10.1111/j.1469-0691.2008.02261.x

Prevalence of infection with *Rickettsia helvetica* in feeding ticks and their hosts in western Poland

J. Stańczak[1], M. Racewicz[1], J. Michalik[2], S. Cieniuch[1], B. Sikora[2] and M. Skoracki[2]

[1]Department of Tropical Parasitology, Medical University of Gdańsk, Gdynia and [2]Department of Animal Morphology, Adam Mickiewicz University, Poznań, Poland

INTRODUCTION

In Poland, *Ixodes ricinus* plays an important role as the vector of the TBE virus, *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* and *Babesia* spp. Moreover, recent studies have shown that it can also serve as a vector for *Rickettsia helvetica*, the newly recognised human pathogen of the spotted fever group rickettsiae (SFG), which is widely distributed in questing ticks, with the prevalence ranging from 1.3% to 11.4% [1]. However, reports are still lacking on its occurrence in naturally infected wild animals and ticks feeding on them. So, this study was designed to detect rickettsial organisms in blood samples of various tick hosts that may serve as reservoirs of infection, as well as in *I. ricinus* parasitising them.

MATERIAL AND METHODS

Investigations were conducted in the Landscape Park 'Puszcza Zielonka' in the Wielkopolska province, western Poland.

EDTA–blood samples from 24 roe deer (*Capreolus capreolus*), seven red deer (*Cervus elaphus*) and 13 fallow deer (*Dama dama*) were collected during the 2005 hunting season. Blood was obtained from the chest cavities of freshly killed animals sampled at two local tagging stations. Blood samples from live-trapped rodents, *Apodemus flavicollis* (n = 112) and *Clethrionomys glareolus* (n = 37), as well as from 130 net-captured birds of diverse species were collected in 2006 (permission no. DOPog-4201-03-158/03/al). All collected specimens were released at the collection site. Blood samples were kept frozen at −20°C until analysis.

Ixodes ricinus ticks recovered from infested deer were kept frozen (−20°C) or preserved in 70% ethanol for DNA extraction.

Total DNA was extracted from blood samples and from feeding *I. ricinus* using commercial kits: Genomic Mini AX Blood and Sherlock AX, respectively (A&A Biotechnology, Gdynia, Poland). Extraction of nucleic acids from male ticks and unfed specimens was carried out by lysis in NH_4OH. Obtained extracts were stored at −20°C and then used as templates in a regular PCR assay.

DNA amplification was assayed using the primer pair RpCs.877p and RpCs.1258n derived from the citrate synthase-encoding gene (*gltA*), which has conserved regions shared by all known *Rickettsia* species [2]. DNA of *R. helvetica* from positive reactions obtained in our previous investigations and confirmed by the analysis of sequences of PCR products [1] was used as positive control and double-distilled water as negative control.

The PCR methodology was as previously described [2]. All PCR reactions were carried out in a GeneAmp®PCR System 9700 (Applied Biosystems 850, Foster City, USA.) Amplification products were analysed after electrophoresis in a 2% agarose gel stained with ethidium bromide. DNA bands of 381 bp were considered positive results.

PCR products of chosen positive samples were purified and sequenced with an ABI PRISM 3100 genetic Analyser (Applied Biosystem 850) according to the manufacturer's protocol. Sequences were edited and compared with gene sequences deposited in the GenBank database using the NCBI BLAST program (U. S. National Institutes of Health, Bethesda, Maryland).

RESULTS

None of the 323 blood samples collected from 130 birds, 149 rodents and 44 wild cervids was PCR positive for *Rickettsia* spp.

A total of 518 immature and adult *I. ricinus* were collected from three deer species. The proportion of deer carrying ticks was 68.2%. The mean infestation intensity was 11.3 per deer, being highest in *C. capreolus* (19.1), followed by *C. elaphus* (5.3) and *D. dama* (1.7). Proportions of nymphs vs. females were: 6.4 vs. 9.9 in roe deer, 1.4 vs. 0.2 in fallow deer and 0 vs. 3.3 in red deer.

Altogether 393 *I. ricinus* were examined individually for the presence of *Rickettsia* spp. (Table 1). Positive PCR products were detected in 50 (12.7%) specimens. The tick infection level ranged from 10.8% to 19.0%, depending on the deer species. The percentage of infected females (16.7%) was two times higher than that observed in males and nymphs.

Corresponding author and reprint requests: Joanna Stańczak, Department of Tropical Parasitology, Medical University of Gdańsk, 9B Powstania Styczniowego str., 81-519 Gdynia, Poland
E-mail: astan@amg.gda.pl

No conflicts of interest declared.

Table 1. Prevalence of infection with *Rickettsia helvetica* in *Ixodes ricinus* ticks collected from hunter-killed wild cervids in the Landscape Park 'Puszcza Zielonka' (Wielkopolska province, western Poland) in 2005

Deer species	*Ixodes ricinus* (no. of collected/no. of infected (% infected))			
	Nymphs	Females	Males	Total
Cervus elaphus	0/0 (0.0)	23/4 (17.4)	14/0 (0.0)	37/4 (10.8)
Capreolus capreolus	89/7 (7.9)	178/28 (15.7)	68/7 (10.3)	335/42 (12/5)
Dama dama	18/2 (11.1)	2/2 (100)	1/0 (0.0)	21/4 (19.0)
Total	107/9 (8.4)	203/34 (16.7)	83/7 (8.4)	393/50 (12.7)

Sequencing of PCR products was performed with 10/50 positive samples. All sequences obtained were 100% similar to each other (GenBank acc. no. EU779822) and 100% homologous to *R. helvetica* sequences deposited in GenBank: DQ910785, EF392725.

CONCLUSIONS

The results, demonstrating the presence of *R. helvetica* in ticks feeding on wild cervids, support the previous findings that in Poland infection of *I. ricinus* with this bacterium is frequent. However, its association with the wide range of vertebrates that serve as tick hosts remains unknown.

None of the blood samples collected from birds (including species frequently and abundantly parasitised by ticks: *Turdus merula*, *T. philomelos*, *Sturnus vulgaris*), small rodents or deer were positive for *Rickettsia* spp. No roe deer blood samples in Denmark were positive for *R. helvetica* by PCR either [3]. An explanation for these findings may be that the natural tick hosts analysed were not rickettsiemic at the time of sampling, as this phase may only last for short periods. The detection of *R. helvetica* in nymphs and adult *I. ricinus* ticks feeding on non-rickettsiemic deer hosts suggests that they primarily acquired infection via transstadial transmission, which is common among ticks infected with *Rickettsia* spp. However, recent evidence of *R. helvetica* infection in spleen samples of roe deer in Slovakia [4] and in peripheral blood of Sika deer in Japan [5] indicates that wild cervids may serve as reservoir hosts of *R. helvetica*. Thus, further investigations are necessary in order to understand their role and that of other vertebrates in the natural circulation of this bacterium.

ACKNOWLEDGEMENTS

This study was partially supported by the state committee for Scientific Research (grant no. 2P04C 111 29).

REFERENCES

1. Stańczak J, Racewicz M, Michalik J, Buczek A. Distribution of *Rickettsia helvetica* in *Ixodes ricinus* tick populations in Poland. *Int J Med Microbiol* 2008; **298**, S.1**:** 231–234.
2. Regnery RL, Spruill CL, Plikyatis BD. Genotypic identification of rickettsiae and estimation of interspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol* 1991; **173:** 1576–1589.
3. Skarpédinsson S, Jensen PM, Kristiansen K. Survey of tickborne infection in Denmark. *Emerg Infect Dis* 2005; **11:** 1055–1061.
4. Stefanidesova K, Kocianova E, Boldis V *et al*. Evidence of *Anaplasma phagocytophilum and Rickettsia helvetica* infection in free-ranging ungulates in Central Slovakia. *Eur J Wildl Res* 2008; **54:** 519–524.
5. Inokuma H, Seino N, Suzuki M *et al*. Detection of *Rickettsia helvetica* DNA from peripheral blood of Sika deer (*Cervus nippon yesoensis*) in Japan. *J Wildl Dis* 2008; **44:** 164–167.