Occurrence of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii* in the *Ixodes ricinus* ticks from Eastern Slovakia

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Abstract. A total of 2816 unfed adults nymphs of *Ixodes ricinus* ticks were collected from vegetation in Košice (Eastern Slovakia) from 1994 to 1997. Prevalence of *Borrelia burgdorferi* s. 1. in *I. ricinus* ticks, detected by dark field microscopy, varied and depended upon the year and the habitat of the collected ticks. The lowest prevalence was observed in 1994 (4.8%). During 1995 it increased to 17.2% and during the next two years decreased to 15.5% and 14.2%. The rate of infection varied from 2.1 to 23.3% within 10 examined habitats of the Košice area. A different value of relative density of ticks was observed in various habitats. It ranged from 9–212 ticks per collecting hour within one flagged area (600 m²) which is 1.5–35.5 ticks per 100 m². Eight isolates were obtained from the infected ticks. Electrophoresis and immunoblotting with 6 monoclonal antibodies were used for the identification of *Borrelia* strains. Three tick isolates were identified as *B. burgdorferi* s. s. and the other three isolates were found to be *B. garinii*. One strain reacted as a mixed culture of *B. burgdorferi* s. s., and *B. garinii*. The strain originated from the Vihorlat Mountains habitat and was detected by PCR-SSCP as *B. burgdorferi* s. s. with a small amount of *B. afzelii*. The obtained results emphasize the epidemiological importance not only of *B. garinii* and *B. afzelii* but also of *B. burgdorferi* s. s. in Central Europe.

Key words: *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, Isolations, *Ixodes ricinus*, Prevalence, Ticks

Introduction

Since its discovery in 1982, *Borrelia burgdorferi* s. 1. [1] the etiological agent of Lyme borreliosis (LB), has been the subject of many epidemiological and epizootical studies. Many strains of *B. burgdorferi* have been isolated from ticks, humans and reservoir hosts. Five genomic groups or genomospecies of *B. burgdorferi* s. 1. have been found in Europe: *B. burgdorferi* s. s., *B. garinii*, *B. afzelii* [2–6] *B. valaisiana* (VS116) [7] and *B. lusitaniae* (PoTiB2) [8]. The European isolates are more heterogeneous with respect to their antigenic profiles than American ones [4, 5, 9].

In natural foci, LB is transmitted primarily by blood-sucking arthropods, especially by ticks of the genus *Ixodes: I. ricinus* L. in Europe, *I. persulcatus* Schulze in Eurasia, *I. pacificus* Cooley et Kohls and *I. scapularis* Say in North America [10, 11]. The infected ticks frequently occur in peripheral forest parks in the neighbourhood of large cities in Central Europe. Therefore, we were interested in the occurrence and overinfestation of ticks by this dangerous zoonosis in suburban parks and forest parks around the largest town of Eastern Slovakia – Košice, during 1994–1997.

Litle is known about the prevalence of *Borrelia* spirochetes in ticks in Slovakia. The occurrence of *B. burgdorferi* in ticks in this region was studied by Kmety et al. [12], Prokopčáková et al. [13], Drgonova and Řeháček [14], Pet’ko et al. [15] and Mateička et al. [16]. Serological diagnostic of LB in dogs has been studied by Štefančíková et al. [17].

In the present study we focused our attention on the distribution of *B. burgdorferi* s. 1. in Eastern Slovakia and on the occurrence of various genomospecies in the tick population.

Materials and methods

Isolation and growth of Borreliae

The density of tick populations and their overinfection with *B. burgdorferi* was studied in different habitats of Eastern Slovakia from 1994 to 1997. *I. ricinus* ticks (n=2816) were collected from May to September by flagging low vegetation. The collections were carried out primarily in the places most frequently visited by people in recreational areas around the city of Košice with a predominance of oak and hornbeam, namely: Čermel’, Tahanovce, Zelený dvor, Furča, Bankov, Hradová, Myslava, Perín, Sečovská, Popradská (habitats 1–10). The relative density of active ticks in the vegetation was expressed as the number of ticks collected within one flagged area of approximately 600 m² per hour.

The ticks were immersed in 70% ethanol for 5 min to reduce surface contamination, rinsed in sterile
saline, and placed on watchglasses. Midguts from ticks or from pool samples (5 ticks) were dissected out in a drop of sterile saline and examined for the presence of mobile spirochetes by dark-field microscopy. In the case of positive results, the other part of the positive samples was cultured in BSK-H medium (Sigma) supplemented with 6% rabbit serum, containing phosphomycin (170 μg/ml), amicacin (8 μg/ml) and sulphamethoxazol (13 μg/ml) to prevent bacterial contamination. Antibiotics were added in the form of antibiotic discs (Sensi Discs, Becton Dickinson). Cultures were incubated at 33 °C and examined for the presence of spirochetes every seven days during two months.

Antigen preparation
Two-week-old cultures (4th passage) were harvested by centrifugation at 10,000 g, for 30 min and washed three times in phosphate-buffered saline (pH 7.2) containing 5 mM MgCl_2. The whole cells of spirochetes suspended in saline were sonicated (Sonic Dymembrator, Dynatech, UK) at 20 kHz for 3 min, 20–30 W and the final protein concentration was estimated by the method of Bradford [18].

SDS-PAGE and immunoblotting
The sonicated antigen was dissolved in a sample buffer (containing β-mercaptoethanol as a reducing agent), boiled for 5 min and subjected to SDS-PAGE (12% polyacrylamide gel and 4% acrylamide stacking gel) using the system of Laemmli [19]. Low-range-molecular-mass standard (BioRad) was used in each gel. The high passage strains: B31 (B. burgdorferi s. s.), NE462 (B. garinii) and NE632 (B. afzelii) were used as references. Electrophoresis was carried out at a constant current of 25 mA for 1 hour until the marker dye migrated down to an optimized point in the gel. Coomassie brilliant blue G250 (Sigma) was used to visualize the proteins in the gels. Proteins separated in the other gel were then transferred to a nitrocellulose membrane using the system of Towbin [20]. The nitrocellulose membranes were cut into 3 mm wide strips. One part of each membrane was stained with amido black, to assess the efficiency of transfer, the other one was used to react with monoclonal antibodies.

After a 10 min wash with TBS (Tris-buffered saline, pH 7.5), the strips were blocked with 3% gelatin in TTBS (TBS with an additional 0.05% Tween 20) for 1 hour, then incubated for 3 hours with MAb D6. After three additional washes the substrate (4-chloro-1-naphtol, Sigma) was added and the protein bands were visualized. The reaction was stopped by deionized water [21].

Results
A total of 2816 nymph and adult I. ricinus ticks were collected in the suburbs of Košice from 1994 to 1997. A different value of the relative density of ticks was observed in various habitats. This ranged from 9–212 ticks per collecting hour within one flagged area (600 m²). In 1996 and 1997 the relative density of I. ricinus evaluated in the various locations was on average, 34.8. No correlation between the relative density of ticks and the prevalence of infected ticks with B. burgdorferi s. 1. was found (Figure 1).

In 1994 moving spirochetes, morphologically resembling bacteria from B. burgdorferi cultures, were observed in 4.8% of I. ricinus, however, in 1995, 17.2% of I. ricinus harboured borreliae as detected by dark field microscopy. The prevalence of infected adults increased during 2 years from 5 to 19.3% and nymphs from 4 to 11.6% respectively. The following rates of the prevalence of B. burgdorferi in ticks were observed: 15.6% for 1996 and 14.2% for 1997 (Table 1). Infected ticks were collected in every habitat with great local variability of prevalence from 2.1 to 28.4%. Adult ticks were infected more often than nymphs and the females more often than males.

In 1996, 40 nymphs and adults of I. ricinus ticks were collected in the Vihorlat Mountains – Morské oko. The ticks were dissected in pools (five ticks) and 62.5% of the pools were detected as being positive for borreliae by dark-field microscopy. The minimum infection rate, calculated according to Kahl et al. [22], was 12.5%, though the actual prevalence of borreliae in ticks could be higher.

Out of 25 isolation attempts, 8 strains were obtained: Ir105, Ir107, Ir110, Ir112 and Ir113 from females; Ir108 and V123 from males and Ir103 from nymphs of I. ricinus ticks. Seven strains: Ir103, Ir105, Ir107, Ir108, Ir110, Ir112 and Ir113 originated from the woodland park in the city of Košice and the strain V123 from the Vihorlat Mountains.

![Figure 1. Relative density (RD) of Ixodes ricinus ticks and prevalence of Borrelia burgdorferi s. 1. in Eastern Slovakia (Košice forest-park) in 1996 and 1997.](image)