Spotted fever group *Rickettsia* spp. in questing *Ixodes ricinus* ticks in north-eastern Poland

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Introduction & aim of the study

The genus *Rickettsia* (Rickettsiales; Rickettsiaceae) includes Gram-negative, obligate, intracellular bacteria that are transmitted by arthropod vectors (Fournier and Raoult, 2007). In Europe, one of the reservoirs and vectors of *Rickettsia* spp. pathogenic for humans, included in the spotted fever group (SFG), are *Ixodes ricinus* ticks. In humans, the most common clinical symptoms of tick-borne rickettsiosis are fever, headache, myalgia, and rash (Azagi et al. 2020).

The aim of the study was:

- assessment the prevalence of *Rickettsia* spp. in questing *I. ricinius* ticks in north-eastern Poland
- determination of the species diversity of the *Rickettsia* genus in the study area
- examining the impact of conditions related to the subregion, biotope and year of study on the frequency of *Rickettsia* spp.

Material & methods

Study area and ticks collection

Rickettsia spp. were detected in a total of 4090 *I. ricinus* ticks, including 381 females, 450 males and 3260 nymphs (652 pools of 5 specimens). The questing ticks were collected between April and June 2016-2017 in Warmia and Mazury region using the standard flagging method. The tick collection sites represented the western, central and eastern part of the region and two types of habitats: forest landscapes and ecotones (Fig. 1).

Fig. 1. Tick collection sites located in western, central and eastern subregions of north-eastern Poland.

Molecular analysis

Total DNA was extracted using the ammonia method (Rijpkema et al., 1996). The presence of *Rickettsia* spp. in tick genomic DNA samples was confirmed by the PCR method using set of primers (CS409/Rp1258) (Roux et al. 1997) specific to the citrate synthase (*gltA*) gene. The Rickettsia species were identified by sequencing the PCR products (Macrogen Europe, Amsterdam, the Netherlands). The nucleotide sequences obtained were compared with the data registered in the GenBank database using the BLAST-NCBI.

Statistical & phylogenetic analysis

A chi-square test and 95% confidence intervals (95% CI) were used to compare the infection rate between sex of ticks, regions, habitats and years of study. The analysis was conducted using the software package SPSS version 27.0 for Windows (SPSS Inc., Chicago, IL). In all analyses, p-values below 0.05 were considered statistically significant. The phylogram was constructed using the Maximum Likelihood method based on the Kimura 2-parameter model. The topology of the phylogenetic tree was evaluated using the bootstrap method with 1,000 replicates. Phylogenetic analysis was conducted using MEGA X software (https://www.megasoftware.net).

Results

- Rickettsial DNA was detected in 11.3% of females, 11.8% of males and in at least 6.5% (MIR, minimum infection rate) of nymphs of *I. ricinus* (Tab. 1).
- The proportion of *Rickettsia*-positive ticks was significantly higher in 2016 (8.4%) than in 2017 (6.6%).
- The highest infection rate (9.5%) was recorded in the central subregion of Warmia and Mazury.
- The type of habitat did not affect the *Rickettsia spp.* prevalence, which was 7.3% in forest areas and 7.7% in ecotones.

Table 1. Prevalence of *Rickettsia* spp. in *I. ricinus* ticks by developmental stage, year, subregion and habitat, north-eastern Poland

		No. of tested ticks	*Rickettsia*-positive n/% * (95% CI)	p-value**
Stage	Nymphs	3259	212/6.5[a] (5.68-7.41)	<0.001
	Females	381	43/11.3[b] (8.29-14.90)	
	Males	450	53/11.8[b] (8.95-15.12)	
Year	2016	2188	183/8.4[a] (7.24-9.60)	0.030
	2017	1902	125/6.6[b] (5.50-7.78)	
Subregion	West	1563	104/6.7[a] (5.47-8.00)	0.003
	Central	1351	129/9.5[b] (8.03-11.24)	
	East	1173	75/6.4[a] (5.05-7.93)	
Habitat	Forest	1750	127/7.3[a] (6.09-8.57)	0.567
	Ecotone	2340	181/7.7[a] (6.68-8.89)	
Total		4090	308/7.5 (6.74-8.38)	

* – for nymphs Minimum Infection Rate (MIR) is given (5 nymphs per isolate); ** – chi2 test, $p<0.05$; a, b – different letters mean significant differences (post -hoc Bonferroni test)

Results

- Sequence analysis of the fragment of *gltA* gene (~730 bp) showed the presence of *R. helvetica* and *R. monacensis*. Both *Rickettsia* species are known as human pathogens.
- All obtained nucleotide sequences of *R. helvetica* (n=45) were similar and showed 100% identity to isolate C9P9 of *R. helvetica* (GenBank: U59723). The same nucleotide sequence was also detected from *I. ricinus* in north-eastern (GenBank: OM927745) and central (GenBank: MH018978) Poland as well as from Serbia (GenBank: MH618386) and Italy (GenBank: MN226407) (Fig.2).
- One obtained sequence identified as *R. monacensis* showed 100% identity with gltA sequences of the *R. monacensis* strain IrR/Munich from Germany (GenBank: LN794217) and from questing *I. ricinus* ticks from north-eastern (GenBank: MW595238) and central Poland (GenBank: MH018982) (Fig.2).

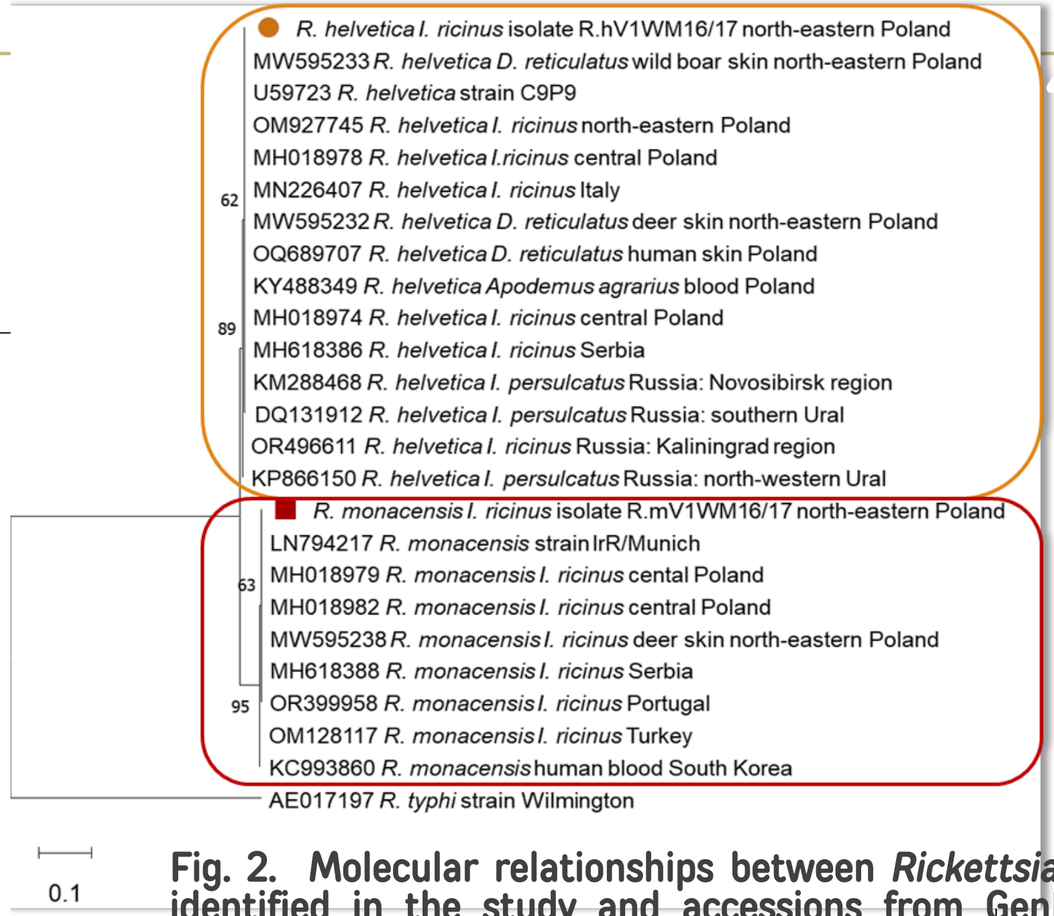

Fig. 2. Molecular relationships between *Rickettsia* spp. identified in the study and accessions from GenBank, based on the sequences of the *gltA* gene. The sequences obtained in this study are labelled with symbols.

Conclusion

The current study has confirmed the presence of *Rickettsia* species included in the spotted fever group (SFG) in the population of *I. ricinus* ticks in north-eastern Poland. The prevalence of *R. helvetica* and *R. monacensis* is relatively low, however indicates that spotted fever rickettsioses should not be excluded in the diagnosis of tick-borne diseases in people bitten by ticks in this area. The risk of infection with these pathogens, determined based on infected ticks, varied significantly depending on the year of study and the subregion of north-eastern Poland.