

Circulation of *Rickettsia* spp. and *Coxiella burnetii* in central part of Slovakia



L. Berthová & Eva Špitalská

Institute of Virology, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava



Introduction

Geographic distribution and activity of infected ticks are important in the epidemiology of tick-borne rickettsioses. Ticks are main reservoir of SFG (spotted fever group) rickettsiae. Humans are accidental hosts that become infected when ticks containing virulent rickettsiae in their salivary glands take blood meal and inject the rickettsiae into the feeding site. Domestic ruminants (cattle, sheep, and goats) are considered as the main reservoirs for *C. burnetii*. They are often asymptomatic carriers. Many wild mammals and birds have been found to be hosts of the infectious microorganism. Among ectoparasites, ticks are considered to be the natural primary reservoirs of *C. burnetii* (Porter et al. 2011).

The aim of study

The aim of this study was to investigate presence of rickettsiae and *Coxiella burnetii* in ticks, rodents and birds in natural forest habitat in the central part of Slovakia during 2012 and 2013.

Material and methods

All material was collected during 2012 and 2013 in Prievidza, central part of Slovakia (48.79 N 18.59 E). Questing ticks were collected by dragging of white blanket over vegetation. Rodents were live-trapped in two lines (50 traps per line), each line was exposed during two consecutive nights. Blood, ear biopsies and spleens were stored in 70% ethanol. Birds were mist-netted nine times during two years. Blood samples were taken by puncture of *vena branchialis*. Ticks from hosts were removed by fine tweezers and stored in 70% ethanol as well as questing ticks. DNA from all samples was extracted using NucleoSpin® Tissue kit (Macherey-Nagel). *Rickettsia* spp. and *C. burnetii* were detected in all samples using PCR-based methods amplifying partial regions of the *gltA* and *comI* gene (Regnery et al. 1991, Špitalská et al. 2003). For detection of rickettsiae in blood samples of birds qPCR targeting *gltA* and 16S rRNA genes of *Rickettsia* sp. and 23S rRNA of *R. helvetica* (Boretti et al. 2009, Melničáková et al. 2003) were used.

Results

Rodent trapping yielded 57 rodents (45 *Apodemus flavicollis* and 12 *Myodes glareolus*), which were infested with a total of 92 ticks. From 167 birds of 35 species caught 95 ticks were removed. Only two species of ticks were found on hosts - *Ixodes ricinus* and only one larva of *Haemaphysalis concinna* (on female *A. flavicollis*).

By dragging of white blanket over vegetation 605 ticks of five species (323 *I. ricinus*, 181 *Dermacentor marginatus*, 95 *H. concinna*, 5 *H. inermis* and 1 nymph *Rhipicephalus truncatus*) were collected.

Rickettsial infection was determined in 7.9% (48/605) in questing ticks (39 *D. marginatus*, 9 *I. ricinus* ticks) (Fig. 1). The presence of *Rickettsia* spp. was confirmed also in 6.5% (6/92) ticks from rodents, 8.4% (8/95) ticks from birds, 9.6% (16/167) bird blood samples (Fig. 2). The presence of *C. burnetii* was not identified in our samples. Overview of rickettsial infection in different types of samples is shown in Tab. 1.

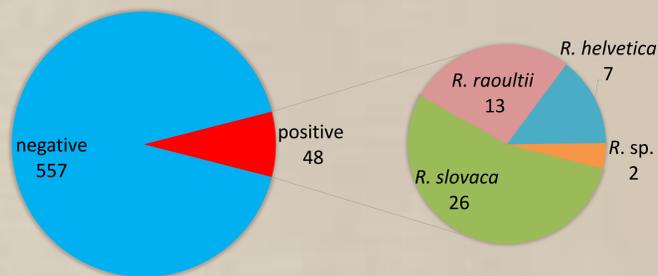


Fig. 1 Rickettsiae in questing ticks

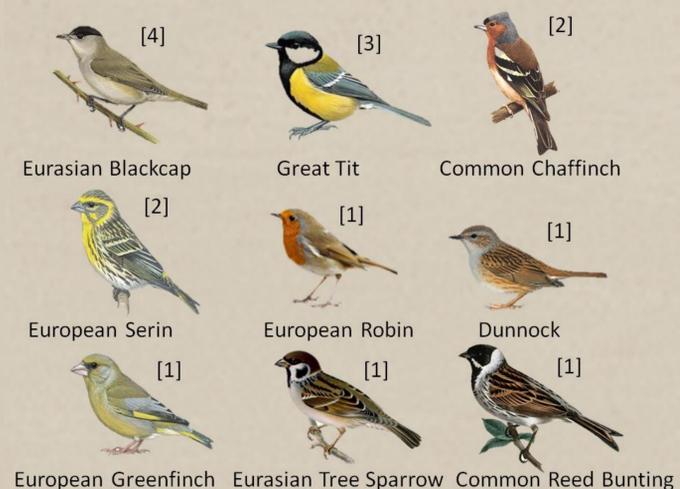


Fig. 2 Bird species with rickettsia-positive blood [number of positive individuals]

Tab. 1 Overview of all examined samples

	number of samples	<i>Rickettsia</i> spp.– positive samples
questing ticks	605	7.9% <i>R. helvetica</i> , <i>R. raoultii</i> , <i>R. slovacica</i> , <i>R. sp.</i>
blood of birds	167	9.6% <i>R. helvetica</i>
blood of rodents	55	0%
infestation of birds	95 ticks / 167 birds	0,6 ticks per host
ticks from birds	95	8.4% <i>R. helvetica</i>
infestation of rodents	92 ticks / 57 rodent	1,6 ticks per host
ticks from rodents	92	6.5% <i>R. helvetica</i> , <i>R. monacensis</i>

Conclusion

Our results confirmed circulation of rickettsiae in a model study area. We identified presence of five species: *R. slovacica*, *R. raoultii*, *R. helvetica* and two unidentified *Rickettsia* sp. Considering our data we suppose that birds can serve as reservoirs of rickettsiae but we approved neither the role of birds and rodents nor of questing ticks as reservoirs of *C. burnetii*.

References

- Boretti FS, Perreten A, Meli MM, Cattori V, Willi B, Wengi N, Hornok S, Honegger H, Hegglin D, Woelfel R, Reusch CE, Lutz H, Hofmann-Lehmann R (2009) Molecular investigation of *Rickettsia helvetica* infection in dogs, foxes, humans, and *Ixodes* ticks. *Appl Environ Microbiol* 75:3230–3237.
 Melničáková J, Derdáková M, Barák I (2013) A system to simultaneously detect tick-borne pathogens based on the variability of the 16S ribosomal genes. *Parasites Vectors* 6:269.
 Porter SR, Czaplíckí G, Mainil J, Guattéo R, Melničáková J, Derdáková M, Barák I (2013) A system to simultaneously detect tick-borne pathogens based on the variability of the 16S ribosomal genes. *Parasites Vectors* 6:269.
 Regnery RL, Spruill CL, Plikaytis BD (1991) Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol* 173: 1576–1589.
 Špitalská, E, Kocianová E (2003) Detection of *Coxiella burnetii* in ticks collected in Slovakia and Hungary. *European Journal of Epidemiology* 18: 263–266.

Acknowledgements: The study was supported by VEGA 2/0061/13, FP7 project EDENext (No. 261504), and SRDA-0280-12.