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Diversity and prevalence of spotted-fever group rickettsiae in ixodid ticks across Slovakia, Central Europe

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ABSTRACT

Habitats with the sympatric occurrence of several ixodid tick species are significant from an epidemiological perspective. These habitats can influence the diversity and prevalence of tick-borne pathogens, and their monitoring can help estimate the risk of infection. A total of 1260 questing ticks from five species (*Ixodes ricinus, Dermacentor marginatus, Dermacentor reticulatus, Haemaphysalis concinna*, and *Haemaphysalis inermis*) were collected from vegetation using the flagging method in three different habitats in eastern Slovakia. Additionally, 900 rodent-attached ticks of six species (*I. ricinus, Ixodes trianguliceps, D. marginatus, D. reticulatus, H. concinna*, and *H. inermis*) collected from 149 small mammals belonging to seven species (*Apodemus flavicollis, Apodemus agrarius, Microtus arvalis, Myodes glareolus, Micromys minutus, Crocidura leucodon*, and *Crocidura suaveolens*) were selected for molecular analyses. DNA obtained from rodent-attached and questing ticks was tested by nested PCR targeting the *gltA* gene to determine the presence of *Rickettsia* spp. The *ompA, ompB*, and *sca4* genes were amplified and sequenced to identify rickettsiae species. The overall prevalence of rickettsiae in questing and rodent-attached ticks was 12.5 % and 20.0 %, respectively. Overall studied localities, the most diverse spectrum of rickettsiae species, including *R. helvetica, R. monacensis, R. raoultii*, and *R. slovaca*, was recorded in questing and rodent-attached ticks in the natural habitat of the Slovak Karst. The dominant species, *R. helvetica* (62.9 %), was identified in two species of questing and rodent-attached ticks, specifically *I. ricinus* and *H. concinna,* and in rodent-attached *D. reticulatus* ticks. *Rickettsia raoultii* (20.4 %) was identified in questing and rodent-attached *D. marginatus, D. reticulatus,* and in questing *H. concinna*. Additionally, this study provides the first input of pathogenic *R. raoultii* in *I. trianguliceps* obtained from the striped field mouse (*A. agrarius*). *Rickettsia monacensis* (9.0 %) was detected in questing and rodent-attached *I. ricinus*, while *Rickettsia slovaca* (3.6 %) was found in questing and rodent-attached *D. marginatus* ticks. Natural biotopes with different tick species and hosts significantly affect the diversity and prevalence of rickettsiae.

1. Introduction

Rickettsiae are a diverse group of obligate intracellular bacteria, characterized by small genomes and a gram-negative cell wall structure. Rickettsiae from the spotted fever group (SFG) are often transmitted through arthropod vectors and can cause various human infections. Although primarily detected in ticks, they can also be found in fleas and mites (Raoult and Roux, 1997; Abdad et al., 2011; Portillo et al., 2017; Adem, 2019; Heglasová et al., 2020a). Some species, such as *R. helvetica* and *R. felis,* are identified in a broad spectrum of arthropods. However, the primary vectors of *R. helvetica* and *R. felis* are *I. ricinus* ticks and *Ctenocephalides felis* fleas, respectively (Abdad et al., 2011; Heglasová et al., 2020a).

Rickettsial diseases are typically zoonotic, affecting both animals and humans, with mammals occasionally serving as reservoir hosts for the pathogens. In particular, small mammals are important hosts for the immature stages of ixodid ticks and may contribute to the maintenance and circulation of rickettsiae in enzootic cycles. Several members of the genus *Rickettsia* are responsible for significant human diseases. These infections may manifest with a broad spectrum of clinical symptoms, ranging from mild febrile illness to severe, life-threatening multisystem involvement. The global burden of these diseases is often

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underestimated due to misdiagnosis, as symptoms can mimic other febrile illnesses. However, rickettsial diseases are an important public health concern, particularly in areas where vector populations are expanding. In Slovakia, *R. slovaca, R. raoultii, R. monacensis, R. helvetica,* and *R. felis* have been identified in arthropods and hosts (e.g., small mammals) using various molecular methods (Boldiš et al., 2008; Sekeyová et al., 2013; Špitalská et al., 2015; Minichová et al., 2017; Heglasová et al., 2018). Additionally, cases of human rickettsiosis have been confirmed in the region (Mittermayer et al., 1980; Kováčová et al., 2006; Zubriková et al., 2022).

Ixodes ricinus is the most common and widely distributed tick species in Slovakia (Hanincová et al., 2003; Derdáková et al., 2014; Švehlová et al., 2014; Stanko et al., 2022). It is a recognized vector of multiple infectious agents, including tick-borne encephalitis virus (TBEV), *Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., and *Babesia* spp. *Dermacentor* spp. are vectors of a wide range of pathogens, such as viruses, bacteria (e.g., *Rickettsia* spp., *Anaplasma* sp., *Francisella tularensis*), and parasites of the genus *Babesia* (Nosek, 1972; Sekeyová et al., 1998; Zivkovic et al., 2007; Špitalská et al., 2012; Garcia-Vozmediano et al., 2020; Boldiš et al., 2008; Földvári et al., 2013; Jongejan et al., 2015, 2016). In the study by Heglasová et al. (2018), *R. slovaca* was detected in wild rodents but only in habitats with the sympatric occurrence of *D. marginatus* and *D. reticulatus* ticks. *Dermacentor* spp. are known vectors of *R. raoultii* and *R. slovaca*. The presence of *R. slovaca* is expected, especially in sites with *Dermacentor* spp. ticks. These tick species prefer inundated forests and forest-steppe areas in southwestern and southeastern Slovakia (Černý, 1972; Nosek, 1972; Stanko et al., 2022).

Haemaphysalis concinna, H. punctata, and *H. inermis* are mostly found in the southern and central parts of the country (Stanko et al., 2022). *Haemaphysalis inermis* ticks are considered potential vectors of different bacterial pathogens, such as *R. helvetica, R. raoultii,* and *Borrelia miyamotoi* (Hornok et al., 2010; Heglasová et al., 2020b; Ouarti et al., 2022). In *H. concinna*, members of the genera *Babesia, Theileria, Borrelia*, and *Rickettsia*, as well as *Anaplasma phagocytophilum*, have been detected (Hamšíková et al., 2016; Kazimírová et al., 2018; Dwużnik-Szarek et al., 2021). However, the vector competence of *Haemaphysalis* tick species to transmit *Rickettsia* spp. remains unknown (Minichová et al., 2017; Ouarti et al., 2022).

In the present study, we aimed to evaluate the presence and diversity of rickettsiae in a wide spectrum of questing and host-attached ticks collected from different habitat types over three years. Long-term monitoring can help increase awareness, especially in areas with a high incidence of infected ticks.

2. Materials and methods

2.1. Tick sampling

Questing and rodent-attached ticks were collected from three sampling sites in eastern Slovakia during 2014–2016. The first site represents an urban habitat, specifically a botanical garden with deciduous forest vegetation, located in the center of Košice (208 m a.s.l.; 48°44′46.84″N; 21°14′16.14″ E). The second site, Čermeľ Valley, is characterized by mixed forest vegetation, predominantly beech, hornbeam, and spruce (220–410 m a.s.l.; 48°45′46.67″N; 21°8′8.17″E), representing a suburban habitat. The third site, located in the Slovak Karst National Park near the village of Hrhov (200–220 m a.s.l.; 48°34′53.9″N; 20°46′44.4″ E), features mixed forest vegetation with a predominance of beech, oak, and hornbeam and represents a natural habitat.

Questing ticks were collected along small mammal-trapping transects by flagging a 1 m^2 white wool blanket over the vegetation for one hour during May–July and September–October in the years 2014 to 2016. Trapped rodents and rodent-attached ticks were collected at the same three study sites in eastern Slovakia as the questing ticks.

Live trapping of small mammals was conducted in May–June and September–October from 2014 to 2016. Rodent sampling methods and results were described in detail by Heglasová et al. (2018). The captured small mammals were euthanized according to licenses from the Ministry of Environment of the Slovak Republic (No. 4874/2011–2.2 and No 4559/2015–2.3). The rodents and insectivores were identified to species level based on morphological characteristics (e.g., body size, tail, ears, legs), using standard identification keys by Pelikán et al. (1979), Sládek and Mošanský (1985), and Anděra and Horáček (2005). In addition, sex and age class (juvenile, subadult, adult) were recorded for each individual.

Each small mammal was checked for the presence of ticks. All collected ticks were stored in 70 % ethanol until identification. The species and developmental stages of the ticks were determined based on morphological characteristics under a light microscope using the identification keys by Filippova (1977), Slovák (2014), and Estrada-Peña et al. (2017).

2.2. DNA extraction and molecular analyses

The ticks were washed with sterile water, air-dried, transferred to individual tubes, and crushed with a sterile scalpel. The genomic DNA of questing *I. ricinus* ($n = 1164$) was extracted from each tick individually using alkaline hydrolysis (Rijpkema et al., 1996). Total DNA from rodent-attached *I. ricinus* ($n = 619$), and other species of rodent-attached and questing *I. trianguliceps* ($n = 24$), *D. marginatus* ($n = 131$), *D. reticulatus* ($n = 99$), *H. concinna* ($n = 93$), and *H. inermis* ($n = 30$) was extracted from each tick individually using the DNeasy Blood & Tissue Kit (Qiagen®, Germany) according to the manufacturer's instructions. All DNA samples were stored at −20 °C for further processing.

Subsequently, all DNA samples were screened for the presence of *Rickettsia* spp. by nested PCR amplification of a fragment of the citrate synthase gene (*gltA*), using the genus-specific primers RpCS.877p and RpCS.1258n (Regnery et al., 1991) and the inner primer pair RpCS.896p and RpCS.1233n (Choi et al., 2005), resulting in a 337-bp PCR product. To further characterize *Rickettsia* species, selected *gltA*-positive amplicons were analyzed for the presence of fragments of the gene loci (*ompA, ompB*, and *sca4*) encoding outer membrane proteins A, B, and surface cell antigen 4, as previously described (Regnery et al., 1991; Roux et al., 1996; Roux and Raoult, 2000; Sekeyová et al., 2001), with primers RR190.70F, RR190.701R, 120–2788, 120–3599, and D1390r, D767f, respectively.

Rickettsia helvetica, R. monacensis, R. raoultii, and *R. slovaca* were identified by the amplification of the *gltA, ompA, ompB*, and *sca4* genes followed by sequencing (Roux et al., 1996; Roux and Raoult, 2000; Sekeyová et al., 2001; Portillo et al., 2017). For each PCR, a sample with DNA from previously sequenced *Rickettsia* spp. was used as a positive control, and nuclease-free water was used as a negative control. Several randomly chosen PCR-positive samples from questing and rodent-attached ticks were purified using the Isolate II PCR and Gel Kit (Bioline) and sequenced (Sanger sequencing, dye terminator method) in both directions using the same primers as for the PCR amplification at the University of Veterinary Medicine and Pharmacy in Košice, Slovakia.

2.3. Accession numbers of obtained nucleotide sequences

Nucleotide sequences of the 337 bp fragments of the *gltA* gene obtained in this study from questing and rodent-attached ticks were submitted to the GenBank database under the following accession numbers respectively: *R. helvetica* MK792533-MK792584, *R. monacensis* MK792585-MK792594, *R. raoultii* MK792595-MK792604, *R. slovaca* MK792605 and *R. helvetica* MK875680-MK875725, *R. monacensis* MK875726-MK875734, *R. raoultii* MK875735-MK875757, *R. slovaca* MK875758-MK875762. Additionally, fragments of *ompB* gene: *R. monacensis* PQ644884-PQ644886, PQ644895; *R. slovaca* PQ644888; *R. raoultii* PQ644887; *ompA* gene: *R. monacensis* PQ644894; *R. slovaca*; and *sca4* gene: *R. monacensis* PQ644890-PQ644892; *R. raoulti* PQ644893.

2.4. Statistical analysis

The differences in the prevalence of rickettsial infection for ticks from vegetation and rodent-attached ticks between habitats were tested with the χ2 and Fisher's exact test. GraphPad Prism, version 8.1.0 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com) was used for statistical analysis.

3. Results

3.1. Ticks and small mammals collection

A total of 1260 questing ticks from five species (*I. ricinus* ($n = 1164$), *D. marginatus* ($n = 12$), *D. reticulatus* ($n = 12$), *H. concinna* ($n = 45$), and *H. inermis* ($n = 27$) were collected by flagging vegetation during spring and summer (May/June/July) as well as autumn (September/October) of 2014–2016 (Table 1). Additionally, 250 small mammals representing 11 species (*Apodemus agrarius, Apodemus flavicollis, Apodemus uralensis, Myodes glareolus, Crocidura leucodon, Crocidura suaveolens, Micromys minutus, Microtus arvalis, Microtus subterraneus, Neomys fodiens,* and *Sorex minutus*) were captured in natural, suburban, and urban habitats in eastern Slovakia. Of the 250 trapped small mammals, 149 (59.6 %) were infested with ticks.

Totally, 1102 ixodid ticks (920 larvae and 182 nymphs) were collected from small mammals. Of these, 900 ticks (868 larvae and 32 nymphs) representing six species (*I. ricinus, I. trianguliceps, D. marginatus, D. reticulatus, H. concinna,* and *H. inermis*) were further processed for molecular analysis in the present study (Table 2). Small mammals were examined in the previous study by Heglasová et al. (2018). The selected rodent-attached ticks were dominated by *I. ricinus* (68.8 %), comprising 594 larvae and 25 nymphs. This was followed by *D. marginatus* (13.2 %, 119 larvae), *D. reticulatus* (9.7 %, 87 larvae), *H. concinna* (5.3 %, 48 larvae), *I. trianguliceps* (2.7 %, 18 larvae and 6 nymphs), and *H. inermis* (0.3 %, 2 larvae and 1 nymph).

Of the captured small mammals, 27 were rickettsia-positive (Heglasová et al., 2018). Six individuals of the three species of rickettsia-positive rodents (*M. glareolus, A. flavicolis*, and *A. agrarius*) from suburban and natural habitats carried rickettsia-positive ticks. *Rickettsia helvetica* was identified in the larva of *I. ricinus* from *Rickettsia* sp.-positive *M. glareolus* from the suburban area - Čermeľ. We also detected pathogenic *R. raoultii* in the larva of *I. trianguliceps* obtained from a *Rickettsia*-negative striped field mouse (*A. agrarius*) captured in Čermeľ. Other *Rickettsia* spp. from rodent-attached ticks collected from rickettsia-positive rodents could not be identified species level due to the low concentration of DNA.

The highest diversity of questing tick species was recorded in the sylvatic/natural habitat (*I. ricinus* ($n = 423$), *D. marginatus* ($n = 12$), *D. reticulatus* ($n = 12$), *H. concinna* ($n = 45$), and *H. inermis* ($n = 27$). In this habitat, 116 small mammals of seven species (*A. agrarius, A. flavicollis, A. uralensis, M. glareolus, C. leucodon, M. arvalis, S. minutus*) were captured, and six species of rodent-attached ticks (*I. ricinus, I. trianguliceps, D. marginatus, D. reticulatus, H. concinna, H. inermis*) were collected.

In the suburban habitat, only *I. ricinus* ($n = 546$) was recorded on vegetation. A total of 126 small mammals of six species (*A. agrarius, A. flavicollis, M. glareolus, M. minutus, M. subterraneus, N. fodiens*) were captured, and two tick species (*I. ricinus, I. trianguliceps*) were collected from the animal hosts. In the urban habitat, questing *I. ricinus* dominated ($n = 195$). Eight rodents of three species (*A. agrarius, A. flavicollis,* and *C. suaveolens*) were captured in this habitat, but only one tick species (*I. ricinus*) was found on the animals.

3.2. The presence of SFG rickettsia spp. in questing and rodent-attached ticks

All 1260 questing ticks were individually analyzed for the presence of SFG *Rickettsia* spp. The overall prevalence of rickettsiae in ticks from vegetation was 12.5 % ($n = 158/1260$). DNA of *Rickettsia* spp. was detected in all developmental stages of four ixodid tick species from all study sites (Table 1). *Haemaphysalis inermis* ticks only the adult tested negative for the presence of rickettsiae. The overall prevalence of rickettsial infection was 14.5 % in the natural habitat, 13.3 % in the urban habitat, and 10.4 % in the suburban habitat (Table 1), with no significant differences noted ($\chi^2 = 4.04$, df = 2, $p = 0.133$).

A total of 900 randomly chosen rodent-attached ticks were individually screened for the presence of *Rickettsia* spp. Rickettsial DNA was detected in 20.0 % ($n = 180/900$) of the ticks collected from rodents. The prevalence of rickettsial infection was 21.5 % in the natural habitat and 11.3 % in the suburban habitat (Table 2). The prevalence of rickettsiae was not calculated for rodent-attached ticks from the Botanical Garden sampling site due to the low number of ticks tested ($n = 6/22$). A significant difference was confirmed between the natural and suburban habitats (Fisher's exact test, $p = 0.004$); the urban habitat was not included in the test because the overall number of ticks was too small.

Out of a total of 338 *Rickettsia* spp. *gltA* PCR-positive DNA samples obtained from rodent-attached and questing ticks, more than half ($n = 181$), were selected for sequencing. This subset ($n = 181$) included representatives of each tick species and developmental stage that tested positive for *Rickettsia*, selected from all study localities. A total of 168 nucleotide sequences were successfully obtained. Based on the sequencing of four genetic markers (*gltA, ompA, ompB*, and *sca4*), we confirmed the presence of *R. helvetica* ($n = 105$) in *I. ricinus, H. concinna,* and *D. reticulatus*. Additionally, *R. raoultii* was confirmed ($n = 34$) in *D. reticulatus, D. marginatus, H. concinna,* and *I. trianguliceps; R. monacensis* ($n = 16$) in *I. ricinus; R. slovaca* ($n = 6$) in *D. marginatus*; and *Rickettsia* sp. ($n = 7$) in *I. ricinus* and *H. inermis* ticks (Tables 3 and 4).

BLAST analysis of the *gltA* and *sca4* gene fragments of questing and rodent-attached ixodid ticks showed the 99.1–100 % identity with the

Table 1
Prevalence[a] of *Rickettsia* spp. in questing ticks in three habitat types in Slovakia.

Habitat /tick stage	*I. ricinus*		*D. reticulatus*		*D. marginatus*		*H. concinna*		*H. inermis*		Total
Slovak Karst (Natural)	M	10/67	M	1/7	M	4/4	M	0/3	M	0/10	75/519 (14.5 %)[a]
	F	8/44	F	1/5	F	4/8	F	0/2	F	0/17	
	N	37/245	N	0	N	0	N	0/10	N	0	
	L	5/67	L	0	L	0	L	5/30	L	0	
Čermeľ (Suburban)	M	8/62									57/546 (10.4 %)
	F	15/92									
	N	34/383									
	L	0/9	–		–		–		–		
Botanical garden (Urban)	M	6/22									26/195 (13.3 %)
	F	2/13									
	N	13/106									
	L	5/54	–		–		–		–		
Total		143/1164		2/12		8/12		5/45		0/27	158/1260 (12.5 %)

No. of *Rickettsia*-positive/no. of tested ticks.

Table 2
Prevalence[a] of *Rickettsia* spp. in rodent-attached juvenile ticks in three habitat types in Slovakia.

Habitat /tick stage	*I. ricinus*		*I. triangulicpes*		*D. reticulatus*	*D. marginatus*	*H. concinna*	*H. inermis*	Total
Slovak Karst (Natural)	N	5/20	N	0/2	N -	N -	N -	N 1/1	158/736 (21.5 %)[a]
	L	84/441	L	0/16	L 20/87	L 46/119	L 1/48	L 1/2	
Čermeľ (Suburban)	N	0/5	N	0/4					16/142 (11.3 %)
	L	15/131	L	1/2	–	–	–	–	
Botanical garden (Urban)	N	0/0							6/22
	L	6/22	–		–	–	–	–	
Total		110/619		1/24	20/87	46/119	1/48	2/3	180/900 (20.0 %)

No. of *Rickettsia*-positive/no. of tested tick.

Table 3
Rickettsia spp. in questing ticks.

Ticks	No. of *Rickettsia*-positive ticks/no. of tested ticks	*Rickettsia* spp.
Ixodes ricinus	143/1164	*R. helvetica* ($n =$ 56) *R. monacensis* ($n =$ 6) *Rickettsia* sp. ($n =$ 2)
Dermacentor reticulatus	2/12	*R. raoultii* ($n = 2$)
Dermacentor marginatus	8/12	*R. slovaca* ($n = 1$) *R. raoultii* ($n = 6$)
Haemaphysalis concinna	5/45	*R. raoultii* ($n = 3$) *R. helvetica* ($n = 1$)
Haemaphysalis inermis	0/27	–

Table 4
Rickettsia spp. in ticks from small mammals.

Ticks	No. of *Rickettsia*-positive ticks/no. of tested ticks	*Rickettsia* spp.
Ixodes ricinus	110/619	*R. helvetica* ($n = 46$) *R. monacensis* ($n =$ 10) *Rickettsia* sp. ($n = 3$)
Ixodes trianguliceps	1/24	*R. raoultii* ($n = 1$)
Dermacentor reticulatus	20/87	*R. raoultii* ($n = 12$) *R. helvetica* ($n = 1$)
Dermacentor marginatus	46/119	*R. raoultii* ($n = 10$) *R. slovaca* ($n = 5$)
Haemaphysalis concinna	1/48	*R. helvetica* ($n = 1$)
Haemaphysalis inermis	2/3	*Rickettsia* sp. ($n = 2$)

nucleotide sequences of *R. helvetica* (e.g., MF673863, MF673861, KY231194-KY231199, KM288466-KM288468, MF163040). BLAST analysis of the *gltA, ompB,* and *sca4* gene fragment sequences confirmed the presence of *R. monacensis* in both questing and rodent-attached *I. ricinus*. The sequences showed 100 % identity with *R. monacensis* isolates from ixodid ticks across Europe (e.g., KU961970, KU961543, LN794217, KC137254, LN794217).

The nucleotide sequences of the *gltA, ompB,* and *sca4* gene fragments from questing and rodent-attached *D. marginatus* showed 99.1–100 % identity with *R. slovaca* sequences from the GenBank database (e.g., KY940286, KY940286, MF002538, CP003375, HQ232242, MG973876, MF002539, CP003375). Amplification of the *ompA* gene fragment of *R. slovaca* and *R. monacensis* from questing and rodent-attached ticks failed.

Ten nucleotide sequences of the *gltA, ompA,* and *ompB* genes from *H. concinna, D. marginatus,* and *D. reticulatus* showed 99.7–100 % identity with *R. raoultii* isolates from *Dermacentor* spp. ticks across Europe, Russia, Asia, and China (e.g., MK792595-MK792600, MK792603, KR131756, MF166732, MF166730, MG973878, HQ232252-HQ232258, EU036984, and others).

4. Discussion

In the present study, a total of 2160 specimens belonging to several species of questing and rodent-attached ticks from different habitat types (a botanical garden - urban; Čermeľ Valley - suburban; and the Slovak Karst National Park, near the village Hrhov - natural habitat) in Eastern Slovakia were screened for the presence of *Rickettsia* spp. Rickettsial DNA was found in 20.0 % ($n = 180/900$) of rodent-attached ticks and 12.5 % ($n = 158/1260$) of questing ticks. The results of this study confirmed the presence of *Rickettsia* in six tick species, namely *I. ricinus, I. trianguliceps, D. marginatus, D. reticulatus, H. concinna*, and *H. inermis*, collected at the sampling sites in eastern Slovakia.

The highest prevalence of *Rickettsia* spp. was in questing (14.5 %) and rodent-attached ticks (21.5 %) from the natural habitat of the Slovak Karst region, followed by an urban habitat and a suburban habitat for both questing and rodent-attached ticks. Only *I. ricinus* were found in the urban habitat, and this species appears to be the most significant vector of rickettsiae in this area. Based on the sequencing of four genetic markers (*gltA, ompA, ompB*, and *sca4*), *R. helvetica, R. monacensis, R. raoultii, R. slovaca*, and *Rickettsia* sp. were found in ixodid ticks. The most diverse spectrum of rickettsiae, with four identified species — *R. helvetica, R. monacensis, R. raoultii*, and *R. slovaca* — were recorded in questing ticks from the natural habitat of the Slovak Karst region. In the suburban habitat, the presence of two species, *R. helvetica* and *R. monacensis*, was detected, and in the urban habitat, only *R. helvetica* was confirmed in ticks. A similar species representation of *Rickettsia* occurred in rodent-attached ticks, with *R. helvetica, R. monacensis, R. raoultii*, and *R. slovaca* in the natural habitat; *R. helvetica* and *R. raoultii* in the suburban habitat; and *R. helvetica* in the urban habitat.

In the studied areas, the most significant rodent hosts of the ticks are predominantly *Apodemus* spp. and *Myodes* spp. (Minichová et al., 2017; Heglasová et al., 2018). These rodent species are among the most abundant in Slovakia (Martiniaková et al., 2010). The highest prevalence of *Rickettsia* spp. in rodents was detected in the Čermeľ (16.0 %) (Heglasová et al., 2018). *Rickettsia helvetica* was identified in *I. ricinus* from *M. glareolus* and *R. raoultii* in *I. trianguliceps* obtained from *A. agrarius*, both trapped in Čermeľ. Areas with a higher diversity of tick species exhibited a greater variety of *Rickettsia* species. In contrast, sites with lower tick species diversity tend to harbor a narrower spectrum of bacteria. For example, a higher prevalence of *R. helvetica* and *R. monacensis,* typically associated with *I. ricinus,* was observed in areas where this tick species is predominant. *Rickettsia helvetica* dominates in rodent hosts in locations with the significant occurrence of its vector, *I. ricinus* (Heglasová et al., 2018). Thus, the composition of *Rickettsia* species in ticks parasitizing rodents in various habitats (natural, suburban, urban) is largely shaped by the specific tick species present.

In our study, the sympatric occurrence of six tick species — *I. ricinus, I. trianguliceps, D. marginatus, D. reticulatus, H. concinna*, and *H. inermis* — were recorded in the natural habitat of the Slovak Karst region. Similarly, a relatively wide spectrum of *Ixodes* spp., *Dermacentor* spp., and *Haemaphysalis* spp. was reported in this location in the past (Nosek, 1971, 1972; Hubálek, 1986, and others). In this study, *R. slovaca* was found in questing and rodent-attached *D. marginatus* ticks only in the natural environment. In a previous study in this region, one *A. flavicollis* tested positive for *R. slovaca* (Heglasová et al., 2018). The results confirm long-term circulation of *R. slovaca* in the Slovak Karst region, with *Dermacentor* spp. as a potential reservoir. Similarly, pathogenic *R. slovaca* is found in *Dermacentor* spp. ticks in Eurasian areas (Sekeyová et al., 1998; Raoult et al., 2002; Špitalská et al., 2012; Földvári et al., 2013; Parola et al., 2009; Minichová et al., 2017, and others). Based on available data, the occurrence of *R. slovaca* is primarily associated with habitats where *Dermacentor* spp. ticks are present. The occurrence of *Dermacentor* ticks in the environment may indicate a risk of human infection. *Rickettsia slovaca* and *R. raoultii* were described as potential etiological agents of TIBOLA/DEBONEL/SENLAT disease in humans, which is associated with a tick bite, an inoculation eschar, and cervical adenopathies (Parola et al., 2009).

Rickettsia raoultii was detected in questing *D. marginatus, D. reticulatus*, and *H. concinna* ticks only in the natural habitat of the Slovak Karst. *Rickettsia raoultii* is usually associated with *Dermacentor* spp. (Špitalská et al., 2012; Švehlová et al., 2014). Evidence of *R. raoulti* in *H. concinna* ticks is interesting and represents the first data on the presence of the bacterium in this tick species. Previously published data revealed the presence of *R. raoultii* in *D. reticulatus* and *H. inermis* ticks in the same study area in the Slovak Karst National Park in southeastern Slovakia (Ouarti et al., 2022). From ticks attached to small mammals, *R. raoultii* was recorded in larvae of *D. marginatus* and *D. reticulatus* in the natural habitat, and one *I. trianguliceps* larva from the Čermeľ Valley, representing the suburban habitat. This is the first evidence of *R. raoultii* infection in the endophilic tick species *I. trianguliceps*. However, it is not known whether *H. concinna* and *I. trianguliceps* are involved in the life cycle of *R. raoultii*.

Rickettsia monacensis was identified in larvae and nymphs of *I. ricinus* in both rural and suburban habitats. These rickettsiae have also been detected in *Ixodes arboricola* and *H. concinna* from Romania (Mărcuțan et al., 2016) and recently also in the flea *Ctenophthalmus agyrtes* in Lithuania (Radzijevskaja et al., 2018).

The most prevalent rickettsial species, *R. helvetica* was detected in all developmental stages of *I. ricinus, D. reticulatus*, and *H. concinna* at all studied sites in eastern Slovakia. The most common tick species, *I. ricinus*, represents a vector and natural reservoir of *R. helvetica*, which is responsible for human rickettsioses diagnosed in Slovakia (Parola et al., 2005; Sekeyová et al., 2012; Švehlová et al., 2014; Špitalská et al., 2014, 2016; Minichová et al., 2017). In many other European countries and Japan, *R. helvetica* has also been confirmed in the genera *Ixodes, Haemaphysalis*, and *Dermacentor* (Fournier et al., 2002; Parola et al., 2005; Nijhof et al., 2007; Dobec et al., 2009; Hornok et al., 2010; Tijsse-Klasen et al., 2013; Tomassone et al., 2013; Mărcuțan et al., 2016). In the study by Heglasová et al. (2020a), *R. helvetica* was identified in *C. agyrtes* fleas collected from *A. agrarius* captured in the same suburban area. Additionally, *R. helvetica* was confirmed in small mammals belonging to the genera *Apodemus, Myodes*, and *Microtus* in natural and suburban habitats of Eastern Slovakia (Heglasová et al., 2018).

In the forest area of the Čermeľ Valley, six species of small mammals and two tick species (*I. ricinus, I. trianguliceps*) were identified. Previously published studies from this area reported on the presence of several species of pathogens (e.g., *R. felis, R. helvetica, B. miyamotoi*) in rodents, ticks, and fleas (Špitalská et al., 2015; Heglasová et al., 2018, 2020a, 2020b). The least diversity of hosts and vectors was found in the Botanical Garden, which represents an urban area. In this area, *B. miyamotoi* was identified in questing *I. ricinus* and *R. felis* in fleas collected from the small mammals (Heglasová et al. 2020b). The widely distributed *R. helvetica* and *R. felis* can be expected in different groups of vectors.

The highest prevalence and diversity of *Rickettsia* species were recorded in the Slovak Karst National Park, which represents a natural habitat and a unique locality with a sympatric occurrence of six tick species. Similarly, the largest representation of small mammal hosts was recorded in the same region. Previously, several species of rickettsiae (*R. slovaca, R. helvetica, R. raoultii,* and *Rickettsia* sp.) have been identified in rodents, fleas, rodent-attached ticks, and questing ticks from vegetation sampled in the Slovak Karst area (Radzijevskaja et al., 2015; Heglasová et al., 2018, 2020a; Ouarti et al., 2022). Additionally, another pathogen, *B. miyamotoi*, was identified in *Ixodes* spp. and *Haemaphysalis* spp. ticks, as well as in one rodent host species in areas of eastern Slovakia (Heglasová et al., 2020b). The high density and diversity of vectors and hosts in the natural environments provide suitable conditions for the co-circulation of several species of pathogens.

5. Conclusions

Human pathogens such as *R. helvetica, R. slovaca, R. raoultii,* and *R. monacensis* were detected in six species of questing and rodent-attached ixodid ticks in Eastern Slovakia. The most prevalent, *R. helvetica,* was detected in *I. ricinus, D. reticulatus,* and *H. concinna* in the rural habitat. *Rickettsia helvetica* was detected at all studied sites, but exclusively in *I. ricinus* ticks.

Rickettsia slovaca was found in questing and rodent-attached *D. marginatus* ticks only in natural environments. *Rickettsia raoultii* was detected in *D. marginatus, D. reticulatus,* and *H. concinna* in rural areas and in one *I. trianguliceps* larva in a suburban habitat. This is the first evidence of *R. raoultii* infection in the endophilic tick species *I. trianguliceps. Rickettsia monacensis* was identified in larvae and nymphs of *I. ricinus* in rural and suburban habitats.

Our results confirmed the highest prevalence and diversity of *Rickettsia* spp. in natural habitats with a sympatric occurrence of six tick species. Biotopes with a higher diversity of vectors and hosts influence the diversity and prevalence of rickettsiae (and other vector-borne pathogens). Monitoring these areas helps identify high-risk regions and enables targeted control strategies to reduce the incidence of infections.

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Consent to participate and publish

All authors consent to participate in this study and agree to the publication of the manuscript in Ticks and Tick-borne Diseases.

CRediT authorship contribution statement

Ivana Heglasová: Formal analysis, Data curation, Conceptualization. **Bronislava Víchová:** Conceptualization. **Michal Stanko:** Conceptualization.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

Abdad, M.Y., Stenos, J., Graves, S., 2011. *Rickettsia felis*, an emerging flea-transmitted human pathogen. Emerg. Health Threats J. 4, 7168. https://doi.org/10.3402/ehtj.v4i0.7168.

Adem, P.V., 2019. Emerging and re-emerging rickettsial infections. Semin. Diagn. Pathol. 36, 146–151. https://doi.org/10.1053/j.semdp.2019.04.005.

Anděra, M., Horáček, I., 2005. Poznávame naše savce. 2. Přepracované vydání. Sobotáles, Praha.

Boldiš, V., Kocianová, E., Štrus, J., Tušek-Žnidarič, M., Sparagano, O.A., Štefanidesová, K., Špitalská, E., 2008. Rickettsial agents in Slovakian ticks (acarina, ixodidae) and their ability to grow in vero and L929cell lines. Ann. N. Y. Acad. Sci. 1149, 281–285. https://doi.org/10.1196/annals.1428.090.

Černý, V., 1972. The tick fauna of Czechoslovakia. Folia Parasitol. (Praha) 19, 87–92.

Derdáková, M., Václav, R., Pangrácova-Blaňárová, L., Selyemová, D., Koči, J., Walder, G., Špitalská, E., 2014. *Candidatus* Neoehrlichia mikurensis and its co-circulation with *anaplasma phagocytophilum* in *Ixodes ricinus* ticks across ecologically different habitats of Central Europe. Parasit. Vectors 7, 160. https://doi.org/10.1186/1756-3305-7-160.

Dobec, M., Golubic, D., Punda-Polic, V., Kaeppeli, F., Sievers, M., 2009. *Rickettsia helvetica* in *Dermacentor reticulatus* ticks. Emerg. Infect. Dis. 15, 98–100. https://doi.org/10.3201/eid1501.080815.

Dwużnik-Szarek, D., Mierzejewska, E.J., Alsarraf, M., Alsarraf, M., Bajer, A., 2021. Pathogens detected in the tick *Haemaphysalis concinna* in western Poland: known and unknown threats. Exp. Appl. Acarol. 84, 769–783. https://doi.org/10.1007/s10493-021-00647-x.

Estrada-Peña, A., Mihalca, A.D., Petney, T.N., 2017. Ticks of Europe and North Africa: a guide to species identification. Springer, Switzerland.

Filippova, N.A., 1977. Ixodid ticks of the subfamily ixodinae. Fauna SSSR, new ser.

Földvári, G., Rigó, K., Lakos, A., 2013. Transmission of *Rickettsia slovaca* and *Rickettsia raoultii* by male *Dermacentor marginatus* and *Dermacentor reticulatus* ticks to humans. Diagn. Microbiol. Infect. Dis. 76, 387–389. https://doi.org/10.1016/j.diagmicrobio.2013.03.005.

Földvári, G., Široký, P., Szekeres, S., Majoros, G., Sprong, H., 2016. *Dermacentor reticulatus*: a vector on the rise. Parasit. Vectors 9, 314. https://doi.org/10.1186/s13071-016-1599-x.

Fournier, P.E., Fujita, H., Takada, N., Raoult, D., 2002. Genetic identification of rickettsiae isolated from ticks in Japan. J. Clin. Microbiol. 40, 2176–2181. https://doi.org/10.1128/jcm.40.6.2176-2181.2002.

Garcia-Vozmediano, A., Giglio, G., Ramassa, E., Nobili, F., Rossi, L., Tomassone, L., 2020. *Dermacentor marginatus* and *Dermacentor reticulatus*, and their infection by SFG Rickettsiae and *Francisella*-like endosymbionts, in mountain and periurban habitats of Northwestern Italy. Vet. Sci. 7, 157. https://doi.org/10.3390/vetsci7040157.

Hamšíková, Z., Kazimírová, M., Haruštiaková, D., Mahríková, L., Slovák, M., Berthová, L., Kocianová, E., Schnittger, L., 2016. *Babesia* spp. In ticks and wildlife in different habitat types of Slovakia. Parasit. Vectors 9, 292. https://doi.org/10.1186/s13071-016-1560-z.

Hanincová, K., Tarageľová, V., Koči, J., Schäfer, S.M., Hails, R., Ullmann, A.J., Piesman, J.P., Labuda, M., Kurtenbacg, K., 2003. Association of *Borrelia garinii* and *B. valaisiana* with songbirds in Slovakia. Appl. Environ. Microbiol. 69, 2825–2830. https://doi.org/10.1128/AEM.69.5.2825-2830.2003.

Heglasová, I., Rudenko, N., Golovchenko, M., Zubriková, D., Miklisová, D., Stanko, M., 2020b. Ticks, fleas and rodent-hosts analyzed for the presence of *Borrelia miyamotoi* in Slovakia: the first record of *Borrelia miyamotoi* in a *Haemaphysalis inermis* tick. Ticks. Tick. Borne Dis. 11, 101456. https://doi.org/10.1016/j.ttbdis.2020.101456.

Heglasová, I., Víchová, B., Kraljik, J., Mošanský, L., Miklisová, D., Stanko, M., 2018. Molecular evidence and diversity of the spotted-fevergroup *rickettsia* spp. In small mammals from natural, suburban and urban areas of Eastern Slovakia. Ticks. Tick. Borne Dis. 9, 1400–1406. https://doi.org/10.1016/j.ttbdis.2018.06.011.

Heglasová, I., Víchová, Stanko, M., 2020a. Detection of *rickettsia* spp. In fleas collected from small mammals in Slovakia, Central Europe. Vector. Borne Zoonotic. Dis. 20, 652–656. https://doi.org/10.1089/vbz.2019.2567.

Hornok, S., Meli, M.L., Perreten, A., Farkas, R., Willi, B., Beugnet, F., Lutz, H., Hofmann-Lehmann, R., 2010. Molecular investigation of hard ticks (Acari: ixodidae) and fleas (Siphonaptera: pulicidae) as potential vectors of rickettsial and mycoplasmal agents. Vet. Microbiol. 140, 98–104. https://doi.org/10.1016/j.vetmic.2009.07.013.

Hubálek, Z., 1986. Comparative biogeography of Bhanja virus in Europe. Acta *Sc.* Nat. Brno. 20, 1–50.

Choi, Y.J., Jang, W.J., Ryu, J.S., Lee, S.H., Park, K.H., Paik, H.S., Koh, Y.S., Choi, M.S., Kim, I.S., 2005. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg. Infect. Dis. 11, 237–244. https://doi.org/10.3201/eid1102.040603.

Jongejan, F., Ringenier, M., Putting, M., Berger, L., Burgers, S., Kortekaas, R., Lenssen, J., van Roessel, M., Wijnveld, M., Madder, M., 2015. Novel foci of *Dermacentor reticulatus* ticks infected with *Babesia canis* and *Babesia caballi* in The Netherlands and in Belgium. Parasit. Vectors 8, 232. https://doi.org/10.1186/s13071-015-0841-2.

Kazimírová, M., Hamšíková, Z., Špitalská, E., Minichová, L., Mahríková, L., Caban, R., Sprong, H., Fonville, M., Schnittger, L., Kocianová, E., 2018. Diverse tick-borne microorganisms identified in free-living ungulates in Slovakia. Parasit. Vectors 11, 495. https://doi.org/10.1186/s13071-018-3068-1.

Kováčová, E., Sekeyová, Z., Trávniček, M., Bhide, M.R., Mardzinová, S., Čurlik, J., Španelová, D., 2006. Monitoring of humans and animals for the presence of various Rickettsiae and *coxiella burnetii* by serological methods. Ann. N. Y. Acad. Sci. 1078, 587–589. https://doi.org/10.1196/annals.1374.117.

Mărcuţan, I.D., Zsuzsa, K., Ionică, A.M., D'Amico, G., Mihalca, A.D., Vasile, C., Sándor, A. D., 2016. Spotted fever group rickettsiae in ticks of migratory birds in Romania. Parasit. Vectors 9, 294. https://doi.org/10.1186/s13071-016-1565-7.

Martiniaková, M., Omelka, R., Jančová, A., Stawarz, R., Formicki, G., 2010. Heavy metal content in the femora of yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) from different types of polluted environment in Slovakia. Environ. Monit. Assess. 171, 651–660. https://doi.org/10.1007/s10661-010-1310-1.

Minichová, L., Hamšíková, Z., Mahríková, L., Slovák, M., Kocianová, E., Kazimírová, M., Škultéty, Ľ., Katarína Štefanidesová, K., Špitalská, E., 2017. Molecular evidence of *rickettsia* spp. In ixodid ticks and rodents in suburban, natural and rural habitats in Slovakia. Parasit. Vectors 10, 1–12. https://doi.org/10.1186/s13071-017-2094-8.

Mittermayer, T., Brezina, R., Úrvolgyi, J., 1980. First report of an infection with *R. slovaca*. Folia Parasitol. (Praha) 27, 373–376.

Nijhof, A.M., Bodaan, C., Postigo, M., Nieuwenhuijs, H., Opsteegh, M., Franssen, L., Jebbink, F., Jongejan, F., 2007. Ticks and associated pathogens collected from domestic animals in The Netherlands. Vector. Borne Zoonotic. Dis. 7, 585–595. https://doi.org/10.1089/vbz.2007.0130.

Nosek, J., 1971. The ecology, bionomics and behaviour of *Haemaphysalis* (*Aboimisalis*) *punctata* tick in central Europe. Z. Parasitenkd. 37, 198–210. https://doi.org/10.1007/BF00259499.

Nosek, J., 1972. The ecology and public health importance of *dermacentor marginatus* and *D. reticulatus* ticks in central Europe. Folia Parasitol. (Praha) 19, 93–102.

Ouarti, B., Hamzaoui, B.E., Stanko, M., Laroche, M., Mediannikov, O., Parola, P., Sekeyová, Z., 2022. Detection of *rickettsia raoultii* in *dermacentor reticulatus* and *haemaphysalis inermis* ticks in Slovakia. Biologia (Bratisl) 77, 1611–1617. https://doi.org/10.1007/s11756-021-00789-8.

Parola, P., Paddock, C.D., Raoult, D., 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin. Microbiol. Rev. 18, 719–756. https://doi.org/10.1128/cmr.18.4.719-756.2005.

Parola, P., Rovery, C., Rolain, J.M., Brouqui, P., Davoust, B., Raoult, D., 2009. *Rickettsia slovaca* and *R. raoultii* in tick-borne rickettsioses. Emerg. Infect. Dis. 15, 1105–1108. https://doi.org/10.3201/eid1507.081449.

Pelikán, J., Gaisler, J., Rődl, P., 1979. Naši Savci. Academia, Praha.

Portillo, A., de Sousa, R., Santibáñez, S., Duarte, A., Edouard, S., Fonseca, I.P., Marques, C., Novakova, M., Palomar, A.M., Santos, M., Silaghi, C., Tomassone, L., Zúquete, S., Oteo, J.A., 2017. Guidelines for the detection of *rickettsia* spp. Vector. Borne Zoonotic. Dis. 17, 23–32. https://doi.org/10.1089/vbz.2016.1966.

Radzijevskaja, J., Kaminskienė, E., Lipatova, I., Mardosaitė-Busaitienė, D., Balčiauskas, L., Stanko, M., Paulauskas, A., 2018. Prevalence and diversity of *Rickettsia* species in ectoparasites collected from small rodents in Lithuania. Parasit. Vectors 11, 375. https://doi.org/10.1186/s13071-018-2947-9.

Radzijevskaja, J., Paulauskas, A., Aleksandraviciene, A., Jonauskaite, I., Stanko, M., Karbowiak, G., Peťko, B., 2015. New records of spotted fever group rickettsiae in Baltic region. Microbes. Infect. 17, 874–878. https://doi.org/10.1016/j.micinf.2015.09.006.

Raoult, D., Lakos, A., Fenollar, F., Beytout, J., Brouqui, P., Fournier, P.E., 2002. Spotless rickettsiosis caused by *Rickettsia slovaca* and associated with *Dermacentor* ticks. Clin. Infect. Dis. 34, 1331–1336. https://doi.org/10.1086/340100.

Raoult, D., Roux, V., 1997. Rickettsioses as paradigms of new or emerging infectious diseases. Clin. Microbiol. Rev. 10, 694–719. https://doi.org/10.1128/cmr.10.4.694.

Regnery, R.L., Spruill, C.L., Plikaytis, B.D., 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J. Bacteriol. 173, 1576–1589. https://doi.org/10.1128/jb.173.5.1576-1589.1991.

Rijpkema, S., Golubic, D., Moelkenboer, M., Verbeek-De Kruif, N., Schellekens, J., 1996. Identification of four genomic groups of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic region of northern Croatia. Exp. Appl. Acarol. 20, 23–30. https://doi.org/10.1007/BF00051474.

Roux, V., Fournier, P.E., Raoult, D., 1996. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR amplified DNA of the gene encoding the protein rOmpA. J. Clin. Microbiol. 34, 2058–2065. https://doi.org/10.1128/jcm.34.9.2058-2065.1996.

Roux, V., Raoult, D., 2000. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (ompB). Int. J. Syst. Evol. Microbiol. 50, 1449–1455. https://doi.org/10.1099/00207713-50-4-1449.

Sekeyová, Z., Mediannikov, O., Subramanian, G., Kowalczewska, M., Quevedo-Diaz, M., Kocianová, E., Raoult, D., 2012. Isolation of *Rickettsia helvetica* from ticks in Slovakia. Acta Virol. 56, 247–252. https://doi.org/10.4149/av_2012_03_247.

Sekeyová, Z., Roux, V., Raoult, D., 2001. Phylogeny of *Rickettsia* spp. inferred by comparing sequences of 'gene D', which encodes an intracytoplasmic protein. Int. J. Syst. Evol. Microbiol. 51, 1353–1360. https://doi.org/10.1099/00207713-51-4-1353.

Sekeyová, Z., Roux, V., Xu, W., Řeháček, J., Raoult, D., 1998. *Rickettsia slovaca* sp. nov., a member of the spotted fever group rickettsiae. *Int. J.* Syst. *Bacteriol* 48, 1455–1462. https://doi.org/10.1099/00207713-48-4-1455.

Sekeyová, Z., Socolovschi, C., Špitalská, E., Kocianová, E., Boldiš, V., Diaz, M.Q., Berthová, L., Bohacsová, M., Valáriková, J., Fournier, P.E., Raoult, D., 2013. Update on rickettsioses in Slovakia. Acta Virol. 57, 180–199. https://doi.org/10.4149/av_2013_02_180.

Sládek, J., Mošanský, A., 1985. Cicavce okolo nás. Osveta, Martin.

Slovák, M., 2014. Obrázkový kľúč lariev a nýmf kliešťov (Acari: ixodida) fauny Slovenska. Entomofauna carpathica 26, 12–18.

Stanko, M., Derdáková, M., Špitalská, E., Kazimírová, M., 2022. Ticks and their epidemiological role in Slovakia: from the past till present. Biologia (Bratisl.) 77, 1575–1610. https://doi.org/10.1007/s11756-021-00845-3.

Špitalská, E., Boldiš, V., Derdáková, M., Selyemová, D., Rusňáková Tarageľová, V., 2014. Rickettsial infection in *ixodes ricinus* ticks in urban and natural habitats of Slovakia. Ticks. Tick. Borne Dis. 5, 161–165. https://doi.org/10.1016/j.ttbdis.2013.10.002.

Špitalská, E., Boldiš, V., Mošanský, L., Sparagano, O., Stanko, M., 2015. *Rickettsia* species in fleas collected from small mammals in Slovakia. Parasitol. Res. 114, 4333–4339. https://doi.org/10.1007/s00436-015-4713-7.

Špitalská, E., Stanko, M., Mošanský, L., Kraljik, J., Miklisová, D., Mahríková, L., Bona, M., Kazimírová, M., 2016. Seasonal analysis of *rickettsia* species in ticks in anagricultural site of Slovakia. Exp. Appl. Acarol. 68, 315–324. https://doi.org/10.1007/s10493-015-9941-0.

Špitalská, E., Štefanidesová, K., Kocianová, E., Boldiš, V., 2012. *Rickettsia slovaca* and *Rickettsia raoultii* in *dermacentor marginatus* and *dermacentor reticulatus* ticks from Slovak Republic. Exp. Appl. Acarol. 57, 189–197. https://doi.org/10.1007/s10493-012-9539-8.

Švehlová, A., Berthová, L., Sallay, B., Boldiš, V., Sparagano, O.A.E., Špitaská, E., 2014. Sympatric occurrence of *Ixodes ricinus, Dermacentor reticulatus* and *Haemaphysalis concinna* ticks and *Rickettsia* and *Babesia* species in Slovakia. Ticks. Tick. Borne Dis. 5, 600–605. https://doi.org/10.1016/j.ttbdis.2014.04.010.

Tijsse-Klasen, E., Hansford, K.M., Jahfari, S., Phipps, P., Sprong, H., Medlock, J.M., 2013. Spotted fever group rickettsiae in *Dermacentor reticulatus* and *haemaphysalis punctata* ticks in the UK. Parasit. Vectors 6, 212. https://doi.org/10.1186/1756-3305-6-212.

Tomassone, L., Grego, E., Auricchio, D., Iori, A., Giannini, F., Rambozzi, L., 2013. Lyme borreliosis spirochetes and spotted fever group rickettsiae in ixodid ticks from Pianosa Island, Tuscany Archipelago, Italy. Vector. Borne Zoonotic. Dis. 13, 84–91. https://doi.org/10.1089/vbz.2012.1046.

Zivkovic, Z., Nijhof, A.M., de la Fuente, J., Kocan, K.M., Jongejan, F., 2007. Experimental transmission of *anaplasma marginale* by male *Dermacentor reticulatus*. BMC. Vet. Res. 3, 32. https://doi.org/10.1186/1746-6148-3-32.

Zubriková, D., Heglasová, I., Antolová, D., Blaňarová, L., Víchová, B., 2022. A case report of *rickettsia*-like infection in a human patient from Slovakia. Biologia (Bratisl) 77, 1641–1644. https://doi.org/10.1007/s11756-021-00813-x.