BORRELIA BURGDORFERI SENSU LATO IN THE IxODES RICINUS Ticks IN SOUTHERN POLAND

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Abstract: In May 1996, 164 pooled and single samples containing 913 larvae, nymphs and adults of Ixodes ricinus from urban, suburban and wooded biotopes of southern Poland were examined for the presence of Borrelia burgdorferi sensu lato. Ticks were collected by dragging a flag over the vegetation and B. burgdorferi was detected in pooled tick samples by darkfield microscopy or isolated by cultivation in BSK-II medium. Minimum infection rates of nymphs, males and females in the area were 8.2, 9.8 and 10.3%, respectively. Infection of adult I. ricinus ticks on 10 localities fluctuated from 4.0 to 15.0% and over 10% of adult ticks in forests and in some urban and suburban parks of the city Katowice were infected. Five Borrelia strains were isolated from the adult I. ricinus ticks. Relative density of ticks ranged from 20-65 ticks per one collecting hour and from 3.3-10.8 ticks per 100 m², respectively. Frequent occurrence of ticks was also observed in urban parks and residential districts of the city Katowice. The results refer to the risk of human and animal infections by borreliae in the areas of big cities in southern Poland.

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INTRODUCTION

Since its discovery in 1981, Borrelia burgdorferi sensu lato [3], etiological agent of Lyme borreliosis (LB), has been the subject of many epidemiological and epizootiological studies. This polysystemic disease of humans and animals has been recently regarded as the most common human tick-borne disease in the Holarctic. 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 [1, 7, 23]. Infected ticks frequently occur in peripheral and central town parks in the territories of large cities in Central Europe. Therefore, we were interested in the occurrence and overinfestation of ticks by the agent of this dangerous zoonosis in the area of southern Poland with high concentration of large cities.

Little is known about the prevalence of Borrelia spirochetes in ticks in Poland. The most complete data was reported from the northeast provinces by the Institute of Maritime and Tropical Medicine in Gdynia [6, 24, 25, 26].
Ticks of the species *I. ricinus* and *Dermacentor reticulatus* were examined. Most of positive results were obtained from *I. ricinus*; the spirochetes *Borrelia* sp. have been observed recently also in *D. reticulatus* (Wegner, personal information).

Moreover, Siński et al. [20] published the results of a study on the *Borrelia burgdorferi* infection of ticks in Urwita in Masuria Region and several places in the southeast Poland (including Katowice and Szczyglice near Kraków). Tylewska - Wierzbanska et al. [22] examined ticks collected from vegetation, humans and animals in different provinces all over Poland. *I. ricinus* and *D. reticulatus* mostly and occasionally *Argas reflexus* were examined in the above cited works. Positive results were obtained only for *I. ricinus*.

The occurrence of *Borrelia burgdorferi* in ticks in the closely neighbouring area of the Carpathians in Slovakia was studied by Kmety et al. [13], Prokopčaková et al. [19], Peško et al. [15] and in the bordering area of the West Carpathians in Moravia by Hubálek et al. [9, 10] and Chmela [4].

This paper presents the studies on the occurrence of Lyme borreliosis agent in ticks in the areas of large cities in southern Poland over the year 1996.

**MATERIAL AND METHODS**

**Studied area and collection of ticks.** The density of tick populations and their overinfestation with LB agent, spirocheta *Borrelia burgdorferi* sensu lato, was studied in ten localities of southern Poland in May 1996 (Fig. 1). Ticks were collected by dragging a light woolen flag (60 × 90 cm) over the plant and bush vegetation. The collections were carried out primarily in the urban and suburban biotopes of the city Katowice (Fig. 2), in the places most frequently visited by people, e.g. in parks, sporting areas and peripheral villa districts. Some ticks were collected in wooded areas at places with low intensity of anthropogenic damaging (Chobot, Wal Ruda - Fig. 3). The area dragged with one flag in one hour measured approximately 600 m² in pastures and forests with shrubs, 800 m² in meadows and parks. Ticks collected from the particular localities were placed in test tubes with a few blades of grass and preserved at 4°C until investigation. The relative density of active ticks was expressed as the number of ticks collected with one flag per one hour, as well as a number of ticks per 100 m² (Tab. 1).

**Table 1.** Material of *Ixodes ricinus* ticks collected in southern Poland in May 1996.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Date</th>
<th>No. of ticks</th>
<th>Time dragged&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Area dragged&lt;sup&gt;2&lt;/sup&gt;</th>
<th>RD&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>males females nymphs total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>May 24</td>
<td>45 55 3 103</td>
<td>3</td>
<td>2400</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>May 24</td>
<td>71 56 5 132</td>
<td>3</td>
<td>1800</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>May 29</td>
<td>37 36 52&lt;sup&gt;d&lt;/sup&gt; 125</td>
<td>3</td>
<td>1800</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>May 30</td>
<td>52 47 20 119</td>
<td>3</td>
<td>2400</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>May 30</td>
<td>31 39 5 75</td>
<td>3</td>
<td>2400</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>May 30</td>
<td>32 41 27 100</td>
<td>2.25</td>
<td>1350</td>
<td>44</td>
</tr>
<tr>
<td>7</td>
<td>May 30</td>
<td>30 43 10 83</td>
<td>2.25</td>
<td>1350</td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>May 25</td>
<td>13 7 - 20</td>
<td>1</td>
<td>600</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>May 25</td>
<td>35 30 - 65</td>
<td>1</td>
<td>600</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>May 25</td>
<td>39 52 - 91</td>
<td>2</td>
<td>1200</td>
<td>46</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>385 406 <strong>122&lt;sup&gt;d&lt;/sup&gt;</strong> 913</td>
<td>23.50</td>
<td>15900</td>
<td>40.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of ticks per one hour of dragging.<br><sup>b</sup>Relative density of active ticks (number of ticks / hour of dragging).<br><sup>c</sup>Including 6 larvae.
Localities:

1. Mikołów near Katowice, in the “Barbara” coal mine area, meadow with mixed shrubs and trees.
2. Katowice, Ligota, oak-hornbeam forest and surrounding pastures with shrubs and dewberry.
3. Katowice, Kokociniec, forest and pastures with shrubs near railway.
5. Katowice, Park Tadeusza Kościuszki, old park with local occurrence of shrubs.
6. Katowice, Ochojec, forest near railway and residential area.
7. Katowice, Murcki, forest in surroundings of sports area.
8. Chobot (Kraków province), Niepołomicka Forest, old oak-hornbeam forest.
9. Borzęcin (Tarnów province), pastures with mixed shrubs and trees.
10. Wał Ruda (Tarnów province), pastures and meadows near forest with predominance of oaks and pines.

Investigation of ticks. Nymphs, males and females were investigated in pools of 5 specimens each. Moreover, some specimens were examined individually. Ticks were examined for the presence of *Borrelia* spp. by dark field microscopy, according to Wilske *et al.* [27] and by cultivation according to Preac-Mursic *et al.* [18] and Tresová *et al.* [21]. The samples were examined with a Nikon microscope at 400 × magnification. Minimum infection rate of pools was calculated according to Kahl *et al.* [12].

A total of 164 pooled samples containing 820 specimens of *I. ricinus* were examined for the presence of *Borrelia burgdorferi* sensu lato. Another 93 ticks were investigated individually (Tab. 2).

RESULTS

In 1996, 913 larvae, nymphs and adult ticks *I. ricinus* were collected during a 23.5-hour vegetation flagging in southern Poland. Their relative density in 10 examined localities ranged between 20–65 ticks per one flag in one hour, or 2.8–12.3 ticks per 100 m², respectively (Tab. 1). Higher relative densities were observed on pastures in a

<table>
<thead>
<tr>
<th>Locality</th>
<th>No. of pools</th>
<th>Positive pools</th>
<th>Infection rate (%)</th>
<th>Isolated Borrelia strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>11</td>
<td>8</td>
<td>17.8</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>9</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>0</td>
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<td>9</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>13.3</td>
</tr>
</tbody>
</table>

*a pool - 5 specimens, *b* dark field examination, *c* calculated minimum infection rate, *d* cultivation.
Borrelia burgdorferi in the ticks in southern Poland

Moving spirochetes, morphologically resembling bacteria from Borrelia cultures, were observed in 49.3% of male and 51.4% of female I. ricinus pools. The lower values for nymphs (40.9%) were statistically insignificant ($t = 0.690$ and $t = 0.866$, respectively). Minimum infection rate of I. ricinus nymphs, males and females was balanced (8.2–10.3%) in the entire studied area. Individually investigated ticks showed the proportion of infected females to be more than twice as high as for males. Single samples of larvae and nymphs were not evaluated because of a too small set (Tab. 2).

Borreliae were found in ticks from 9 localities (Tab. 3). The absence of infected ticks on locality 8 is probably due to the small number of examined specimens. Minimum infection rate of I. ricinus adult ticks at studied localities fluctuated from 4.0–15.0% without correlation with relative density of ticks (Fig. 4). A relative high infestation was observed in some suburban meadows and forests of the cities Katowice and Mikołów (loc. 1, 2), as well as in the urban park (loc. 5), which was higher than that in wooded rural areas (loc. 9 and 10).

Minimum infection rate of males and females at studied localities showed differences. At localities 2, 3 and 6, females were infected more frequently, at other localities it was males. Five strains of Borrelia spp. were isolated from tick pooled samples at localities 1, 4, 5 and 9. The strain marked as P142 comes from males, the others from females (Tab. 3). Immunochemical investigations of the isolated strains are published in other paper [21].

DISCUSSION

Relatively high densities of ticks were found in May 1996 in the central and peripheral parks of the city of Katowice. Finding of ticks in central parks of large cities

![Figure 3](image-url) - Tick collection localities in the southwestern region of Tarnów province: 8) Chobet, 9) Bazorze, 10) Wal Roda.

![Figure 4](image-url) - Relative density and minimum infection rate of Ixodes ricinus adults with Borrelia species in southern Poland.
The occurrence of ticks in towns is connected with establishing new parks in original forest areas or with building new residential districts on the peripheries of towns close to forests. Persistence of ticks is determined by their possibility to accomplish their life cycle, which depends on the presence of suitable hosts for all developmental stages. The presence of hosts is important for adult ticks. In Central Europe these hosts are represented mainly by squirrels (Sciurus vulgaris), both species of hedgehogs (Erinaceus europaeus and E. concolor) and local stray dogs and cats. A sporadic occurrence of ticks in isolated parks can be attributed to the transmission by birds.

The occurrence of ticks has a mosaic-like character and their infection with borreliae at different places of an area with the presence of LB is highly variable, depending on the place and time of investigation [28]. Cases with seasonal alteration were observed in local populations of ticks [9, 13, 15].

Long-term observations under Central European conditions showed an impressive local fluctuation in the infestation of ticks in the individual years of study: 6.5–11.4% [10]; 1.9–22.0% [16]; 0.9–18.6% [17]; 2.1–41.7% [15]. These variations in tick infestation are most likely reflecting the periodical occurrence of borreliae in ticks, which was pointed out in Slovakia by Kmety et al. [13]. In the Carpathian region of Slovakia this appears as a 4–5 year cycle, with the last minimum recorded in 1994 and maximum in 1992–1993 [15]. In comparing the values of the LB prevalence at different places of this area, the phase of epizootiological cycle should also be considered.

The high variability of Borrelia infection in ticks at individual developmental stages is probably associated with the periodicity of LB. Adult ticks become generally more frequently infected than nymphs, females more frequently than males. Kahl et al. [12] reported a minimum 2.5% infection rate of nymphs, 5.3% of males and 10.2% of females from the territory of Berlin in 1986. Percentage of ticks infected by spirochetes of Borrelia spp. examined in Poland varied. In Olsztyn province it has ranged from 2.9–35.7% (average 11.5%) [25], in Urszulin in Masuria region 3.5% and in examined localities in southeast Poland from 13–58.3% (in Szczylglice near Kraków 19.2%) [20]. Among I. ricinus specimens from different parts of Poland tested by Tylewska-Wierzbowska et al. [22] only 0.75% of ticks were infected by B. burgdorferi. In southern Germany, Wilske et al. [27] found an average 1% infection in larvae, 10% in nymphs and 20% in adult ticks, with no difference observed between the infection rate in males and females. Nymphs are less frequently infected than adult ticks [20, 25, 26], as was observed at several localities in 1992 [8, 15]. A variation in the prevalence of infected adults is generally preceded by the change in the infection rate of nymphs in the previous year. In both mentioned cases, an increased prevalence of infected adults occurred during the next year or the next two years. These differences may reflect the presence of Borrelia donors (animals at the stage of bacteraemia) and of naturally immunized hosts of I. ricinus, which may cause either the increase in the occurrence of borreliae [11] or their loss in ticks [14].

The proportion between the prevalence of borreliae in nymphs and adults and the absolute prevalence values in May 1996 may serve as a basis for estimating the phase of epizootiological cycle and for prognosing the epidemiological situation for LB in the region. A significant local variability of infected tick prevalence on a relatively small territory points out the wide spectrum of ecological factors influencing the occurrence of borreliae in nature.

**CONCLUSION**

The preliminary results indicate the hazard of human infection elicited by the agent of Lyme borreliosis - a grave natural focal zoonosis in the urban and suburban verdure of large cities in southern Poland, which should not be underestimated. Relatively high prevalence of infected ticks and the balanced proportion of nymph and adult infestation with borreliae in southern Poland suggests the culmination of cycle and its possible persistence in the year 1997.

**Acknowledgement**

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Borrelia burgdorferi in the ticks in southern Poland


