MOLECULAR EVIDENCE FOR ANAPLASMA PHAGOCYTOPHILUM AND BORRELIA BURGDORFERI SENSU LATO IN IXODES RICINUS TICKS FROM EASTERN SLOVAKIA

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Abstract: Ixodes ricinus ticks (20 males, 20 females and 20 nymphs) collected in Košice, Slovakia were examined for the presence of Anaplasma phagocytophilum and Borrelia burgdorferi sensu lato (s.l.) by PCR. 38.3% of the tested ticks carried single infection of B. burgdorferi s.l. and 8.3% were infected with A. phagocytophilum. Double infection of both pathogens was detected in 5% of tested ticks. These results indicate that both B. burgdorferi s.l. and A. phagocytophilum co-circulate in the enzootic sites of Eastern Slovakia and may cause co-infection in humans.

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INTRODUCTION

Over the last decades, due to the climatic and urban changes in the environment, ticks and tick-borne diseases have become an emerging problem in temperate regions in Europe [7]. In Slovakia, the most common tick-transmitted bacterial disease is Lyme borreliosis with the incidence of 12.5 cases per 100,000 inhabitants [3]. The causative agent of Lyme borreliosis is spirochete forming a complex of species: B. burgdorferi sensu lato (s.l.) that is characterized by a high level of genetic heterogeneity. The following genospecies have been detected in different areas of Slovakia: B. afzelii, B. garinii, B. burgdorferi sensu stricto (s.s.), B. valaisiana and B. lusitaniae [6, 9, 15].

Recently, Anaplasma (Ehrlichia) phagocytophilum, another important bacterial pathogen transmitted by Ixodes ricinus ticks, claims the attention of public health professionals in Europe. Based on phylogenetic analyses, A. phagocytophilum belongs to the newly-reorganized genus Anaplasma which also encopasses the former Ehrlichia equi and humane granulocytic ehrlichiae (HGE) agent [5]. It is a Gram-negative, intracytoplasmic bacteria that infects granulocytes. In Europe, it has been detected in ticks [1, 8, 10, 11] animals [11] as well as patients [12].

The main aim of this study was to evaluate if Anaplasma phagocytophilum is present in Ixodes ricinus ticks collected from suburban park of the town of Košice in Eastern Slovakia where Lyme borreliosis is highly endemic.
**MATERIAL AND METHODS**

**Collection of ticks.** In spring 2002, 63 ticks (20 males, 20 females and 23 nymphs) were collected by flagging the vegetation in a hornbeam deciduous suburban forest near an apartment complex area in the eastern part of Košice (latitude 48° 59’ 5” N and longitude 14° 28’ 5” E). Ticks were immediately immersed in 70% ethanol.

**DNA extraction.** *I. ricinus* DNA was obtained using DNA easy tissue kit (Qiagen, Valencia, CA) according to a previously described modified protocol [2].

**PCR.** For all PCR reactions, MasterTaq DNA polymerase kit (Eppendorf, Westbury, N.Y.) was used. A total of 2.5 µl of template DNA were added to a PCR master mix containing 10.4 µl of deionized water, 5 µl of 5 X Taq Master PCR Enhancer, 2.5 µl of 10 X Taq buffer containing 10.4 µl of MgSO4, 1.5 µl of a 25 mM solution of Mg2+ and visualized with a UV transilluminator.

**Detection of *A. phagocytophilum*.** Primers EHR 521 (5’-TGT AGG CGG TTC GGT AGG TTA AAG-3’) and EHR 747 (5’-GCA CTC ATC GTT TAC AGC GTG-3’) were used to amplify the 247bp fragment of 16rDNA from *A. phagocytophilum*. PCR was performed according to the previously described protocol of Pancholli et al. [13].

**Detection of *B. burgdorferi* s.l.** Primers IGSa (5’-CGA CCT TCT TCT TAA ACG -3’) and IGSb (5’-AGC TCT TAT TCG CTG ATG GTA -3’) were used to amplify the 250bp fragment of 5S-23S *B. burgdorferi* s.l. intergenic spacer region. PCR conditions were as described by Derdáková et al. [4]. The PCR products were electrophoresed on a 2% agarosed gel, stained with ethidium bromide and visualized with a UV transilluminator.

**Detection of *I. ricinus* gender and stage.**

<table>
<thead>
<tr>
<th>Males</th>
<th>Females</th>
<th>Nymphs</th>
<th>Total</th>
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<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
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<tr>
<td>Total examined ticks</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><em>A. phagocytophilum</em> positive only</td>
<td>2 (10)</td>
<td>3 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> s.l. positive only</td>
<td>7 (35)</td>
<td>8 (40)</td>
<td>8 (40)</td>
</tr>
<tr>
<td><em>A. phagocytophilum</em> positive</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> s.l. positive</td>
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**DISCUSSION**

*I. ricinus* represents the most common tick in broad-leaved and mixed forests of Central Europe. It is an important vector of Tick-Borne Encephalitis virus, *B. burgdorferi* s.l., *A. phagocytophilum* and *B. microti*. In this preliminary study, we report for the first time that *A. phagocytophilum*, the causative agent of the HGE, is present in ticks collected in eastern Slovakia. This finding is not surprising since *A. phagocytophilum* had already been detected in ticks collected in western parts of Slovakia [14]. The 13.3% positivity for *A. phagocytophilum* detected in our study is comparable to results obtained by Greszuck et al. [8] in northeastern Poland, where 16% of ticks were infected. The authors of the above-mentioned study reported significantly higher infectious rate in adults (19.5%) than in nymphs (1.4%). This is in accordance with our results. However, in Norway the prevalence of HGE agent was highest in nymphs [10]. This may indicate different enzootic cycles of *A. phagocytophilum* in reservoir hosts from different geographic areas. Co-infections with *B. burgdorferi* s.l. was detected in 3 ticks. Double infections with these two pathogens were previously reported by other authors [8, 10] and indicates the possible co-infection of patients with both agents. In previous studies, the overall prevalence of *B. burgdorferi* s.l. in *I. ricinus* from the same area during three consecutive years was 16.9% [15]. We report higher infectious prevalence of *B. burgdorferi* s.l. (43.3%). The difference between the findings can be explained by the use of different methods of dark-field microscopy versus PCR in our case.

The results of this pilot study show the presence of *A. phagocytophilum*, as well as confirm the high prevalence...
of *B. burgdorferi* s.l. in *I. ricinus* ticks in Eastern Slovakia. This finding should be noted by physicians, especially in suspected Lyme borreliosis patients with a history of tick-bite and clinical symptoms but negative laboratory findings. Since the co-infection of both pathogens was detected, it is possible that patients may suffer from more than one tick-borne infection.

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**REFERENCES**


