Study on tick-borne rickettsiae in eastern Poland. I. Prevalence in *Dermacentor reticulatus* (Acari: Amblyommidae)

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### Abstract

*Rickettsia* spp. transmitted by ticks are classified mostly in the Spotted Fever Group Rickettsiae (SFGR). Numerous species of this group have been identified in Eurasia as emerging pathogens, but still little is known about their occurrence, effects on human health, and co-incidence with other tick-borne pathogens. The aim of the presented study was to determine the prevalence of *Rickettsia* spp. in adult *Dermacentor reticulatus* (Acari: Amblyommidae) ticks collected in Lublin province of eastern Poland using the PCR method. The infection rate of *D. reticulatus* with *Rickettsia* spp. was 53.0%. All except one rickettsial isolates showed 100% homology with *Rickettsia raurultii*. A high prevalence of *R. raurultii* in *D. reticulatus* ticks from eastern Poland suggests that the SFGR species should be considered as potential causative agents of tick-borne diseases in this area.

### Key words

Spotted Fever Group Rickettsiae, *Rickettsia raurultii*, *Dermacentor reticulatus*, prevalence, PCR

### INTRODUCTION

*Rickettsia* spp. are Gram-negative bacteria mainly transmitted by various arthropod vectors, such as fleas, ticks, and lice. *Rickettsia* spp. transmitted by ticks are classified mostly within the Spotted Fever Group Rickettsiae (SFGR). These are tick-borne intracellular bacteria of which numerous species have been identified in recent decades in Eurasia as emerging pathogens [1, 2, 3, 4, 5]. These potential pathogens comprise such species as: *Rickettsia helvetica*, *R. raurultii*, *R. slovaca*, *R. conorii*, *R. felis*, *R. sibirica*, *R. monacensis*, *R. massiliae*, *R. hoogstraalii*, *R. japonica* [1, 4, 6, 7]. They may cause in humans a spotted fever, influenza-like disease, lymphadenopathy, perimyocarditis, aortic valve disease, and other disorders [8, 9, 10, 11]. Forest workers exposed to tick bite show the presence of antibodies against SFGR and other disorders [8, 9, 10, 11]. *

### MATERIALS AND METHODS

#### Collection of ticks

A total of 528 questing *Dermacentor reticulatus* ticks were collected in Lublin province by dragging a woolen flag over vegetation. Only adult ticks (females and males) were collected.

**DNA isolation and detection of *Rickettsia* spp. DNA by PCR.** Bacterial DNA was isolated from 528 *D. reticulatus* ticks according to Rijpkema et al. [15]. The isolates obtained from *Dermacentor reticulatus* ticks were examined for the presence of *Rickettsia* spp. DNA using amplification by polymerase chain reaction (PCR) with primers specific for a gene encoding the citrate synthase gene *gltA* (RpCS.887p: 5'-GGG GGC CTG CTC ACG GCG G-3' and RpRD.1258n: 5'-CCT TTA TTT AGC TTT ATG CTA GA-3') [16]. Each PCR reaction was carried out in a 50 μl reaction volume which contained the following mix of reagents: 1 U Taq DNA polymerase (Qiagen, USA), 1×PCR buffer containing 15 mM MgCl₂, 2 mM dNTP (final concentration 0.25 mM) (Fermentas, Lithuania), 1 μl 10 μM each of primer (Eurogentec, Seraing, Belgium), 5 μl of DNA and nuclelease-free water (Applied Biosystems, USA). DNA isolated from antigen of Spotted Fever Group Rickettsiae LPS (Fuller Laboratories, CA, USA) was used as a positive control and nuclelease-free water as a negative control. The size of amplified DNA fragment was 381 bp pairs (bp). The amplification was carried out in C1000 Thermal Cycler (BioRad, USA) according to Stańczak [17]. Products of amplification were identified in 2% agarose gel, after electrophoresis in standard conditions and staining with ethidium bromide solution (2 μg/ml).

**DNA sequencing and sequence comparison.** DNA sequencing was performed with ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA, USA) using ABI Prism Big Dye Terminator v. 3.1. Cycle Sequencing Kits and Big Dye X Terminator Purification Kit (Applied Biosystems). The results were compared with sequences in GenBank database using the BLAST server at the National...
Dermacentor reticulatus ticks, 280 (53.0%) were found to be infected with *Rickettsia* spp. The infection rates of males and females detected in the presented study were high – 53.8% (112/208) and 52.5% (168/320), respectively (Tab. 1). The rates were significantly dependent on the collection locality (p<0.001).

Table 1. Presence of the gene fragment gltA of *Rickettsia* spp. in individual life stages of *Dermacentor reticulatus* collected in various localities of the Lublin province.

<table>
<thead>
<tr>
<th>Collection Locality</th>
<th>Life stages of Dermacentor reticulatus</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I/E (%)</td>
<td>I/E (%)</td>
<td>I/E (%)</td>
<td></td>
</tr>
<tr>
<td>Suchawa</td>
<td>33/37 (89.2%)</td>
<td>61/83 (73.5%)</td>
<td>94/120 (78.3%)</td>
<td></td>
</tr>
<tr>
<td>Okuninka</td>
<td>25/55 (45.4%)</td>
<td>42/99 (42.4%)</td>
<td>67/154 (43.5%)</td>
<td></td>
</tr>
<tr>
<td>Ostrów Lubelski</td>
<td>27/67 (40.3%)</td>
<td>31/81 (38.3%)</td>
<td>58/148 (39.1%)</td>
<td></td>
</tr>
<tr>
<td>Sobibir</td>
<td>16/25 (64.0%)</td>
<td>10/23 (43.5%)</td>
<td>26/48 (54.2%)</td>
<td></td>
</tr>
<tr>
<td>Nieszów</td>
<td>3/9 (33.3%)</td>
<td>4/8 (50.0%)</td>
<td>7/17 (41.2%)</td>
<td></td>
</tr>
<tr>
<td>Poleski National Park</td>
<td>4/6 (66.7%)</td>
<td>11/14 (78.6%)</td>
<td>15/20 (75.0%)</td>
<td></td>
</tr>
<tr>
<td>Wilków</td>
<td>4/9 (44.4%)</td>
<td>9/12 (75.0%)</td>
<td>13/21 (61.9%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>112/208 (53.8%)</td>
<td>168/320 (52.5%)</td>
<td>280/528 (53.0%)</td>
<td></td>
</tr>
</tbody>
</table>

I/E, Infected/Examined.

Sequence analysis of the samples positive for *Rickettsia* spp. proved that the amplified products showed 100% homology with the sequence of partial cds citrate synthase (gltA) gene of: *Rickettsia raoultii* strain Khabarovsk (accession number DQ365804) and strain Marne (Accession No. DQ365803). Only one sequence obtained from one *D. reticulatus* tick showed less homology with *R. raoultii*, amounting to 99%. This sequence, classified as belonging to *Rickettsia* sp. closely related to *R. raoultii* (Fig. 1), was deposited in the GenBank under the Accession No. JX402775.

**DISCUSSION**

The results of the presented study show a high prevalence, exceeding 50%, of *Rickettsia raoultii* infection in *Dermacentor reticulatus* ticks collected from vegetation in the Lublin Province of eastern Poland. This suggests the presence of natural foci of this pathogen in the studied area. These findings are in accordance with other authors who reported high prevalence of SFGR in *Dermacentor reticulatus* ticks collected on the territory of north-eastern Poland. Chmielewski *et al.* [10] found that 56.7% of the examined *D. reticulatus* ticks were infected with *Rickettsia raoultii*, while Stańczak [17] found that 40.7% of ticks belonging to this species were infected with SFGR species. The prevalence of SFGR species in *I. ricinus* ticks collected in Poland was lower and ranged from 2.9–18.2% [10, 20].

The presence of SFGR species, most commonly belonging to *Rickettsia helvetica*, was demonstrated in many other European countries. In Germany, the recorded SFGR prevalence was 5.3–14.7% in *Ixodes ricinus* [2, 3, 21, 22, 23–24], 20–23% in *Dermacentor reticulatus* [2, 25] and 31% in *Dermacentor marginatus*. Their prevalence in *I. ricinus* ticks in Sweden was 1.5–17.3% [26, 27, 28, 29], in France 1.4–6% [3, 30], in Denmark 4.7% [31], in Austria 5.7–33.3% [32, 33],
References


