Prevalence of Anaplasma phagocytophilum in ticks and rodents along an urban–natural gradient in SW Slovakia

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Introduction
Anaplasma phagocytophilum is a medically and veterinary important emerging tick-borne pathogen in Europe. It is a gram-negative bacterium localized in the blood cells (neutrophils, monocytes, macrophages and erythrocyths) or endothelial cells of blood vessels and causes febrile disease in humans (human granulocytic anaplasmosis – HGA) and animals (pasture fever, equine and canine granulocytic anaplasmosis). The principal vector is Ixodes ricinus. The bacterium has been detected in a broad range of vertebrate species including rodents, however, reservoir competence of rodents is unclear. Monitoring of A. phagocytophilum prevalence in I. ricinus and rodents in various habitat types of Slovakia may contribute to the knowledge about the epidemiology of anaplasmosis in Central Europe.

The aim of the study
1. Comparison of infection rates of A. phagocytophilum in questing I. ricinus ticks from habitats in SW Slovakia that are differently affected by human intervention
2. Assessment of the role of rodents in the transmission cycle of A. phagocytophilum
3. Evaluation of the effect of wildlife density in the study area on prevalence of A. phagocytophilum in questing ticks.

Material and Methods
The study area is located in the Small Carpathian Mountains (SW Slovakia) and comprises two different sites: the recreational area of the Bratislava forest park, which is strongly influenced by human activities and a non-fragmented woodland at Figelka (about 40 km from Bratislava), affected by human activities to a lower degree (Fig. 1). In each site three 100 m long transects were selected. Questing ticks were dragged with a 1 m-sized blanket in monthly intervals during the periods of highest activity of I. ricinus: April – June and September – October 2011–2013. Rodents were trapped with live traps in 2012–2014. Captured rodents were anesthetized and sacrificed humanely, then examined for ectoparasites. Ticks and rodent organ/tissues were stored in 70% ethanol or in a freezer at −40 °C until DNA extraction using Macherey-Nagel NucleoSpin® Tissue kit. The samples were screened for the presence of A. phagocytophilum DNA with a real-time PCR targeting a 77-bp long fragment of the ms2 gene.

Table 1 Prevalence of A. phagocytophilum in I. ricinus per site in 2011–2013

<table>
<thead>
<tr>
<th>Site</th>
<th>2011 % (pos./neg.)</th>
<th>2012 % (pos./neg.)</th>
<th>2013 % (pos./neg.)</th>
<th>Total % (pos./neg.)</th>
<th>y²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bratislava</td>
<td>7.0 (4/58)</td>
<td>5.3 (2/37)</td>
<td>7.1 (3/42)</td>
<td>6.5 (11/175)</td>
<td>0.002</td>
<td>0.95</td>
</tr>
<tr>
<td>Females</td>
<td>15.6 (2/124)</td>
<td>9.7 (1/106)</td>
<td>13.1 (2/15)</td>
<td>11.9 (4/341)</td>
<td>0.001</td>
<td>0.99</td>
</tr>
<tr>
<td>Males</td>
<td>10.7 (19/178)</td>
<td>6.4 (11/172)</td>
<td>5.8 (8/145)</td>
<td>7.2 (36/495)</td>
<td>0.785</td>
<td>0.39</td>
</tr>
<tr>
<td>Total</td>
<td>8.9 (49/585)</td>
<td>7.2 (28/392)</td>
<td>7.1 (13/187)</td>
<td>7.7 (90/1164)</td>
<td>0.001</td>
<td>0.99</td>
</tr>
<tr>
<td>Figelka</td>
<td>0.8 (3/379)</td>
<td>0.6 (2/338)</td>
<td>1.1 (3/280)</td>
<td>0.9 (8/351)</td>
<td>0.970*</td>
<td>0.36</td>
</tr>
<tr>
<td>Females</td>
<td>6.0 (3/50)</td>
<td>5.3 (4/76)</td>
<td>7.1 (4/56)</td>
<td>5.9 (13/282)</td>
<td>0.003</td>
<td>0.96</td>
</tr>
<tr>
<td>Males</td>
<td>9.1 (15/164)</td>
<td>8.9 (16/184)</td>
<td>9.3 (10/110)</td>
<td>9.0 (31/351)</td>
<td>0.140</td>
<td>0.72</td>
</tr>
<tr>
<td>Total</td>
<td>2.4 (33/1383)</td>
<td>1.7–3.2</td>
<td>2.2–2.9</td>
<td>2.3 (60/2648)</td>
<td>0.027</td>
<td>0.22</td>
</tr>
</tbody>
</table>

(y²), number of positive/number of examined; 95% CI, confidence interval; y², goodness-of-fit test; P, significance level

* Fisher’s exact test was used to compare prevalence in females among years because the condition of y² goodness-of-fit test was not fulfilled

Results
1. Overall prevalence of A. phagocytophilum in questing I. ricinus was significantly higher in the urban/suburban habitat (7.2%, 95% CI 6.1–8.3%) compared to the natural habitat (3.1%, 95% CI 2.5–3.9%) (y² = 14.6, P < 0.001). Significant differences in infection rates in questing ticks were also found among years at both sites (Table 1, Fig. 2). In addition, significant local differences in prevalence of infected questing ticks were found among transects within each habitat as well as between seasons.

2. A total of 407 and 191 rodents were caught in 2012 and 2014, respectively, whereas only 8 individuals were caught in 2013. The trapped rodents belonged to six species (Figs 4, 5). Apodemus flavicollis and Clethrionomys glareolus prevaile in both habitats, Microtus arvalis, Micromys minutus and Microtus subterraneus were present only in the natural habitat. A. phagocytophilum was detected in skin of three rodent specimens (0.5%: 3/606) and only in one spleen sample (0.2%: 1/606). All positive samples came from bank voles trapped in Bratislava Forest Park. In one specimen, the bacterium was detected in both skin and spleen simultaneously. I. ricinus comprised 96.3% of the rodent-attached ticks, the rest were Haemophasys cDN, Ixodes trianguliceps and Dermacentor reticulatus (Fig. 3). In total, 0.9% (5/553) and 0.2% (1/445) of tested engorged ticks were positive for A. phagocytophilum from rodents captured in Bratislava and Figelka, respectively. Only I. ricinus carried the bacterium.

3. Prevalence of A. phagocytophilum in questing I. ricinus did not correlate significantly with relative abundance of ticks or with abundance of wildlife in the area. Positive but not significant correlation of A. phagocytophilum prevalence with abundance of roe deer was found (r = 0.576, P = 0.104). Negative non-significant correlations were found between prevalence and density of the other selected tick maintenance and potential reservoir hosts (red deer: r = –0.610, P = 0.081; fallow deer: r = –0.136, P = 0.728; moufflon: r = –0.492, P = 0.179), whereas negative significant correlation was found for the density of wild boar (r = –0.695, P = 0.038).

Conclusion
The study confirms that urban I. ricinus populations are infected with A. phagocytophilum in a higher rate than in a natural habitat of south-western Slovakia and suggests that rodents are not the main reservoirs of the bacterium in the investigated area.

Acknowledgments
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