	16.66
70-79	1	5.26	0	0
80-890	1	5.26	1	8.33
Total	19	100.00	12	100.00

According to the results of anamnesis from 365 farmers, nobody had Erithema Chronicum Migrans (ECM). 102 (27. 9%) of these people had a story of weakness and fatigue, 50 (13. 7%) of swollen joints and 45 (12. 3%) of tick bites (Table 3).

In WB seropositive cases, 3 individuals among the women had a story of tick bites (42.85%), 4 (57.14%) in men, 5 women (55.55%) and 4 men (44.44%) had pain in joints. Seropositive cases were evaluated in terms of their

stories of tick bites and pain in joints, according to gender (Table 4).

Table 3. Evaluation of anamnesis of 365 people included in the study group, according to gender.

SYMPTOMS	Male	Female	Total
	n	n	n /%
Erithema Chronicum Migrans (ECM)	0	0	0
Weakness and fatigue	41	61	102/27.9
Joint swelling and pain	18	32	50/13.7
Story of tick bites	27	18	45/12.3

Table 4. Evaluation of seropositive cases in terms of their stories of tick bites and pain in joints, according to gender.

Story	WB		
	Gender	n	%
Tick bite story	Female	3	42.85
	Male	4	57.14
Pain in joints	Female	5	55.55
	Male	4	44.44

When positivity rates were statistically evaluated according to WB test results, the difference between the two groups was found statistically insignificant. As a result of the Fisher chi-square test, the chi-square test value was found =0.014. P=1 and since P>0.05 the frequency of Lyme disease experience in women and men was equal in the study. When seropositivity rates according to gender were statistically evaluated, it was understood that seropositivity did not depend on age growth (P>0.05) since there was no IgG (+) by the first 30 years.

Turkey is partially located in Southeastern Europe and partially in Southwestern Asia (that portion of Turkey west of the Bosphorus is geographically part of Europe), bordering the Black Sea, between Bulgaria and Georgia, and bordering the Aegean Sea and the Mediterranean Sea, between Greece and Syria. Turkey is located at the south of the medium climate zone, and is affected by the Mediterranean climate, which is subtropical with dry summers.

Isparta is located in the center of the Lake Region on the high plateaus of the Taurus Chain (Figure 1). Isparta's summers are dry and hot, winters are cold and rainy. Surrounding lakes have considerable effects on climate (Figure 2). Great part of total raining, and snowing, drops in winter and spring months [18]. Therefore, Isparta has a wide range of temperatures from +35 °C in summer to -20 °C in winter. In the study including farmers working under these climatic conditions in Isparta, seropositivity of B.burgdorferi antibodies was found as 3. 28% (Table 1). In studies conducted in various regions of Turkey, seropositivity was reported as 21.5% in individuals living in a mountainous village in Bursa (in Northwestern part of Turkey) with no clinicalal findings and symptoms [19]. In Antalya (Southwestern part of Turkey), seropositivity was reported as 35.9% in 32 blood samples of 89 blood samples [20]. In Elazığ (the east of Turkey), seropositivity was found as 6% in people living in thickly wooded villages [21]. In Kayseri (near the center of Turkey) seropositivity rate was reported as 10% [22]. In patients living in the capital city of Turkey and displaying symptoms similarly to those of Lyme disease, seropositivity was 10.4%, whereas in the control group of the same study it was reported as 1.5% [23]. Seropositivity rate in 90 healthy people living in the coastal area and mountainous areas of Trabzon (Northeastern part of Turkey), who are either interested in cattle dealing or not, was found as 6.6% (6/90) [24]. In the blood samples from totally 95 people (48 of whom were male, 47 of whom were female) living in four different mountainous villages of Denizli district, seropositivity rate was found as 18.9% [25]. In a study conducted in the North of Cyprus, seropositivity rate was 16 (17.6%) out of 91 people in total [26].

The number of annually reported cases of Lyme disease in the United States has increased approximately 25-fold since national surveillance began in 1982; during 1993-1997, a mean of 12451 cases annually were reported by states to CDC (1.2, CDC, unpublished data, 1998). In the United States, the disease is primarily localized to states in the northeastern, mid-Atlantic, and upper North-central regions, and to several areas in Northwestern California [27]. In Europe, the disease was found in almost every country [28].

Data from the literature show number of seropositive results in group at risk (people occupationally exposed to ticks, mainly forestry workers and farmers) [13].

In various European countries, the frequency of antibodies anti-Borrelia burgdorferi in risk group ranged from 0.206 % in Slovenia, 0,135% in Austria, 0.103% in Netherlands, 0.036% in Czech Republic, 0.00034% in Lithuania, 0.026% in Germany, 0,024% in Finland, 0.021% in Latvia, 0.021% in Estonia, 0.016% in Slovakia, 0.013% in Belgium, 0.013% in Bulgaria, 0.0012% in Poland, 0.006% in Norway, 0.012% in Hungary, 0.0094% in France, 0.0011 in England, 0.0019% in Scotland, 22% in Switzerland, 26% in Sweden, 15% in Ireland, 9.3% in Romania, 0.000001% in Italy and 1.1% in Greece. In general population, the values of positive results were low and ranged from 0.000001% in Italy to 0.206% in Slovenia [29-32].

It is seen that in studies conducted in Turkey Antalya has higher seroprevalance of anti-B.burgdorferi antibodies, when compared to the other regions (Figure 3). In Antalya

district, a high seroprevalance rate of anti-B.burgdorferi antibodies (35.9%) was reported [20].

Reimer et.al. (1990) stated that Lyme disease had a two-fold prevalance in men when compared to women in general population of Southeastern Baviera in Germany and that prevalance usually increased depending on age and outdoor activities [33].

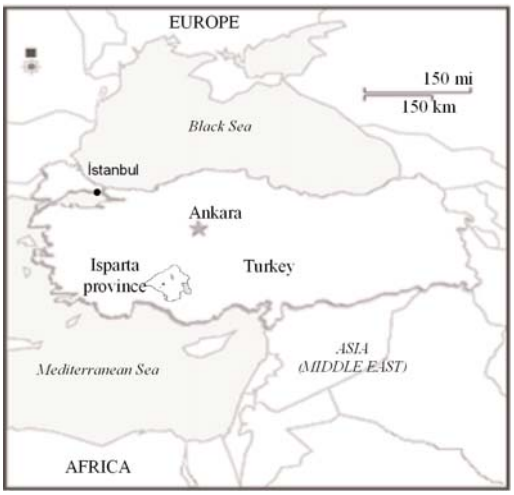

Figure 3. The samples of study areas in Turkey.

In a study conducted in the United States, it was reported that Lyme disease was often found in male population (52.5%) [33]. In an epidemiological study conducted in Denizli district, including 95 cases in total (47 women, 48 men), serpositivity was found as 22.9% for men and 14.9% for women. The reason of the high rate was the fact that men were more actively dealing with cattles than women [25]. Moreover, the reason why seropositivity is high in Antalya and Denizli, which are near Isparta Region, is that more moderate climate and higher number of green areas, when compared to Isparta district, affect existence and lifespan of ticks in these areas positively.

Conclusion

In our study, seroprevalance of anti-B.burgdorferi antibody was 3.28% among the farmers in Isparta region. Moreover, there was no statistically significant difference between man and woman (P>0.05). The highest seropositivity was seen in the age group of 40-46.

n the light of these data, it is necessary to bear similar symptoms of Lyme disease in mind as diagnostic value, since there is risk of Lyme disease in almost every part of Turkey. In order to evaluate immunological tests in a sound way, epidemiological studies on Lyme disease and determination of parts where the disease is endemic are essential. In studies conducted, different values were determined in different regions of Turkey, which show that factors like living conditions and climatic conditions have a positive effect on seropositivity of Lyme disease.

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