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

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*De part ment of In fec tious Dis eases, Uni ver sity Hos pi tal Cen ter Ri je ka, Ri je ka, Croa tia; <sup>1</sup>In sti tute of Mi cro bi ology and Im mu nol ogy, Uni ver sity of Ljubljana, Ljubljana, Slo ve nia; <sup>2</sup>Ri je ka In sti tute of Pub lic Health, Ri je ka, Croa tia; and <sup>3</sup>Labin Health Cen ter, Labin, Croa tia*

**Aim.** Clin i cal forms of Lyme dis ease in Gorski Kotar have oc curred only spo rad i cally, in con trast to the north west ern Croa tia and the neigh bor ing ar eas of Slo ve nia, which are well-known Lyme borreliosis en demic re gions. Our aim was to as sess the level of sero-prevalence of *Borrelia burgdorferi sensu lato* in a high-risk pop u la tion of for estry work ers in the moun tain ous re gion of Gorski Kotar, Croa tia, and com pare it with the sero-prevalence in the res i dents of that area and the neigh bor ing lit o ral re gion.

**Methods.** A sero-epidemiological study was con ducted on 520 healthy sub jects, di vided in 3 groups: the first group in cluded 234 for estry work ers, res i dents of Gorski Kotar, the sec ond 100 res i dents of var i ous pro fes sions in the same re gion, and the third 186 sub jects of var i ous pro fes sions from the neigh bor ing lit o ral re gion. The sera were col lected dur ing the win ters of two suc ces sive years, 1997 and 1998. Lyme borreliosis se rol ogy was per formed by in di rect im mu no flu o res cence as say. Sera from 10 hunt ing dogs from Gorski Kotar were also an a lyzed.

**Results.** The IgG an ti bod ies to *B. burgdorferi sensu lato* were found in 11 ex am i nees (4.7%) from the group of for estry work ers, in 3 (3%) from the sec ond group, and in 5 (2.7%) from the third group. Four out of 10 dogs (40%) had IgG an ti bod ies against *B. burgdorferi*.

**Conclusion.** Our re sults show that the for est and moun tain ous area of Gorski Kotar, Croa tia, has the char ac ter istics of a low sero-prevalence area, in con trast to the en demic neigh bor ing ar eas.

**Key words:** *Borrelia burgdorferi*; Croa tia; Lyme dis ease; prev a lence; se rol ogy

Lyme borreliosis, one of the most common tick-transmitted diseases, is caused by *Borrelia burgdorferi sensu lato* (1-3). Several epidemiological studies have re ported on the spread of the dis ease in an i mals, which are res er voirs and car ri ers of the *B. burgdorferi*, par tic u larly in the en demic re gions of Croa tia and neigh boring Slo ve nia (4-7). In the Primorsko-Goranska County (area 2,774 km<sup>2</sup>; popu lation 339,527) Lyme borreliosis ap pears to be rare (an nual in ci dence rate range is from 1.5 to 5.9/ 100,000) (8), al though Gorski Kotar, a for ested moun tain ous re gion of this county, has an abun dance of the tick popu lation. 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 pa tients show ing a low sero- pre va lence rate (9).

The aim of this study was to assess the sero-prevalence of Lyme borreliosis in the high-risk pop u la

tion of forestry workers, and compare it with the sero-prevalence in other res i dents of Gorski Kotar and the neigh bor ing lit o ral area.

### Sub jects and Methods

#### Study Area

Gorski Kotar is a moun tain re gion (1,273 km<sup>2</sup>) in the west ern part of Croa tia, very sparsely pop u lated (pop u la tion 30,545; 24/km<sup>2</sup>), with no im por tant ur ban cen ters. All the set tle ments are at 300-900 m above the sea level (Fig. 1). The work ing pop u la tion is mostly em ployed in the tim ber and wood-processing in dus try. The cli mate is con ti nental, with the mean an nual tem per a ture vary ing with al ti tude from 8.5°C to 6.3°C. The mean an nual rain fall var ies from 1,723 to 2,468 mm, and the mean an nual hu mid ity is 86%. The area is cov ered with co nif er ous and beech for ests, with rich or nitho fauna and var i ous games. Deer, roe deer, and roe bucks are the most nu mer ous games and their high num bers sup port the de vel op ment of an im por tant pop u la tion of ticks, the Lyme borreliosis vec tors.

#### Subjects

The study in volved 520 healthy sub jects, di vided into 3 groups: the first two groups in cluded 334 res i dents of Gorski Kotar (234 for-

estry workers and 100 of various professions), whereas the third group comprised 186 subjects living outside of Gorski Kotar. A group of 10 hunting dogs, belonging to the hunters from Gorski Kotar, was analyzed separately.

**Group 1** included 234 for estry work ers, whose me dian age was 40 years (range 22-60), all employed in the timber and wood-processing in dus try. Most of them were log gers (n=192; 82%), and the rest per formed a va ri e ty of jobs (7 truck driv ers, 10 trac tor driv ers, 20 saw mill work ers, and 5 for est ers). No form of Lyme borreliosis was de tected in sub jects, al though more than two thirds had a his tory of tick bites.

**Group 2** included 100 sub jects of var i ous pro fes sions, whose me dian age was 40.5 years (range 21-63). They had not spent any sig nif i cant pe riod in wood ed ar eas. Only about one third re ported pre vious tick bites. Data on any of the Lyme borreliosis forms were neg a tive.

**Group 3** com prised 186 sub jects of var i ous pro fes sions and liv ing in the neigh bor ing lit to ral re gion. Their me dian age was 44.2 years (range 17-68). None of the sub jects re ported ei ther a his tory of tick bite or an ac tive or past Lyme borreliosis.

#### Serological Testing

A blood sam ple of 5 mL was taken from each sub ject in a sin gle draw ing dur ing the win ter sea sons of two suc ces sive years, 1997 and 1998. The pres ence of spe cif ic IgM and IgG an ti bod ies against *B. burgdorferi sensu lato* were as sessed by in di rect im mu no fluo res cent assay with out ab sorp tion. *B. afzelii*, which is the most fre quently iso lated agent in Lyme borreliosis pa tients in Eu rope, was used as an ti gen. *Borreliae* were cul ti vated in mod i fied Kelly's me di um for 3 to 4 days, then washed, cen tri fuge d, and smeared on glass sheets. The an ti gen was pre served at -70°C un til test ing. The sera were di luted from 1:64 to 1:512. The an ti body ti ter of  $\geq 256$  was con sid ered pos i tive (10). Each test was per formed with a pos i tive and neg a tive con trol. The test was set up in a high-risk zone of Lyme borreliosis, Slovenia. All the anal yses were per formed at the lab o ra to ries of the Insti tute of Mi cro bi ology and Im mu nol ogy, Uni ver sity of Ljubljana, Slovenia. The re sults were pre sented as titers, and the cut-off value was cal cu lated in re la tion to the healthy sub jects in the en demic re gion. The dogs' sera were also an alyzed with in di rect im mu no fluo res cent as say with out ab sorp tion. Only IgG an ti body was as sessed and the titers of  $>64$  were con sid ered pos i tive, ac cord ing to the lit er a ture (11).

#### Statistical Analysis

The sta tis ti cal soft ware Epi Info 6, ver sion 6.02 from the Cen ters for Dis ease Con trol and Pre ven tion (CDC), USA, and WHO, Geneve, Switzer land, was used to es ti mate the in fec tion risks by means of the prev a lence odds ra tio, with ex act 95% con fi dence in ter vals.

## Results

IgG antibodies in  $\geq 256$  titer were found in 11 (4.7%) out of 234 for estry work ers, and in 3 (3.0%) out of 100 other inhabitants of Gorski Kotar. In the third group, pos i tive IgG an ti body ti ter was found in 5 (2.7%) of 186 sub jects, the res i dents of lit to ral area (Ta ble 1, and Fig. 1). IgM an ti bod ies were not pres ent in any of the sub jects. There was no sig nif i cant dif fer ence in the re la tive prev a lence of *B. burgdorferi sensu lato* IgG an ti bod ies be tween the groups.

Four out of 10 (40%) hunting dogs were sero-positive.

## Discussion

*B. burgdorferi sensu lato* sero-prevalence in spe cif ic pop u la tion groups is of great epi de mi o log i cal sig nif i cance. Our sur vey of the pop u la tion of Gorski Kotar did not show sig nif i cant dif fer ences be tween high-risk groups and the rest of the pop u la tion. Spe cif ic an ti bod ies were de tected in 4.7% of 234 for estry work ers, which is

**Table 1.** Seroprevalence of *B. Burgdorferi* in analyzed population groups<sup>a</sup>

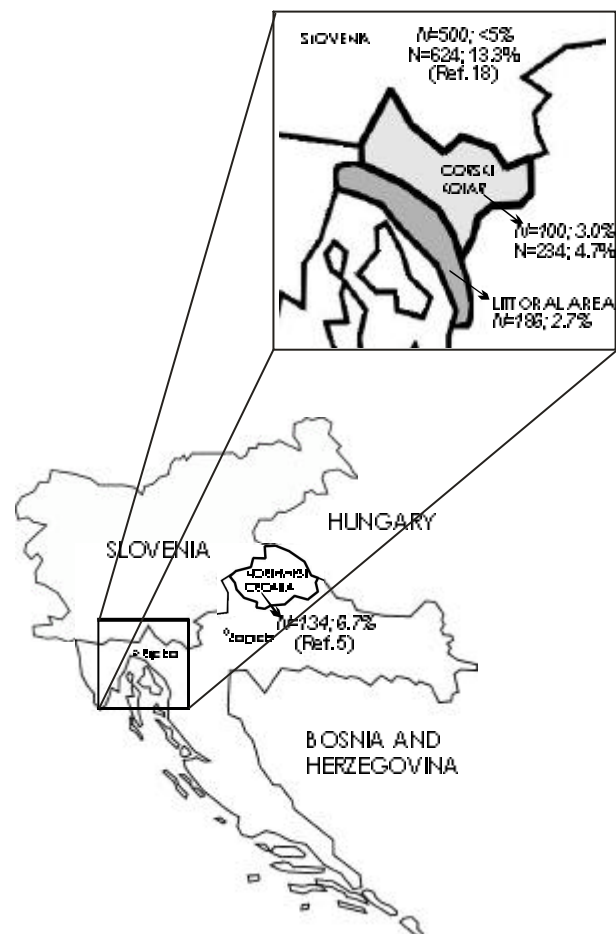
Group	positive (%) <sup>b</sup>	OR <sup>c</sup>	95% CI <sup>d</sup>
Forestry workers	11 (4.7)	1.59	0.41-9.09
Residents of Gorski Kotar	3 (3.0)		
Forestry workers	11 (4.7)	1.79	0.56-6.67
Residents of littoral region	5 (2.7)		
Residents of Gorski Kotar	3 (3.0)	1.12	0.17-5.89
Residents of littoral region	5 (2.7)		

<sup>a</sup>For estry work ers - n=234; res i dents of Gorski Kotar - n=100; res i dents of neigh bor ing (lit to ral) re gion - n=186.

<sup>b</sup>Prev a lence of sero positives among ex po sure groups.

<sup>c</sup>OR - odds ra tio.

<sup>d</sup>95% CI - con fi dence in ter val.



**Figure 1.** Dis tri bu tion of seroprevalence (%) of *B. Burgdorferi* in Gorski Kotar (Croatia) and neighboring areas. Note low sero-prevalence of Lyme borreliosis in the forested, mountainous area of Gorski Kotar, Croatia. Num bers in ital ics, gen eral pop u la tion; bold, for estry work ers.

a much lower per cent age than those reported for the population at risk in European countries (Germany 13.7%, England 25%, the Netherlands 28%, and Switzerland 35%), north western Croatia (26.8% – unpublished data, courtesy of Mišić-Majerus Lj, Department of Infectious Diseases, General Hospital Koprivnica, Koprivnica, Croatia) and the neighboring Slovenia (13,3%) (13-18). This shows that the infection rate may significantly vary even between geographically close regions.

The ratios of sero-prevalence estimated 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 of antibodies to *B. burgdorferi* was 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). It must be pointed out that the interpretation of specific serological findings is highly dependent on a number of subjective and objective factors, i.e., on the non-uniformity of various laboratories and application of serological methods of varying sensitivity and specificity (10). That is why we analyzed our samples in Ljubljana – to be able to compare our data with those from a well-known Lyme borreliosis endemic area. Another explanation for the observed differences in the sero-prevalence may be due to different periodical activities of a natural focus (associated with prevalence of vectors and reservoirs in infection and immunity of local population).

Sero-positivity in dogs is known to correlate significantly with entomological indexes of Lyme borreliosis transmission risk (24). Our sero-epizootiological 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 (dormouse) (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 house hold 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 manifested 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 definition of *B. burgdorferi* genospecies in ticks as well as in humans could explain this inverse entomological-epidemiological picture.

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