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ORIGINAL ARTICLE

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 raoultii*. A high prevalence of *R. raoultii* in *Dermacentor 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 raoultii*, *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. raoultii, 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 [6, 12, 13]. *Ixodes ricinus* is regarded as a main vector of the SFGR in Europe, whereas *Dermacentor reticulatus* and some other species (*Dermacentor marginatus, Rhipicephalus sanguineus, Haemaphysalis punctata, Haemaphysalis sulcata*) are reported as associate vectors [1, 10, 14].

The aim of the presented work was to determine the prevalence of *Rickettsia* spp. in the *Dermacentor reticulatus* ticks collected in Lublin province of eastern Poland.

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 RpCS.1258n: 5'-AAT GCA AAA AGT ACA GTG AAC A-3') [16]. Each PCR reaction was carried out in a 50 µl reaction volume which contained the following mix of reagents: 1U *Taq* DNA polymerase (Qiagen, USA), 1×PCR buffer containing 15 mM $MgCl_2$, 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 nuclease-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 nuclease-free water as a negative control. The size of amplified DNA fragment was 381 base 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 XTerminator Purification Kit (Applied Biosystems). The results were compared with sequences in GenBank database using the BLAST server at the National

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Center for Biotechnology Information (Bethesda, MA, USA). Phylogenetic relationships of the fragment of *Rickettsia* sp. *gltA* gene sequence (accession number JX402775) were inferred by using the MEGA version 5 [19]. The evolutionary history was inferred using the neighbour-joining method. The evolutionary distances were computed using the Tamura 3-parameter method. Differences in the composition bias among sequences were considered in evolutionary comparisons.

Statistical analysis. The data were analysed by χ^2-test with Yates correction, Spearman's rank order test for correlation, and Student's t-test with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA). The value $p<0.05$ was considered significant.

RESULTS

Out of 528 adult *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.

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

I/E, Infected/Examined.
Variability of infection depending on locality, assessed by χ^2 test with Yates correction: males $\chi^2=13.06$, $p=0.042$, dependence significant; females $\chi^2=15.26$, $p=0.018$, dependence significant; total $\chi^2=26.06$, $p=0.00033$, dependence highly significant.
Difference between infection rates in males and females, assessed by Student's t-test: $p=0.653$, difference not significant.

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.

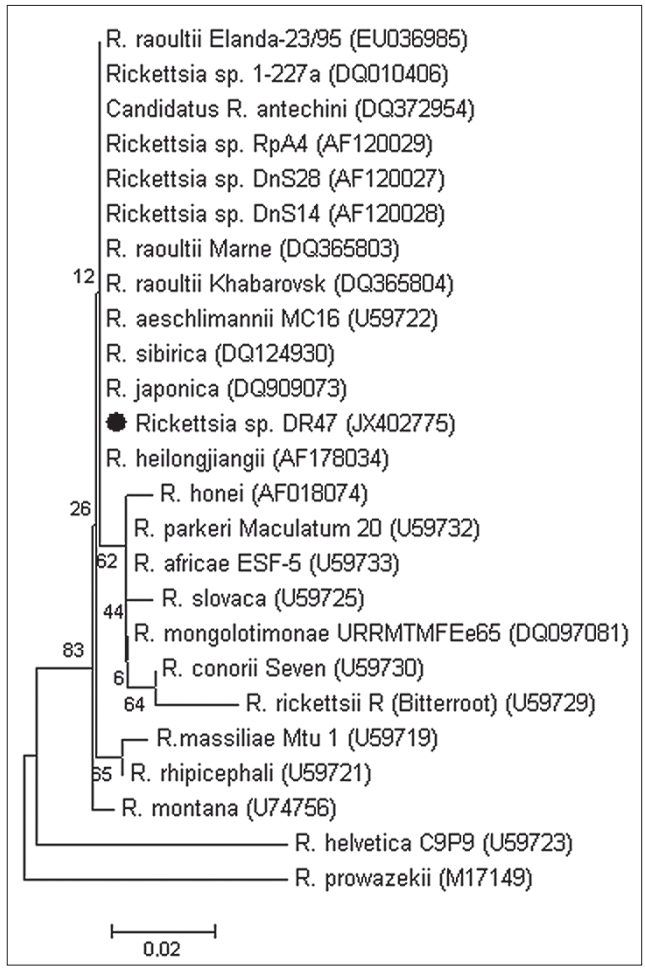

Figure 1. Unrooted dendrogram showing the phylogenetic position of *Rickettsia* sp. (Gen Bank acc. no. JX402775) among *Rickettsia* species inferred from the comparison of *gltA* sequence by the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches. Bar, 2% nucleotide sequence divergence.

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 finding 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],

in Switzerland 12–36% [34]. Moreover, the presence of SFGR was reported from *I. ricinus* ticks collected in Hungary [35], Slovakia [36], and The Netherlands [37, 38].

In the UK [38], the prevalence of *Rickettsia* spp. in *Ixodes ricinus* was 6.5% and in *Dermacentor reticulatus* – 27%. In Serbia, the infection rates of *I. ricinus* ticks with *R. helvetica and R. monacensis* were 7.7 and 15.4%, respectively [39]. In Croatia, the prevalence of SFGR in *D. reticulatus* was 13% [40], while in *Dermacentor marginatus* and *Hyalomma marginatum* ticks it was 36.8% and 12.7%, respectively [41]. In Italy, the infection rate with SFGR was 3.1–6.1% in *I. ricinus* [42, 43], while in *D. marginatus* it was 32.1% with *R. slovaca* and 1.8% with *R. raoultii* [44]. Márquez [1] detected in 11 ticks species collected in southeastern Spain a mean infection rate with rickettsiae equal to 19.5%. *I. ricinus* was infected with *R. monacensis* (27.0%) and *R. helvetica* (2.7%), *D. marginatus* presented *R. slovaca* (24.7%) and *R. raoultii* (59.9%), and ticks belonging to *R. sanguineus* group were infected with *R. massiliae* (15.2%).

In the presented study, sequencing analysis of positive samples showed 99–100% homology to *Rickettsia raoultii*. This species was described, together with *Rickettsia slovaca*, as an etiological agent of TIBOLA/DEBONEL disease [45], and recently a clinical case of spotted fever caused by *R. raoultii* was reported from Poland [46]. *Dermacentor reticulatus* is regarded as a competent vector and reservoir of *Rickettsia raoultii*. Bacteria belonging to this species demonstrated transovarial and transtadial transmission in ticks [47].

The mean infection rate of *D. reticulatus* with *R. raoultii* determined in the prresented study (53.0%) was at the upper limit of SFGR detected in various tick species by Márquez [1], Chmielewski *et al.* [10], and Stańczak *et al.* [17], and markedly higher compared to the infection rates reported by other authors cited above.

To summarize: the high prevalence of *Rickettsia raoultii* in *Dermacentor reticulatus* ticks from eastern Poland suggests that SFGR species should be considered as potential causative agents of tick-borne diseases in this area.

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