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Source / Izvornik: **Croatian Medical Journal, 2000, 41, 433 - 436**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:097858

CROATIAN MEDICAL JOURNAL

41(4):433-436,2000

PUBLIC HEALTH

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 the residents of that area and the neighboring littoral region.

Methods. A sero-epidemiological study was conducted on 520 healthy subjects, divided in 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. The 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 forested 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 km^2; population 339,527) Lyme borreliosis appears to be rare (annual incidence rate range 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 km^2) in the western part of Croatia, very sparsely populated (population 30,545; 24/km^2), with no important urban centers. All the settlements are at 300-900 m above the sea level (Fig. 1). The working population is mostly employed in the timber and wood-processing industry. The climate is continental, with the mean annual temperature varying with altitude 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 beech forests, with rich ornithofauna and various games. Deer, roe deer, and roe bucks are the most numerous games 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 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 an a lyzed sep a rately.

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 ety 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 wooded ar eas. Only about one third re ported pre vi-ous 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 cific IgM and IgG an ti bod ies against *B. burgdorferi sensu lato* were as sessed by in di rect immunofluorescent as say 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 an-ti gen. *Borreliae* were cul ti vated in mod i fied Kelly's me dium for 3 to 4 days, then washed, cen tri fuged, and smeared on glass sheets. The an-ti gen was pre served at –70EC until test ing. The sera were di luted from 1:64 to 1:512. The an ti body ti ter of $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 y ses were per formed at the lab o ra to ries of the In sti tute of Mi cro bi ol ogy 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 a lyzed with in di rect immuno fluo-rescent 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).

StatisticalAnalysis

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, Switzerland, was used to estimate the infection 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 $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 rel a 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-cific pop u la tion groups is of great ep i 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 significant dif fer ences be tween high-risk groups and the rest of the pop u la tion. Spe cific 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[a]

Group	positive (%)[b]	OR[c]	95% CI[d]
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)		

[a]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.
[b]Prev a lence of seropositives among ex po sure groups.
[c]OR – odds ra tio.
[d]95% CI – con fi dence in ter val.

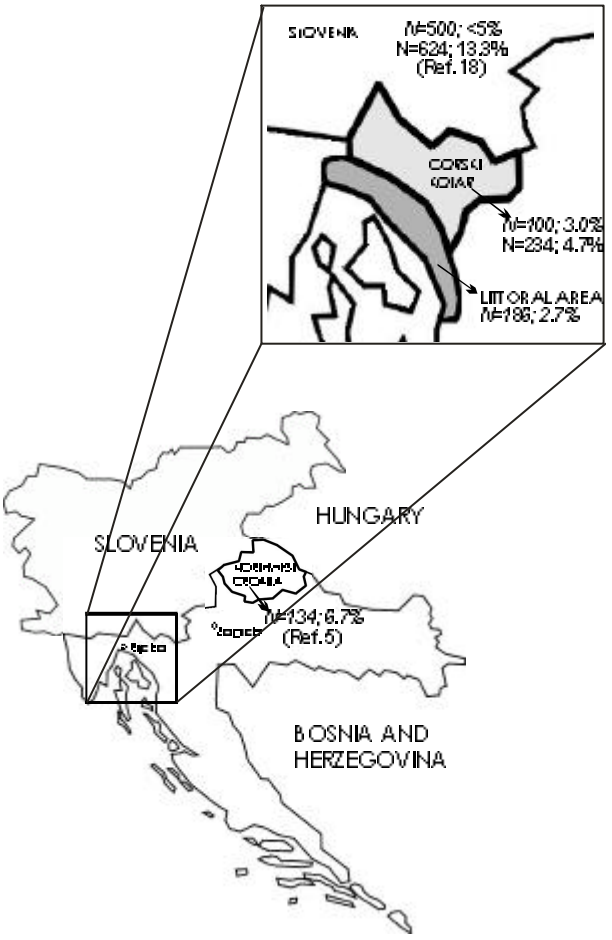

Figure 1. Dis tri bu tion of seroprevalence (%) of *B. Burgdorferi* in Gorski Kotar (Croatia) and neighboring areas. Note low sero-prevalence of Lyme borreliosis in the for ested, moun tain-ous area of Gorski Kotar, Croatia. Num bers in ital ics, gen eral pop u la tion; bold, for estry work ers.

a much lower per cent age than those re ported for the population at risk in European countries (Ger many 13.7%, England 25%, the Netherlands 28%, and Switzerland 35%), north west ern Croatia (26.8% – un pub lished data, cour tesy of Mišiæ-Majerus Lj, De part ment of In fec tious Diseases, General Hospital Koprivnica, Koprivnica, Croatia) and the neigh bor ing Slovenia (13,3%) (13-18). This shows that the in fec tion rate may sig nif i cantly vary even be tween geographical- ly close re gions.

The ra tios of sero-prevalence es ti mated in the in hab itants of Gorski Kotar and in the gen eral pop u la tion of the neighboring littoral area were 3.0% and 2.7%, re spectively. Studies performed in corresponding groups in northwestern Croatia reported higher sero-prevalence of 6.7-9.7% (4,5), which is close to the val ues re corded in the endemic regions of some Western European countries (Swe den 9%, Ger many 17%) (19,20). The sero-prevalence in the gen eral pop u la tion in Slovenia, the en demic re gion well known for many years, is lower than 5%, which is sim i lar to our re sults (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, sim i lar to our in ves ti gated area (19,22,23). It must be pointed out that the in ter pre ta tion of spe cific serological find ings is highly de pend ent on a number of sub jec tive and ob jec tive fac tors, i.e., on the non- uniformity of various laboratories and application of serological meth ods of vary ing sen si tiv ity and spec i fic ity (10). That is why we an a lyzed our sam ples in Ljubljana – to be able to compare our data with those from a well-known Lyme borreliosis endemic area. An other explanation for the observed differences in the sero-prevalence may be due to differentperiodical ac tiv i ties of a natural focus (associated with prevalence of vectors and res er voirs in fec tion and im mu nity of lo cal pop u la tion).

Sero-positivity in dogs is known to cor re late sig nif icantly with entomological indexes of Lyme borreliosis trans mis sion risk (24). Our sero-epi zootiological anal ysis de tected the pres ence of spe cific an ti bod ies in 4 out of 10 hunt ing dogs (40%). The 1994 study in Gorski Kotar revealed sero-positivity in 2 of 20 cows (10%) but in none of 10 wild an i mals (dor mouse) (9). These re sults point to the pres ence of *B. burgdorferi sensu lato* in an imal res er voirs, but the size of our sam ples does not al low more pre cise con clu sions. Cor re spon ding stud ies on cattle and house hold dogs ex am ined by ELISA, con ducted in areas of northwest Croatia endemic for Lyme borreliosis, pro duced com pletely neg a tive re sults. This could also be due to the ap pli ca tion of dif fer ent test ing meth ods (5,6).

In con clu sion, our in ves ti ga tion of the risk of Lyme borreliosis in Gorski Kotar showed that this re gion cannot be regarded as a significant Lyme borreliosis risk zone. In line with this fact is the low in ci dence of clin ically man i fested Lyme dis ease in Gorski Kotar. Even if the number of asymptomatic and unrecognized in fections were taken into account, the incidence of Lyme borre liosis would be far from en demic val ues. Fur ther in ves ti ga tion of dis tri bu tion of in fected ticks and the def i ni tion of *B. burgdorferi* genospecies in ticks as well as in humans could explain this inverse entomological-epidemiological pic ture.

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Re ceived: April 21, 2000
Ac cepted: Oc to ber 15, 2000

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