Low Sero-Prevalence of Lyme Borreliosis in the Forested Mountainous Area of Gorski Kotar, Croatia

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Aim. Clinical forms of Lyme disease in Gorski Kotar have occurred only sporadically, in contrast to the northwestern Croatia and the neighboring areas of Slovenia, which are well-known Lyme borreliosis endemic regions. Our aim was to assess the level of sero-prevalence of Borrelia burgdorferi sensu lato in a high-risk population of forestry workers in the mountainous region of Gorski Kotar, Croatia, and compare it with the sero-prevalence in residents of that area and the neighboring littoral region.

Methods. A sero-epidemiological study was conducted on 520 healthy subjects, divided into 3 groups: the first group included 234 forestry workers, residents of Gorski Kotar, the second 100 residents of various professions in the same region, and the third 186 subjects of various professions from the neighboring littoral region. These sera were collected during the winters of two successive years, 1997 and 1998. Lyme borreliosis serology was performed by indirect immunofluorescence assay. Sera from 10 hunting dogs from Gorski Kotar were also analyzed.

Results. The IgG antibodies to B. burgdorferi sensu lato were found in 11 examinees (4.7%) from the group of forestry workers, in 3 (3%) from the second group, and in 5 (2.7%) from the third group. Four out of 10 dogs (40%) had IgG antibodies against B. burgdorferi.

Conclusion. Our results show that the forest and mountainous area of Gorski Kotar, Croatia, has the characteristics of a low sero-prevalence area, in contrast to the endemic neighboring areas.

Key words: Borrelia burgdorferi; Croatia; Lyme disease; prevalence; serology

Lyme borreliosis, one of the most common tick-transmitted diseases, is caused by Borrelia burgdorferi sensu lato (1-3). Several epidemiological studies have reported on the spread of the disease in animals, which are reservoirs and carriers of the B. burgdorferi, particularly in the endemic regions of Croatia and neighboring Slovenia (4-7). In the Primorsko-Goranska County (area 2,774 km²; population 339,527) Lyme borreliosis appears to be rare (annual incidence rate is from 1.5 to 5.9/100,000) (8), although Gorski Kotar, a forested mountainous region of this county, has an abundance of the tick population. In Gorski Kotar, the first cases of Lyme borreliosis were recorded in the early 1980's (9). The first sero-epidemiological study was published in 1994 and involved multiple sclerosis patients showing a low sero-prevalence rate (9).

The aim of this study was to assess the sero-prevalence of Lyme borreliosis in the high-risk population of forestry workers, and compare it with the sero-prevalence in other residents of Gorski Kotar and the neighboring littoral area.

Subjects and Methods

Study Area

Gorski Kotar is a mountain region (1,273 km²) in the western part of Croatia, very sparsely populated (population 30,545; 24/km²), with no important urban centers. All the settlements are at 300-900 m above the sea level (Fig. 1). The working population is employed in the timber and wood-processing industry. The climate is continental, with mean annual temperature varying with latitude from 8.5°C to 6.3°C. The mean annual rainfall varies from 1,723 to 2,468 mm, and the mean annual humidity is 86%. The area is covered with coniferous and deciduous forests, with rich ornithofauna and various games. Deer, roe deer, and roe bucks are the most numerous game species, and their high numbers support the development of an important population of ticks, the Lyme borreliosis vectors.

Subjects

The study involved 520 healthy subjects, divided into 3 groups: the first two groups included 334 residents of Gorski Kotar (234 forestry workers, and 100 residents of various professions in the same region), and the third group included 186 subjects of various professions from the neighboring littoral region.
were detected in 4.7% of forestry workers, which is significantly less than the rest of the population. Specific antibodies did not show significant differences between high-risk groups and the rest of the population. Our survey of the population of Gorski Kotar did not show significant differences between high-risk groups and the rest of the population. Specific antibodies were detected in 4.7% of 234 forestry workers, which is significantly lower than the rest of the population.

Group 1 included 234 forestry workers, whose median age was 40 years (range 22-60), all employed in the timber and wood-processing industry. Most of them were loggers (n=192; 82%), and the rest performed various jobs (7 truck drivers, 10 tractor drivers, 20 saw mill workers, and 5 forest guards). No form of Lyme borreliosis was detected in subjects, although more than two thirds had a history of tick bites.

Group 2 included 100 subjects of various professions, whose median age was 40.5 years (range 21-63). They had not spent any significant amount of time in wooded areas. Only about one third reported previous tick bites. Data on one of the Lyme borreliosis forms were negative.

Group 3 comprised 186 subjects of various professions and living in the neighboring littoral region. Their median age was 44.2 years (range 17-68). None of the subjects reported either a history of tick bite or an active or past Lyme borreliosis.

**Serological Testing**

A blood sample of 5 mL was taken from each subject in a single drawing during the winter seasons of two successive years, 1997 and 1998. The presence of specific IgM and IgG antibodies against B. burgdorferi sensu lato was assessed by indirect immunofluorescent assay without absorption. B. afzelii, which is the most frequently isolated agent in Lyme borreliosis patients in Europe, was used as an antigen. Borrelias were cut into small pieces and fixed at 4°C until testing. The sera were diluted from 1:64 to 1:512. The antibody titer of 256 was considered positive. Each test was performed with a positive and negative control. The test was set up in a high-risk zone of Lyme borreliosis, Slovenia. All the analyses were performed at the laboratory of the Institute of Microbiology and Immunology, University of Ljubljana, Slovenia. The results were presented as titers, and the cut-off value was calculated in relation to the healthy subjects in the endemic region. The dogs' sera were also analyzed with indirect immunofluorescent assay without absorption. Only IgG antibody was assessed, and the titers of >64 were considered postive, according to the literature (11).

**Statistical Analysis**

The statistical software Epi Info 6, version 6.02 from the Centers for Disease Control and Prevention (CDC), USA, and WHO, Geneva, Switzerland, was used to estimate the infection risks by means of the prevalence odds ratio, with the exact 95% confidence intervals.

**Results**

IgG antibodies in 256 titer were found in 11 (4.7%) out of 234 for forestry workers, and in 3 (3.0%) out of 100 other inhabitants of Gorski Kotar. In the third group, positive IgG antibody titer was found in 5 (2.7%) of 186 subjects, the residents of littoral area (Table 1, and Fig. 1). IgM antibody titers were not present in any of the subjects. There was no significant difference in the seroprevalence of B. burgdorferi sensu lato IgG antibodies between the groups. Four out of 10 (40%) hunting dogs were sero-positive.

**Discussion**

B. burgdorferi sensu lato sero-prevalence in specific population groups is greater epidemiologically significant. Our survey of the population of Gorski Kotar did not show significant differences between high-risk groups and the rest of the population. Specific antibodies were detected in 4.7% of 234 forestry workers, which is significantly lower than the rest of the population.
a much lower percentage than those reported for the population at risk in European countries (Germany 13.7%, England 25%, the Netherlands 28%, and Switzerland 35%), northwestern Croatia (26.8% - unpublished data, courtesy of Misaiajer Lj, Department of Infectious Diseases, General Hospital Kopriwnica, Kopriwnica, Croatia) and the neighboring Slovenia (13.3% -13-18). This shows that the infection rate may significantly vary even between geographically close regions.

The ratio of sero-prevalence stratified in the inhabitants of Gorski Kotar and in the general population of the neighboring littoral area were 3.0% and 2.7%, respectively. Studies performed in corresponding groups in northwestern Croatia reported higher sero-prevalence of 6.7-9.7% (4,5), which is close to the values recorded in the endemic regions of some Western European countries (Sweden 9%, Germany 17%) (19,20). The sero-prevalence in the general population in Slovenia, the endemic region well known for many years, is lower than 5%, which is similar to our results (2.7%). A low sero-prevalence rate is also found in an unselected but non-risk south Estonian population (21). This part of Estonia lies in the proximity of the Lyme borreliosis endemic area, similar to our investigated area (19,22,23). In conclusion, our investigation of the risk of Lyme borreliosis transmission risk (24).

Sero-positivity in dogs is known to correlate significantly with entomological indexes of Lyme borreliosis transmission risk (24). Sero-epizootological analysis detected the presence of specific antibodies in 4 out of 10 hunting dogs (40%). The 1994 study in Gorski Kotar revealed sero-positivity in 2 of 20 cows (10%) but in none of 10 wild animals (dog mouse) (9). These results point to the presence of B. burgdorferi sensu lato in animal reservoirs, but the size of our samples does not allow more precise conclusions. Corresponding studies on cattle and household dogs examined by ELISA, conducted in areas of northwest Croatia endemic for Lyme borreliosis, produced completely negative results. This could also be due to the application of different testing methods (5,6).

In conclusion, our investigation of the risk of Lyme borreliosis in Gorski Kotar showed that this region cannot be regarded as a significant Lyme borreliosis risk zone. In line with this fact is the low incidence of clinically manifest Lyme disease in Gorski Kotar. Even if the number of asymptomatic and unrecognized infections were taken into account, the incidence of Lyme borreliosis would be far from endemic values. Further investigation of distribution of infected ticks and the identification of B. burgdorferi genospecies in ticks as well as in humans could explain this inverse entomological-epidemiological picture.

References


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