Original Article

Wien Klin Wochenschr (2006) 118/23–24: 759–764
DOI 10.1007/s00508-006-0729-4

WIENER KLINISCHE WOCHENSCHRIFT
The Middle European Journal of Medicine
Printed in Austria

Ticks (Ixodidae) from passerine birds in the Carpathian region

Eva Špitalská[1], **Ivan Literák**[2], **Olivier A. E. Sparagano**[3], **Marina Golovchenko**[4], and **Elena Kocianová**[1]

[1] Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic
[2] Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[3] School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, United Kingdom
[4] Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

Krankheitserreger in Ixodes-Zecken von Sperlingsvögeln aus den Karpaten

Zusammenfassung. Vögel wurden wiederholt als Reservoir von Zecken-übertragenen Krankheitserregern identifiziert. Um die diesbezügliche Situation in der Slowakei zu untersuchen, wurden von 3057 mit Netzen gefangenen, ringmarkierten und dann wieder freigesetzten Sperlingsvögeln Zecken gesammelt. Die Fangorte in den Bukovské Vrchy Hügeln, einem Teil der Karpaten in der Nordost-Slowakei, lagen 500 m (im Jahr 2001) und 1000 m (im Jahr 2003) über dem Meeresniveau. Nur 75 Vögel, die 16 Arten angehörten, waren mit subadulten Zecken der Art *Ixodes ricinus* infestiert, was einer Parasitenprävalenz von 2,5% entspricht. Von 31 Vögeln (9 Arten) wurden 62 Larven und von 52 Vögeln (15 Arten) 80 Nymphen entfernt. Die Parasitierung war bei Amsel, *Turdus merula*, Singdrossel, *T. philomelos*, und Heckenbraunelle, *Prunella modularis*, am höchsten. Sechs adulte *Ixodes ricinus*-Zecken wurden von erwachsenen Personen entfernt, die mit den Vögeln arbeiteten und eine weibliche *I. ricinus*-Zecke von ihrem Hund. Die Zecken wurden mittels Polymerase Kettenreaktion auf Anwesenheit von Rickettsien, *Coxiella burnetii, Borrelia burgdorferi* sensu lato und Mitgliedern der Familien Anaplasmataceae und Piroplasmidae untersucht und die nachfolgende Identifizierung mittels Sequenzanalyse durchgeführt. Rickettsien wurden in einer Nymphe vom Rotkehlchen, *Erithacus rubecula*, und in drei adulten Zecken (2 weibliche, 1 männliche) von Menschen und vom Hund nachgewiesen. Eine *Ehrlichia*-ähnliche Art, die „Schotti variant", wurde in einer Nymphe einer Singdrossel nachgewiesen. *Borrelia afzelii* wurde in einer männlichen und *B. garinii* in einer weiblichen Zecke identifiziert, welche beide vom Menschen entfernt worden waren. Aufgrund der niedrigen Ausbeute bleibt die Rolle von Vögeln als Reservoir von Zecken-übertragenen Krankheitserregern weiterhin unklar.

Summary. Birds have been found to be a reservoir host of borrelia. In order to assess the situation in Slovakia ticks were collected from a total of 3057 mist-netted, ringed and released passerine birds in two locations at 500 m (in 2001) and 1000 m (in 2003) above sea level in the Bukovské Vrchy Hills, part of the Carpathian region in the north-east of Slovakia. A total of 75 birds of 16 species were infested with subadult ticks of *Ixodes ricinus* species (prevalence of parasitization 2.5%). Sixty-two larvae from 31 birds of 9 species and 80 nymphs from 52 birds of 15 species were found. The highest intensity of parasitization was observed on blackbirds *Turdus merula*, song thrushes *T. philomelos* and dunnocks *Prunella modularis*. Six *Ixodes ricinus* adult ticks were found on humans working with birds, and one *I. ricinus* female tick on their dog. In ticks, the presence of *Rickettsia* sp., *Coxiella burnetii, Borrelia burgdorferi* sensu lato and members of the Anaplasmataceae and Piroplasmidae, were investigated by polymerase chain reaction, followed by sequence analysis. *Rickettsia* sp. was found in 1 nymph from the European robin *Erithacus rubecula*, in 3 adult ticks (1 male, 2 females) from humans and in the tick from the dog. The closely related *Ehrlichia*-like species "Schotti variant" was detected in 1 nymph from the song thrush. *Borrelia afzelii* was identified in 1 male and *B. garinii* in 1 female tick collected on humans. *Ixodes ricinus* was found to be the vector of a wide spectrum of tick-borne pathogens in a mountainous area of the Carpathians. Because of the low yield of ticks and pathogens the importance of birds as reservoir hosts is still poorly understood.

Key words: Passerine birds, *Ixodes ricinus*, *Borrelia, Rickettsia, Ehrlichia,* Carpathians.

Introduction

The tick *Ixodes ricinus* (Linnaeus, 1758) is a common ectoparasite in Central Europe. It is a well known vector

of many viruses, bacteria and protozoa, causing zoonoses and circulating in natural foci. Birds, mainly passerines (Passeriformes) are often hosts of subadult ticks and could be reservoirs of these pathogens. Tick-borne diseases of humans and animals as rickettsiosis [1–3], coxiellosis [4–6], anaplasmosis caused by *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) [7–10], Lyme borreliosis [8, 10–12] and piroplasmosis (babesiosis) [13–15] are emerging problems in temperate regions of Europe due to climatic and urban changes in the environment [16]. Over 40 species of ticks including *Ixodes* spp., *Dermacentor* spp., *Haemaphysalis* spp. were found to be naturally infected with *Coxiella burnetii* [4]. *Rickettsia spp.* and *Anaplasma phagocytophilum* is transmitted by Ixodidae ticks that act both as vector and reservoir for these bacterial pathogens [3, 17, 18].

In Eurasia, six genospecies of spirochetes have been recorded, three of which, *Borrelia burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* are the causative agents of Lyme borreliosis. The most prevalent genospecies in *I. ricinus* ticks collected and analysed in Slovakia were *B. afzelii, B. garinii* and *B. valaisiana.* Rodents and birds were found as their reservoir hosts [19–22].

The present study reports on *I. ricinus* ticks as a vector of tick-borne diseases caused by *Rickettsia* sp., *C. burnetii, B. burgdorferi* sensu lato, and members of the Anaplasmataceae and Piroplasmidae in mountains of the Central European Carpathians, with passerine hosts used for collecting the ticks. Ticks found on humans working with birds and on one dog were also examined in the study.

Material and methods

Study area, collection of birds and processing of ticks

The birds examined for the presence of ticks were mist-netted for the purpose of ornithological research in two locations in the Bukovské Vrchy Hills. The area is part of the woodland in the north-east Slovakia, and it borders Poland and the Ukraine (49°07´N, 22°21´E). In 2001 birds were monitored at the lower border of the forest in the abandoned village of Ruské in the Ruská Kotlina Valley, 500 m above sea level. In 2003, investigation continued in Kurników Beskid Mountain on the Slovak-Polish border about 3 km from Ruské, 1000 m above sea level. The nets were installed along a narrow grassy meadow on a ridge surrounded with natural woody growths with a dominance of beech (*Fagus sylvatica*). The climate being montane, small temperature variation occurred and humidity ranged from humid to very humid.

Birds were investigated from the end of July to the beginning of October both in 2001 and 2003. About 200 m of mist-nets were used. Engorged and half-engorged ticks were removed from birds using fine forceps. All birds were released after the examination. All collected ticks were preserved in 70% ethanol.

DNA isolation, PCR amplification and sequencing

Ticks were screened by polymerase chain reaction (PCR) and DNA sequencing methods for the presence of tick-borne pathogens – *Rickettsia* sp., *C. burnetii, B. burgdorferi* sensu lato, and members of the Anaplasmataceae and Piroplasmidae families.

DNA from tick samples was extracted using the QIAamp Tissue Extraction Kit according to the manufacturer's recommendation (Qiagen, Germany).

Controls for PCR amplifiability of DNA solutions were carried out using general eukaryotic 28S rDNA primers [23].

The PCR amplifications were performed using the following oligonucleotide primers: RpCS.877p, RpCS.1258n, which amplify portions of the *gltA* gene, encoding the citrate synthetase of *Rickettsia prowazekii*, with a PCR product size of 382 bp [24] and primers CBCOS, CBCOE [6], which amplify portions of *com1* gene, encoding an approximately 27-kDa outer membrane-associated immunoreactive protein of *Coxiella burnetii*; with a PCR product size of 494 bp.

The PCR amplifications of the *gltA* and *com1* genes were performed in a Techne, Progene DNA Thermocycler with the following cycle profile for the *gltA* gene: 5 cycles at 94 °C for 10s, 46 °C for 45s, 65 °C for 45s, followed by 30 cycles at 94 °C for 10s, 47 °C for 45s, 65 °C for 45s, and a final extension step at 65 °C for 10 minutes. The cycle profile for the *com1* gene was: 30 cycles at 95 °C for 40s, 56 °C for 45s, 72 °C for 45s followed by the extension at 72 °C for 10 minutes. Each 50 µl reaction mixture contained 10 pmol of each primer, 200 mM of each dNTP, 1.5 mM $MgCl_2$, 5 µl 10 × reaction buffer, 2 U of *Taq* DNA polymerase (Finnzyme, Finland), template DNA < 1 µg and nuclease-free water to final volume. As a positive control for the amplification of *Rickettsia* sp. DNAs of *Rickettsia slovaca* and *R. africae* were used. As positive controls for the amplification of *C. burnetii* DNA from the Nine Mile and Priscilla strains were used and also DNA extracted from laboratory ticks infected with *C. burnetii*, Nine Mile phase I strain. PCR products were separated on 1.2% agarose electrophoresis gel, stained with ethidium bromide, followed by visualization under UV light.

For detection of borreliae, amplification primers targeting the *ospA* gene encoding the outer surface protein A were used. The primer set SL was used for detection of *B. burgdorferi* sensu lato, resulting in a PCR product of 308 bp; primer set GI for *B. burgdorferi* sensu stricto, resulting in a PCR product of 544 bp; primer set GII for *B. garinii,* resulting in a PCR product of 345 bp; and primer set GIII for *B. afzelii*, resulting in a PCR product of 190 bp [25].

The PCR amplifications of the *ospA* gene were performed in a PTC-200 MJ Research DNA Thermocycler with the following cycle profile: 95 °C for 1 minute followed by 30 cycles of 95 °C for 40s, 54 °C for 45s, 72 °C for 45s and final extension at 72 °C for 10 minutes. The species-specific PCR reactions were conducted in 50 µl with the final composition of 1.5 mM $MgCl_2$, 100 mM dNTPs, 20 pmol of both primers and 0.5 µl of Taq DNA polymerase (5U/µl), (Gibco). DNA of *B. burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii* were used as a template for positive control reactions. In negative controls, template DNA was substituted by Milli Q water. Conditions of DNA preparation, PCR reaction and analysis of positive control, negative control and test samples were identical. PCR products were analysed on a 1.2% agarose electrophoresis gel, stained with ethidium bromide, with the final visualization under UV light.

After electrophoresis the amplified fragments were cut out of the gel and purified using Sephaglas BandPrep Kit (Amersham, Austria). Purified PCR products were cloned into the pCR4-TOPO vector using TOPO TA Cloning Kit for Sequencing (Invitrogen, USA) following the manufacturer protocol. Plasmid DNA from the recombinant clones were purified with QIAprep Spin Miniprep Kit (Qiagen, Germany). Inserts were sequenced in both directions using M13 Reverse, M13 Forward

Table 1. Infestation of birds with *Ixodes ricinus* in Bukovské Vrchy Hills, Slovakia

Species of birds	Location, year					
	Ruské, 2001		Kurników Beskid, 2003		Total	
	No. of birds examined	No. of birds with ticks	No. of birds examined	No. of birds with ticks	No. of birds examined (%)	No. of birds with ticks
Erithacus rubecula	452	23 (5.1%)	534	8 (1.5%)	986 (32.3%)	31 (3.1%)
Sylvia atricapilla	265	3 (1.1%)	126	0	391 (12.8%)	3 (0.8%)
S. communis	53	0	35	1 (2.9%)	88 (2.9%)	1 (1.1%)
Turdus merula	39	9 (23.1%)	24	0	63 (2.1%)	9 (14.3%)
T. philomelos	35	3 (8.6%)	50	2 (4%)	85 (2.8%)	5 (5.9%)
T. torquatus	0	0	32	1 (3.1%)	32 (1.0%)	1 (3.1%)
Pyrrhula pyrrhula	64	7 (10.9%)	25	0	89 (2.9%)	7 (7.9%)
Fringilla coelebs	20	1 (5.0%)	57	1 (1.8%)	77 (2.5%)	2 (2.6%)
Prunella modularis	17	1 (5.9%)	104	4 (3.8%)	121 (4.0%)	5 (4.1%)
Phylloscopus trochilus	19	2 (10.5%)	41	0	232 (7.6%)	2 (0.9%)
P. collybita	379	2 (0.5%)	117	1 (0.9%)	496 (16.2%)	3 (0.6%)
Luscinia luscinia	9	1 (11.1%)	5	1 (20.0%)	14 (0.5%)	2 (14.3%)
Parus palustris	9	1 (11.1%)	1	0	92 (3.0%)	1 (1.1%)
Parus major	195	1 (0.5%)	10	0	205 (6.7%)	1 (0.5%)
Troglodytes troglodytes	36	0	35	1 (2.9%)	71 (2.3%)	1 (1.4%)
Saxicola rubetra	6	0	9	1 (11.1%)	15 (0.5%)	1 (6.7%)
Total	1,852 100.0%	54 2.9%	1,205 100.0%	21 1.7%	3,057 100.0%	75 2.5%

(–20) primers (Invitrogen). DNA sequencing was performed using a CEQ 2000 XL DNA Sequencer (Beckman Coulter, Fullerton, CA, USA) and the CEQ 2000 Dye Terminator Cycle Sequencing Kit (Beckman Coulter) according to manufacturer recommendations. Each fragment was sequenced at least twice in both directions and analyzed with the DNASTAR software (DNASTAR, Ltd., London, UK). Database searches used the BLAST programs of the National Center for Biotechnology Information (Bethesda, MD, USA).

For PCR amplification of the 16S rRNA gene spanning the V1 region, primers 16S8FE and B-GA1B were used as described by Bekker et al. [26] and for of the 18S rRNA gene, primers R2 and F2 covering the hypervariable region 4 [27]. The PCR amplifications of the 16S rRNA gene of Anaplasmataceae and the 18S rRNA of Piroplasmidae were performed in an Eppendorf DNA Thermocycler. Fifty μl of the reaction mixture contained 25 μl of REDExract-N-Amp PCR Ready Mix (Sigma, UK), 0.25 μl of forward primer (0.5 pmol), 0.25 μl of reverse primer (0.5 pmol), 5 μl of DNA extract and Milli Q water to a final volume. The reaction was incubated at 94 °C for 10 min to denature genomic DNA and the thermal cycle reaction programme was as follows: 94 °C for 20s, 67 °C for 30s and 72 °C for 30s for two cycles. During the subsequent two-cycle sets the annealing temperature was lowered by 2 °C until it reached 59 °C following a traditional touchdown programme. Then 30 cycles were performed with the annealing temperature of 57 °C. The PCR reaction was ended by a final extension at 72 °C for 5 min. Samples containing DNA of *A. marginale, E. ovina, E. canis, E. equi, Babesia caballi, Theileria lestoquardi, T. annulata* were used as positive controls. In negative controls template DNA were substituted by Milli Q water. PCR products were visualized on 1.2% agarose gels stained with ethidium bromide with the following visualization under UV light. Sequencing was conducted by Lark Technologies, Inc., Essex. DNA sequencing was performed with an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, UK). Sequences were analyzed on an ABI 3730 × 1 capillary automated DNA sequencer (Beckman) followed by analysis with the ABI sequence-analysis software version 5.0 (Applied Biosystems) and comparisons were made with available databases using the BLAST programs of the National Center for Biotechnology Information (Bethesda, MD, USA).

Results

A total of 3,057 birds were examined (1,852 in Ruské and 1,205 in Kurników Beskid). *Ixodes ricinus* ticks were collected from 75 birds (prevalence = 2.5%), representing 16 species, which were infested by subadult stages (larvae and/or nymphs) of *I. ricinus.* The most frequent bird species (32.3%) was the European robin *Erithacus rubecula* in both localities. Tick infestation prevalence on birds in Ruské was 2.9%, with the highest of 23% (9 from 39 examined) on the blackbird *Turdus merula*. The tick infestation on birds in Kurników Beskid was 1.7%, with the highest being 20% (1 out of 5 examined) in thrush nightingale *Luscinia luscinia* (Table 1).

A total of 62 larvae was collected from 31 birds representing nine species. The average intensity of parasitization (I = number of ticks per bird) was 2.0. A total of 80 nymphs was collected from 52 birds representing 15 species (I = 1.5). Forty-five larvae were picked up from 21 birds representing 6 species in Ruské. The highest intensity (I = 3.0) was on the blackbird. Seventeen larvae from

Table 2. Infestation of birds by subadult *Ixodes ricinus* ticks showing intensity of parasitization

Species	Location					
	No. of collected larvae			No. of collected nymphs		
	Ruské	Kurników Beskid	Total	Ruské Beskid	Kurników	Total
Erithacus rubecula	30/13/2.3	6/6/1.0	36/19/1.9	15/12/1.3	3/3/1.0	18/15/1.2
Sylvia atricapilla	3/2/1.5	0	3/2/1.5	1/1/1.0	0	1/1/1.0
S. communis	0	1/1/1.0	1/1/1.0	0	0	0
Turdus merula	6/2/3.0	0	6/2/3.0	20/9/2.2	0	20/9/2.2
T. philomelos	4/2/2.0	0	4/2/2.0	4/1/4.0	2/2/1.0	6/3/2.0
T. torquatus	0	0	0	0	1/1/1.0	1/1/1.0
Pyrrhula pyrrhula	0	0	0	13/7/1.9	0	13/7/1.9
Fringilla coelebs	0	3/1/3.0	3/1/3.0	2/1/2.0	1/1/1.0	3/2/1.5
Prunella modularis	0	6/1/6.0	6/1/6.0	2/1/2.0	6/4/1.5	8/5/1.6
Phylloscopus trochilus	1/1/1.0	0	1/1/1.0	1/1/1.0	0	1/1/1.0
P. collybita	0	0	0	3/2/1.5	1/1/1.0	4/3/1.3
Luscinia luscinia	1/1/1.0	1/1/1.0	2/2/1.0	1/1/1.0	0	1/1/1.0
Parus palustris	0	0	0	1/1/1.0	0	1/1/1.0
P. major	0	0	0	1/1/1.0	0	1/1/1.0
Troglodytes troglodytes	0	0	0	0	1/1/1.0	1/1/1.0
Saxicola rubetra	0	0	0	0	1/1/1.0	1/1/1.0
Total	45/21/2.1	17/10/1.7	62/31/2.0	64/38/1.7	16/14/1.1	80/52/1.5

No. of ticks/No. of hosts/Intensity (I) of occurrence of ticks on hosts.

10 birds representing 5 species were collected in Kurników Beskid with the highest intensity (I = 6.0) on the dunnock *Prunella modularis* (Table 2). Sixty-four nymphs from 38 birds representing 12 species were collected in Ruské with the highest intensity (I = 4.0) on the song thrush *Turdus philomelos*. Only 16 nymphs from 14 birds representing 8 species were collected in Kurników Beskid, the highest intensity (I = 1.5) was on the dunnock (Table 2). Nymphs to larvae parasitized birds in the ratio 1.3:1.

Fifty-five nymphs and 30 larvae from birds, 1 male and 5 females found on humans, and 1 female tick found on a dog were screened for the presence of tick-borne pathogens (Table 3).

Rickettsia sp. DNA amplicon indicating the presence of was found in 1 nymph from the European robin in Ruské, in 1 adult female tick from a dog and in 3 adult ticks (1 male, 2 females) from humans. DNA of the closely related species *Ehrlichia*-like sp. "Schotti variant" (accession No. in GenBank AF104680.1, similarity 98%) from the Anaplasmataceae family was detected in 1 nymph collected from a song thrush in Kurników Beskid. *B. afzelii* (accession No. U78301.1 in GenBank, similarity 100%) was identified in 1 adult male tick from a human and *B. garinii* (accession No. L19702.1, similarity 99%) in 1 adult female tick also from a human. One adult male tick collected from a human was co-infected with *Rickettsia* sp. and *B. afzelii.* DNA of *C. burnetii* and of piroplasm species was not found.

Discussion

Infections of ticks with tick-borne pathogenic microorganisms in Slovakia have been monitored for many years, but adult ticks of the genera *Ixodes, Haemaphysalis* and *Dermacentor* were usually collected from vegetation

Table 3. Detected tick-borne microorganisms in *I. ricinus* ticks

Ticks	Hosts	*Rickettsia sp.*	*Ehrlichia* "Schotti variant"	*Borrelia afzelii*	*Borrelia garinii*
I. ricinus M	human	1	0	1	0
I. ricinus F	human	2	0	0	0
I. ricinus F	human	0	0	0	1
I. ricinus F	dog	1	0	0	0
I. ricinus N	bird *E. rubecula*	1	0	0	0
I. ricinus N	bird *T. philomelos*	0	1	0	0

M male, *F* female, *N* nymph.

[3, 4, 6, 28, 29]. In this study, subadult stages of *I. ricinus* ticks were found on 2.5% of the birds of 16 passerine species in the post-breeding period in the Carpathians of north-east Slovakia. The frequency of parasitization by these ticks showed difference according to the altitude of 500 and 1,000m above see level, 60.6% and 39.4%, respectively. The most parasitized birds were those feeding on the ground such as the blackbird, song thrush, dunnock, European robin, and thrush nightingale, which are the most likely to come into contact with ticks. Blackbirds and song thrush birds were the most infested by subadult stages of *I. ricinus* ticks as also found in other localities in the lowlands of Slovakia [30].

Passerines were infested either by larvae or nymphs or by both stages simultaneously. Nymphs were more common than larvae. These results are in contrast to those of previous studies involving rodents, in which nymphs feeding on rodents were less abundant than larvae [20]. Whether this could have significance for the horizontal transmission of tick-borne agents in natural foci in the Central Europe, would require further studies.

The finding of *Rickettsia* sp. in 1 nymph from the European robin is the first record of rickettsia in ticks from birds in Slovakia. The species of *Rickettsia* found in this nymph and in adult ticks collected from humans and a dog could be *R. slovaca, R. helvetica, Rickettsia* IRS3 or IRS4, species commonly occurring in the Central Europe [31–33].

Anaplasma phagocytophilum in *I. ricinus* collected from vegetation and in the blood of the small rodents *Apodemus flavicollis* and *Clethrionomys glareolus* were detected in Slovakia in previous studies [3, 8]. Now we report the first record of the presence of the closely related *Ehrlichia*-like species "Schotti variant" in a tick from a bird and the first record of this organism in Slovakia. It was first detected and identified in *I. ricinus* in the Netherlands [10], but Bjoersdorff et al. [7] did not find *I. ricinus* larvae infected with *Ehrlichia* on migrating passerine birds. Birds could carry infected ticks without being competent reservoirs and the reservoir status of birds for *Ehrlichia* spp has not yet been resolved.

B. garinii, B. afzelii, and *B. burgdorferi* s.s. are medically important in Slovakia and have often been recorded in ticks from vegetation, rodents and humans [3, 12, 19, 20, 22]. Recently, Hanincová et al. [21] concluded that passerines are the reservoirs for *B. garinii* and *B. valaisiana* in Central Europe. In their study, which was situated in the lowland of western Slovakia, three of the 17 captured passerine species were infested with spirochaete-infected ticks – blackbird, song thrush and great tit (*Parus major*). We also identified *B. garinii* and *B. afzelii* in *I. ricinus* adults collected from humans working with birds. These humans were infested with infected ticks on the ridge of Bukovské Vrchy Hills at an altitude of 1000 m and the ticks probably originated from vegetation in the study localities. Another study on the tick-borne agents [8] showed a relatively high prevalence of *B. burgdorferi* s.l. (38.3%) and *A. phagocytophilum* (8.3%) in *I. ricinus* collected on vegetation in Eastern Slovakia. In these ticks mixed infections were detected in 5% of tested ticks with both pathogens also. Our results indicate the presence of an endemic focus of borreliae in this region of the Carpathians. The altitude limit of *I. ricinus* occurrence in Central Europe was previously thought to be 700–800 m, but current surveys indicate their occurrence at higher altitudes. Danielová et al. [34] reported *I. ricinus* on vegetation in the Šumava Mountains, Czech Republic, at altitudes ranging from 760 m to 1100 m. Occurrence of blood parasites detected in examined birds in the same season in 2001 and 2003 [35], could have an influence on the frequency and intensity of parasitization as well as on the prevalence of tick-borne agents in vectors. Elucidation of these questions would require further studies.

Migrating passerines could transmit ticks and tick-borne pathogens to new areas, in which ticks could live and adapt to climatic and urban changes in the environment and could spread the diseases of humans and/or animals caused by these pathogens. The importance of birds as reservoir of tick-borne pathogens is still poorly understood.

Acknowledgements

This study was partially supported by a Royal Society/ NATO Postdoctoral Fellowship of Dr. Špitalská, by the Slovak agency project VEGA No. 2/4045 and project MSM 6215712402 from the Ministry of Education, Youth and Sports of the Czech Republic.

References

1. Raoult D, Berbis PH, Roux V, Xu W, Maurin M (1997) A new tick-transmitted disease due to *Rickettsia slovaca*. Lancet 350: 112–113
2. Řeháček J, Kocianová E, Lukáčová M, Stanek G, Khanakah G, Výrosteková V, et al (1997) Detection of spotted fever group rickettsia in *Ixodes ricinus* ticks in Austria. Acta Virol 41: 355–356
3. Špitalská E, Kocianová E (2003) Tick-borne microorganisms in southern Slovakia. Ann N Y Acad Sci 990: 196-200
4. Řeháček J, Úrvölgyi J, Kocianová E, Sekeyová Z, Vavreková M, Kováčová E (1991) Extensive examination of different tick species for infestation with *Coxiella burnetii* in Slovakia. Eur J Epidemiol 7: 299–303
5. Řeháček J, Kocianová E, Kováčová E (1996) Problems of contamination of some areas in Slovakia by *Coxiella burnetii* strains imported from abroad. Vet Med 41: 173–176
6. Špitalská E, Kocianová E (2002a) Detection of *Coxiella burnetii* in ticks collected in Slovakia and Hungary. Eur J Epidemiol 18: 263–266
7. Bjoersdorff A, Bergstrom S, Massung RF, Haemig PD, Olsen B (2001) Ehrlichia-infected ticks on migrating birds. Emerg Infect Dis 7: 877–879
8. Derdáková M, Halanová M, Stanko M, Štefančíková A, Čisláková L, Pet'ko B (2003) Molecular evidence for *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* from Eastern Slovakia. Ann Agric Environ Med 10: 269–271
9. Lotriz-Furlan S, Petrovec M, Avsic-Zupanc T, Nicholson WL, Sumner JW, Childs JE, et al (1998) Human ehrlichiosis in central Europe. Wien Klin Wochenschr 110: 894–897
10. Schouls LM, Van De Pol I, Rijpkema SG, Schot CS (1999) Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. J Clin Microbiol 37: 2215–2222

11. Baumgarten BU, Rollinghoff M, Bogdan C (1999) Prevalence of *Borrelia burgdorferi* and granulocytic and monocytic ehrlichiae in *Ixodes ricinus* ticks from southern Germany. J Clin Microbiol 37: 3448–3451
12. Štefančíková A, Pet'ko B, Rozická I, Šalyová N, Ohlasová D (2001) Epidemiological survey of human borreliosis diagnose in Eastern Slovakia. Ann Agric Environ Med 8: 171–175
13. Edelhofer R, Muller A, Schuh M, Obritzhauser W, Kanout A (2004) Differentiation of *Babesia bigemina, B. bovis, B. divergens* and *B. major* by Western blotting-first report of B. bovis in Austrian cattle. Parasitol Res 92: 433–435
14. Kalman D, Sreter T, Szell Z, Egyed L (2003) *Babesia microti* infection of anthropophilic ticks (*Ixodes ricinus*) in Hungary. Ann Trop Med Parasitol 97: 317–319
15. Zahler M, Gothe R (1997) Endemic risk of *Babesia canis* by *Dermacentor reticulatus* in Germany. An epidemiologic study. Tierärztl Prax Ausg Kleintiere K Heimtiere 25: 666–670
16. Gray JS (2002) Biology of *Ixodes* species ticks in relation to tick-borne zoonoses. Wien Klin Wochenschr 114: 473–478
17. Lakos A, Raoult D (1999) Tick-borne lymphadenopathy (TIBOLA) a *Rickettsia slovaca* infection? 258–261. In: Raoult D, Brouqui P (eds) Rickettsiae and rickettsial diseases at the turn of the 3rd millennium. Elsevier, Paris, pp 58–261
18. Špitalská E, Kocianová E (2002b) Agents of *Ehrlichia phagocytophila* group and other microorganisms co-infecting in soutwestern Slovakia. Acta Virol 46: 49–50
19. Gern L, Chang Min Hu, Kocianová E, Výrosteková V, Řeháček J (1999) Genetic diversity of *Borrelia burgdorferi* s.l. isolates obtained from *Ixodes ricinus* collected in Slovakia. Eur J Epidemiol 15: 665–669
20. Hanincová K, Schafer SM, Etti S, Sewell HS, Taragelová V, Žiak D, et al (2002) Association of *Borrelia afzelii* with rodents in Europe. Parasitol 126: 11–20
21. Hanincová K, Taragelová V, Koči J, Schafer SM, Hails R, Ullmann AJ, etal. (2003) Association of *Borrelia garinii* and *B. valaisiana* with Songbirds in Slovakia. App Environ Microbiol 69: 2825–2830
22. Štěpánová-Tresová G, Pet'ko B, Štefančíková A, Nadzamová D (2000) Occurrence of *Borrelia burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* in the Ixodes ricinus from Eastern Slovakia. Eur J Epidemiol 16: 105–109
23. Werren JH, Windsor D, Guo LR (1995) Distribution of *Wolbachia* among neotropical arthropods. Proc Roy Soc London, Ser B 262: 197–204
24. 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
25. Demaerschalck I, Messaoud AB, De Kesel M, Hoyois B, Lobet Y, Hoet P, et al (1995) Simultaneous presence of different *Borrelia burgdorferi* genospecies in biological fluids of Lyme disease patients. J Clin Microbiol 33: 602–608
26. Bekker CPJ, de Vos S, Taoufik A, Sparagano OAE, Jongejan F (2002) Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Vet Microbiol 89: 223–238
27. Špitalská E, Torina A, Cannella V, Caracappa S, Sparagano OAE (2004) Discrimination between *Theileria lestoquardi* and *T. annulata* in thein vector and hosts by RFLP based on the 18S rRNA gene. Parasitol Res 94: 318–320
28. Řeháček J, Kováčová E, Kováč P (1976) Rickettsiae belonging to the spotted fever group from ticks in the Tríbeč Mountains. Folia Parasitol 23: 69–73
29. Řeháček J, Kováčová E, Lisak V, Rumin W (1990) Occurrence of *Coxiella burnetii*, *Rickettsia slovaca*, and organisms resembling bacillary rickettsiae in their natural foci in Slovakia 20 years after their first detection. Folia Parasitol 37: 285–286
30. Taragel'ová V, Koči J, Hanincová K, Olekšák M (2005) Songbirds as hosts for ticks (Acari, Ixodidae) in Slovakia. Biológia 60: 529–537
31. Sekeyová Z, Roux V, Xu W, Řeháček J, Raoult D (1998) *Rickettsia slovaca* sp. nov., a member of the spotted fever group rickettsie. Int J Syst Bacteriol 48 Pt 4: 1455–1462
32. Sreter T, Sreterne Lancz Z, Szell Z, Egyed L (2005) *Rickettsia helvetica*: an emerging tick-borne pathogen in Hungary and Europe. Orv Hetil 146: 2547–2552
33. Sekeyová Z, Fournier PE, Řeháček J, Raoult D (2000) Characterization of a new spotted fever group rickettsia in *Ixodes ricinus* (Acari: Ixodidae) collected in Slovakia. J Med Entomol 37: 707–713
34. Danielová V, Daniel M, Rudenko N, Golovchenko M (2004) Prevalence of *Borrelia burgdorferi* sensu lato genospecies in host-seeking *Ixodes ricinus* ticks in selected South Bohemian locations (Czech Republic). Centr Eur J Pub Health 12: 151–156
35. Hauptmanová K, Benedikt A, Literák I (2006) Blood parasites in passerine birds in Slovakian East Carpathians. Acta Protozoologica 45: 105–109

Correspondence: Dr. Elena Kocianová, Institute of Virology, Slovak Academy of Sciences, Dúbravska cesta 9, 845 05 Bratislava 45, Slovak Republic, E-mail: virukoc@savba.sk