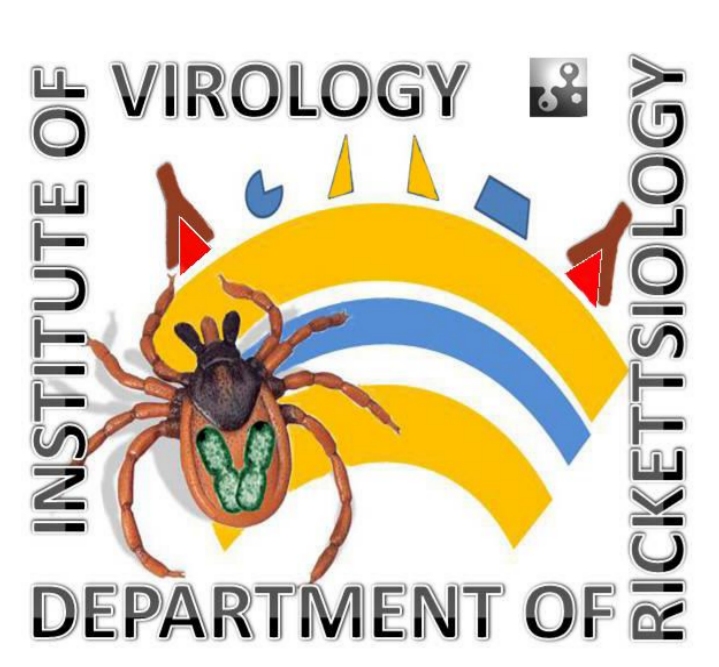


The role of birds in the natural cycle of *Rickettsia* spp. and *Coxiella burnetii* in Slovakia

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Introduction

Ixodid ticks (Acarina: Ixodidae) are known as primary vectors of many pathogens causing diseases of humans and domestic animals. *Ixodes ricinus* is associated with deciduous and mixed forests, but its distribution has significantly expanded over the past decades. Presently, its occurrence in city parks, cemeteries, gardens and peri-urban areas is not surprising. Birds serve as maintenance hosts for tick larvae and nymphs, introducing and maintaining tick-borne pathogens. *Rickettsia* species are Gram negative intracellular bacteria associated with eukaryotic cells within which they live, divide by binary fission and may cause diseases called rickettsioses. Ticks play the role of reservoirs and vectors of rickettsiae. *Coxiella burnetii* is the causative agent of Q fever, a worldwide distributed zoonotic bacterial disease. Domestic ruminants (cattle, sheep, and goats) are considered as the main reservoirs for *C. burnetii*. 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 purpose of this study was to assess tick infestation of birds; to evaluate the role of avian hosts in the circulation and dissemination of tick-borne pathogens like *Rickettsia* species and *Coxiella burnetii* and to evaluate possible role of synanthropic birds (i.e. occupying urban and suburban habitats) in circulation of rickettsiae and *C. burnetii* in suburban and natural areas of Slovakia (Fig. 1).

Material and methods

Birds were mist-netted during 2012 and 2013. Ticks were removed with fine forceps, preserved in 70% ethanol, and stored individually. All birds were blood sampled (ca. 30 µl) from the *vena ulnaris cutanea* and stored in 96% ethanol at 5-7°C until analyses. DNA extractions from ticks and blood samples were performed using NucleoSpin® Tissue (Macherey-Nagel GmbH & Co., Germany) following the manufacturer's recommendation. *Rickettsia* spp. were identified in tick and blood samples by a PCR targeting fragments of *gltA* and *sca4* genes (Regnery et al. 1991; Sekeyová et al. 2001; Labruna et al. 2004) and *C. burnetii* by PCR targeting *com1* gene (Špitalská et al. 2003). *Rickettsia*-positive tick samples were screened for the presence of *R. helvetica* using TaqMan PCR assay targeting 23S rRNA gene (Boretti et al. 2009). The presence of rickettsiae in blood samples was also evaluated by three TaqMan PCRs, targeting parts of the *gltA* and 16S rRNA genes of *Rickettsia* spp., and part of the 23S rRNA gene of *R. helvetica* as described in Boretti et al. (2009) and Melničáková et al. (2013).



Fig. 1. Study areas: 1 suburban, 2 natural forest habitat

Results

From 2012 to 2013, 225 birds belonging to 36 species were caught and examined for ticks. A total of 509 specimens of *I. ricinus* were collected (429 larvae, 79 nymphs, 1 female) (Fig. 2). Altogether 30% of birds were infested by ticks, some birds carried more than one tick. The Song Thrush *Turdus philomelos* (3 infested/3 examined) and the Great Tit *Parus major* (31/37) were the most infested bird species.

Totally, 6.6% and 2.8% of ticks collected from birds were infected with *Rickettsia* spp. and *C. burnetii*, respectively (Tab. 1 and 2). *Rickettsia helvetica* predominated in bird-attached ticks (5.9%), whereas *R. monacensis* (0.5%) was only sporadically detected.

Rickettsia spp. was detected in 8.9% and *R. helvetica* in 4.2% of bird blood samples. The birds with rickettsia-positive blood and ticks carried by them are listed in Tab. 3. *C. burnetii* wasn't detected in any of examined blood samples.

Tab. 3 Lists of birds with *Rickettsia*-positive blood in suburban and natural habitat

habitat	host	collected ticks	<i>Rickettsia</i> -positive ticks	habitat	host	collected ticks	<i>Rickettsia</i> -positive ticks
suburban	<i>Cyanistes caeruleus</i>	0	-	natural forest	<i>Carduelis chloris</i>	0	-
	<i>Cyanistes caeruleus</i>	0	-		<i>Emberiza schoeniclus</i>	0	-
	<i>Erithacus rubecula</i>	12	1		<i>Erithacus rubecula</i>	0	-
	<i>Parus major</i>	20	2		<i>Fringilla coelebs</i>	0	-
	<i>Parus major</i>	9	0		<i>Fringilla coelebs</i>	0	-
	<i>Parus major</i>	19	5		<i>Parus major</i>	0	-
	<i>Parus major</i>	0	-		<i>Parus major</i>	5	0
	<i>Picus viridis</i>	0	-		<i>Parus major</i>	0	-
			<i>Parus major</i>		0	-	
			<i>Passer montanus</i>		0	-	
			<i>Prunella modularis</i>		8	1	
			<i>Serinus serinus</i>		0	-	
			<i>Serinus serinus</i>		0	-	
			<i>Sylvia atricapilla</i>		0	-	
			<i>Sylvia atricapilla</i>		0	-	
			<i>Sylvia atricapilla</i>		0	-	
			<i>Sylvia atricapilla</i>	0	-		
			total	73	9		

Fig. 2 The Eurasian nuthatch with *I. ricinus* female tickTab. 1 List of birds infested with *Rickettsia*-positive ticks

habitat	host	larvae	nymphs	adults	total
natural forest	<i>Fringilla coelebs</i>	4/13	0/2	0/0	4/15
	<i>Erithacus rubecula</i>	1/2	0/0	0/0	1/2
	<i>Turdus merula</i>	0/2	2/16	0/0	2/18
	<i>Prunella modularis</i>	0/7	1/2	0/0	1/9
	total	4/24	3/20	0/0	7/44
suburban	<i>Parus major</i>	21/319	4/33	0/0	25/352
	<i>Sitta europaea</i>	0/2	1/1	1/1	2/4
	total	21/321	5/34	1/1	27/356

Tab. 2 List of birds infested with *C. burnetii*-positive ticks

habitat	host	larvae ex/pos	nymphs ex/pos	adults ex/pos	total ex/pos
suburban	<i>Parus major</i>	9/245	2/14	0/0	11/259
	<i>Erithacus rubecula</i>	0/12	1/2	0/0	1/14
	<i>Sitta europaea</i>	2/22	0/0	0/0	2/22
	total	11/279	3/16	0/0	14/295

Ex/pos = number of examined/number of *C. burnetii* positive

Conclusion

Our study highlights the role of birds in the natural cycle of *Rickettsia* spp. and *C. burnetii* that are of medical and veterinary relevance. According to infection detected in ticks feeding on birds, infection detected in blood samples and our previous results we consider birds as carriers of infected ticks with a role in the geographical spread and maintenance of *Rickettsia* spp. and *C. burnetii* in nature.

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