


Ixodes ricinus L. ticks (n = 913) from southern Poland, near town of Katowice, were examined for the presence of B. burgdorferi s. l., using dark field microscopy (Peňko B., Studa K., Stanko M., Tresová G., Karbowiak G., Fričová J. 1997: Ann. Agric. Environ. Med. 4: 263-269). Minimum infection rate in pooled samples of nymphs, males and females was 8.2-10.3%, respectively. Infection of adult ticks on 10 localities fluctuated from 4.0 to 15.0%. Motile spirochetes, morphologically resembling bacteria from Borrelia cultures, were observed in 49.3% of male and 51.4% of I. ricinus female pools (Peňko et al., op. cit.). The present work is a follow up to our previous study. The aim of the investigation was to characterize electrophoretically and immunologically spirochetal isolates obtained from I. ricinus.

I. ricinus ticks were collected by flagging low vegetation in different localities of Katowice in Poland in May 1996. Ticks were immersed in 70% ethanol for 5 min, rinsed in sterile saline and placed on watch glass. Midguts from ticks (pool 5) were dissected out in a drop of sterile saline and examined for the presence of motile spirochetes by dark field microscopy. Positive samples were cultured in BSK-H medium (Sigma) containing phosphomycin (170 mg/ml), amikacin (8 mg/ml), sulphanamethoxazole (13 mg/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 by dark-field microscopy every seven-ten days for two months. Non-contaminated cultures of spirochetes were cultivated in BSK-H medium supplemented with 6% rabbit serum. Five isolates, out of 45 isolation attempts, from I. ricinus ticks were obtained: P137 (5 female), P142 (5 male), P153 (5 female), P175 (5 female) and P210 (5 female). Sonicated antigens were prepared from the 4th passage of cultures (Matei ěka F. 1992: Rheimatologie 6: 81-86). Antigens were subjected to SDS-PAGE in 10% polyacrylamide gel (Laemmli U.K. 1970: Nature, London, 227: 680-685). The high passage strain B31 - B. burgdorferi s. str., NE462 strain - B. garinii and NE632 strain - B. afzelii were used as reference. Proteins from gel were transferred on nitrocellulose sheets (Towbin H., Staehlin T., Gordon J. 1979: Proc. Natl. Acad. Sci. USA. 76: 4350-4354). Monoclonal antibodies (Mab) used in this study were: H9724 and H5332, dilution 1: 10 (supplied by A. Barbour, Texas, USA), J 8.3 and 1 17.3, dilution 1: 1 000 (supplied by D.A. Postic, Pasteur Institute, Paris, France), D6, dilution 1: 50 (supplied by O. Péter, Sion, Switzerland), H3TS, dilution 1: 500 (supplied by L. Gern, Neuchâtel, Switzerland). For immunodetection and visualisation of the single proteins peroxidase conjugated swine anti-mouse IgG

Electrophoresis and Western immunoblot with 6 monoclonal antibodies (Table 1) were used for identification of tick isolates in species (B. burgdorferi s. l.) and genospecies levels. Monoclonal antibody (Mab) H9724 against 41 kDa flagellar protein recognizes genus-specific epitopes. Beside B. burgdorferi, this Mab reacts with other species of the genus Borrelia but not with Treponema, Leptospira and Spirochaeta (Barbour A.G., Hayes S.F., Heiland R.A., Schrumpf M.E., Tessier S.L. 1986: Infect. Immun. 52: 549-554). Another Mab, H5332, specific for B. burgdorferi s. str., recognizes 31 kDa outer surface protein OspA (Barbour A.G., Tessier S.L., Todd W.J. 1983: Infect. Immun. 41: 795-804). Examined strains (excluding strain P137) reacted with Mab H9724 and Mab H5332. For this reason, 4 strains P142, P153, P175, P210 belong to the genus Borrelia and to the species B. burgdorferi s. l.

Other genospecies specific Mabs used in this study: H3TS recognizes 31 kDa protein of B. burgdorferi s. str. (Baranton et al., op. cit.), J 8.3 and I 17.3 recognize OspA and OspB proteins of B. afzelii (Canica et al., op. cit.). None of the isolates reacted with that 3 Mabs, which suggests that B. burgdorferi s. str. and B. afzelii can be excluded. Identification at genospecies level was supported by an immunoblot analysis of 12 kDa antigen and its reactivity with Mab D6, specifically recognizing B. garinii (Péter O., Bretz A.G. 1992: Zbl. Bakt. 277: 28-33). Two of five strains, P142 and P153, reacted with Mab D6; for this reason they were identified as B. garinii.

As shown in Figure 1, B. burgdorferi s. str. - strain B31 produced 2 major proteins at 31 and 34 kDa (OspA and OspB), B. garinii as exemplified by strain NE462 produced a single predominant OspA - 33 kDa protein. B. afzelii - strain NE632 produced 2 major proteins OspA and OspB located at 32 and 35 kDa. Electrophoretic profiles of the strains obtained seem to be identical, resembling those of B. garinii, because they exhibited a single 33 kDa protein. Definitive classification using PCR method will be the aim of our further study.

One genospecies was identified among isolates originated from Katovice, strains P142 and P153. On the other hand, the presence of all three Lyme borreliosis-associated genospecies in northern Poland was confirmed by genospecies-specific PCR. In the Gdansk and Olsztyn provinces, where overall infection rates of B. burgdorferi in I. ricinus ticks was 10.6 to 21.3%, isolates have been determined as B. afzelii, B. garinii and B. burgdorferi s. str. (Stanczak J., Picken R.N., Wegner Z., Kubicza-Biernat B., Picken M.M. 1996: VII Int. Congr. Lyme Borreliosis, June 16-21, 1996, San Francisco, California. Abstracts C440).

An understanding of the antigenic diversity and regional differences in species and genospecies distribution is important for several reasons. Differences in the clinical manifestations of Lyme borreliosis may be related in part to differences among spirochetal agents from distinct geographic regions.

**Table 1. Reactivity of Borrelia strains with specific monoclonal antibodies using Western immunoblot.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>H9724 flagellin Borrelia</th>
<th>H5332 OspA B.b.s.t.</th>
<th>H3TS OspA B.b.s.t.</th>
<th>D6 12 kDa B.garinii</th>
<th>J 8.3 OspA B.afzelii</th>
<th>117.3 OspB B.afzelii</th>
</tr>
</thead>
<tbody>
<tr>
<td>P142</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P153</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P175</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P210</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P137</td>
<td>nt</td>
<td>nt</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* - B. burgdorferi s. l.
** - B. burgdorferi s. str.
nt - not tested

**Fig. 1.** Coomasie brilliant blue stained SDS-PAGE gel of whole cells Borrelia. Lane 1: B. burgdorferi s. str.; lane 2: B. garinii; lane 3: B. afzelii, lanes 4 to 8: strains P137, P142, P153, P175, P210.


Mapping the presence of different genospecies in various geographic areas in Europe, where Lyme disease occurs, is still inadequate. Our observations have contributed to the knowledge about the spread of B. burgdorferi in I. ricinus ticks in the Carpathian region of Poland.

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