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Small mammals as hosts of vector-borne pathogens in the High Tatra Mountains region in Slovakia, Central Europe

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ABSTRACT

Rodents and insectivores are significant reservoirs of many zoonotic pathogens, contributing to the transmission of diseases affecting human and animal health. This study investigated the prevalence and diversity of vector-borne pathogens in small mammals within the High Tatras region of Slovakia, an area with substantial recreational activity and protected zones. A total of 156 small mammals, comprising ten species, were screened for pathogens such as *Bartonella* spp., *Borrelia* spp., *Anaplasma phagocytophilum*, and *Babesia* spp. The prevalence of vector-borne pathogens in the studied animals reached 74.35%, with *Bartonella* spp. being the most common, identified in 57.7% of the animals, particularly in *Apodemus flavicollis* and *Clethrionomys glareolus*. *Borrelia burgdorferi* (*sensu lato*) was detected in 11.5% of the rodents, with *Borrelia afzelii* identified as the predominant species. *Babesia microti* was found in *A. flavicollis* and *Mus musculus*, with a total prevalence of 3.2%. The lowest was the prevalence of *A. phagocytophilum* reaching 1.9%. This study provides evidence of the significant role of rodents as reservoirs of vector-borne pathogens in protected areas of the High Tatras region and Tatra National Park.

1. Introduction

Approximately 70% of diseases, transmissible from animals to humans originate from wildlife (Hassell et al., 2017). Various domestic and wild animals can act as potential vectors of zoonotic pathogens, with wild and synanthropic small mammals being the most significant amplifiers of pathogens and parasites that pose a substantial threat to human health. In addition, rodents contribute to the spread of these pathogens in various environments, from densely populated urban areas to rural and wildlife areas (Meerburg et al., 2009; Luis et al., 2013; Morand et al., 2015). Synanthropic species such as the black rat (*Rattus rattus*), Norway rat (*Rattus norvegicus*), and house mice (*Mus musculus*) have expanded their habitats due to human activities and often coexist due to their synanthropic behavior. These rodents are associated with the emergence of many diseases.

The role of small mammals in transmitting the causative agents of many diseases is crucial (Akhtar et al., 2023). With 2277 extant species from 33 families, almost 10% of the small mammal population is either a carrier or reservoir of zoonotic pathogens playing a crucial role in the ecology and transmission dynamics of many parasites and pathogens of public health significance including protozoans, fungi, helminths, bacteria, and viruses (Burgin et al., 2018). These disease-causing organisms are transmitted through fleas, mosquitoes, infected tick bites, inhalation of aerosolized rodent excreta (feces, urine), or contact with contaminated surfaces and food. The risk of pathogen spillover increases in environments where wild and synanthropic rodents cohabitate and live close to human settlements (Meerburg et al., 2009; Himsworth et al., 2013).

This study aimed to assess the diversity and prevalence of pathogens such as *Borrelia* spp., *Bartonella* spp., *Anaplasma phagocytophilum*, and

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Babesia spp. in rodent and insectivore species collected in the region of High Tatras, northern Slovakia, Central Europe, a significant part of the Tatra National Park protected area.

2. Materials and methods

The small mammals (rodents and insectivores) used for this study were found dead, road-killed, or deceased during the pest control operation. The carcasses were delivered to the TANAP (Tatra National Park) Research Station and Museum. In total, 156 individuals of 10 species from 7 genera (*Apodemus agrarius*, *A. flavicollis*, *Clethrionomys glareolus*, *Microtus arvalis*, *Microtus* sp., *Mus musculus*, *Rattus norvegicus*, *Crocidura suaveolens*, *Sorex minutus*, and *Sorex araneus*) were screened for the presence of pathogens responsible for zoonotic diseases. The animals were collected from 6 sampling sites (Fig. 1) with altitudes ranging from 684 m a.s.l. (Poprad City) to 1754 m a.s.l. (Skalnatá dolina). Each individual was identified to a species level based on morphological characteristics and then dissected. Spleen and ear tissue were collected from each individual and stored at −20 °C until DNA extraction.

DNA was extracted from spleen samples (about 25 mg tissue sample per specimen) and ears using a commercial DNA extraction kit (NucleoSpin Blood kit, NucleoSpin Tissue kit, Machery Nagel, Germany) and resuspended in a total volume of 100 µl. Lysates were stored at −20 °C before use. DNA of *Bartonella* spp., *Anaplasma* spp., *Borrelia* spp., and *Babesia* spp. was detected using conventional PCR assays.

PCR amplifications were performed in a 25 µl reaction mixture containing 15.8 µl of DNA-free water, 2.5 µl of 10× PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 1 U of Taq DNA polymerase (Qiagen, Hilden, Germany), 0.5 µl of 10 mM dNTP Mix (Promega, Madison, WI, USA), 1 µl of 10 µM concentration of each primer, and 2.5 µl of DNA template. Positive (DNA sample from the known infected rodents or ticks and confirmed by sequencing) and negative controls (sterilized DNA-free water) were used in each PCR reaction. Data for the primers used are provided in Table 1. PCR amplicons were visualized on 2% agarose gels stained with GoodView Nucleic Acid Stain (Beijing SBS Genetech, Beijing, China). Products selected for further analysis were purified using ISOLATE II PCR and Gel Kit (Bioline) and sequenced at the University of Veterinary Medicine and Pharmacy, Košice, in both directions using the PCR primers.

The newly generated sequences were aligned and visually screened for errors such as frameshifts, stop codons within the gene sequence, or unusual amino acid substitutions using MEGA version 11 (Tamura et al., 2021). Representative sequences were deposited in the GenBank database under the accession numbers PQ429062 and PQ429063 (*Bartonella grahamii ssrA* gene), PQ408971 and PQ408970 (*Bartonella* sp. *ssrA* gene), PQ408972 and PQ420800 (*Anaplasma phagocytophilum p44/msp2* gene), PQ408973, PQ420929 and PQ420930 (*Borrelia afzelii* 5S-23S ITS), and PQ423045-PQ423049 (*Babesia microti* and *Babesia* sp. 18S rRNA gene).

3. Results

In total, 10 species of small mammals were studied. The overall prevalence of vector-borne pathogens in the sample of wild small mammals from the Tatra National Park was high across most sites, reaching 74.35% ($n = 116/156$). Representatives of the genera *Apodemus* and *Clethrionomys* carried DNA of all tested microorganisms (*Bartonella* spp., *Borrelia* spp., *Babesia* spp., and *Anaplasma* spp.) (Table 2).

The overall prevalence of *Bartonella* spp. in all examined small mammal species was the highest among all studied pathogens in the sample of small mammals, reaching 57.7% ($n = 90/156$). Exceptionally high prevalence of 100% ($n = 12/12$) and 70% ($n = 42/60$) was observed in *Apodemus agrarius* and *Apodemus flavicollis*, respectively.

Genotyping of several randomly selected PCR-positive samples confirmed the presence of *Bartonella grahamii* and *Bartonella* sp. (Table 3). *Bartonella* spp. was recorded in *Clethrionomys glareolus* (prevalence of 56.3%; 27/48) and *Mus musculus* (prevalence of 21.7%; 5/23). The most prevalent species of *Bartonella* in *C. glareolus* and *M. musculus* were *B. grahamii* (GenBank: PQ429062 and PQ429063) and *Bartonella* sp. (PQ408971 and PQ408970). The new sequences of *B. grahamii* showed a minimum of 96.92% (100% query coverage) similarity to sequences of *B. grahamii* previously isolated from *Dermacentor marginatus* ticks in Slovakia (GenBank: PP230806-PP230808) or fleas from cats and dogs from the UK (GenBank: MK298177).

Borrelia spp. were less prevalent in small mammals than *Bartonella* spp.; however, similarly to *Bartonella* spp., these were confirmed in *Apodemus* spp. and *Clethrionomys* spp. from all sampling sites. The overall prevalence of *Borrelia burgdorferi* (*sensu lato*) in tested animals reached 11.5%. Spirochaete DNA was found in *A. agrarius* (16.7%), *A. flavicollis* (15.0%), and *C. glareolus* (14.6%). Further genotyping

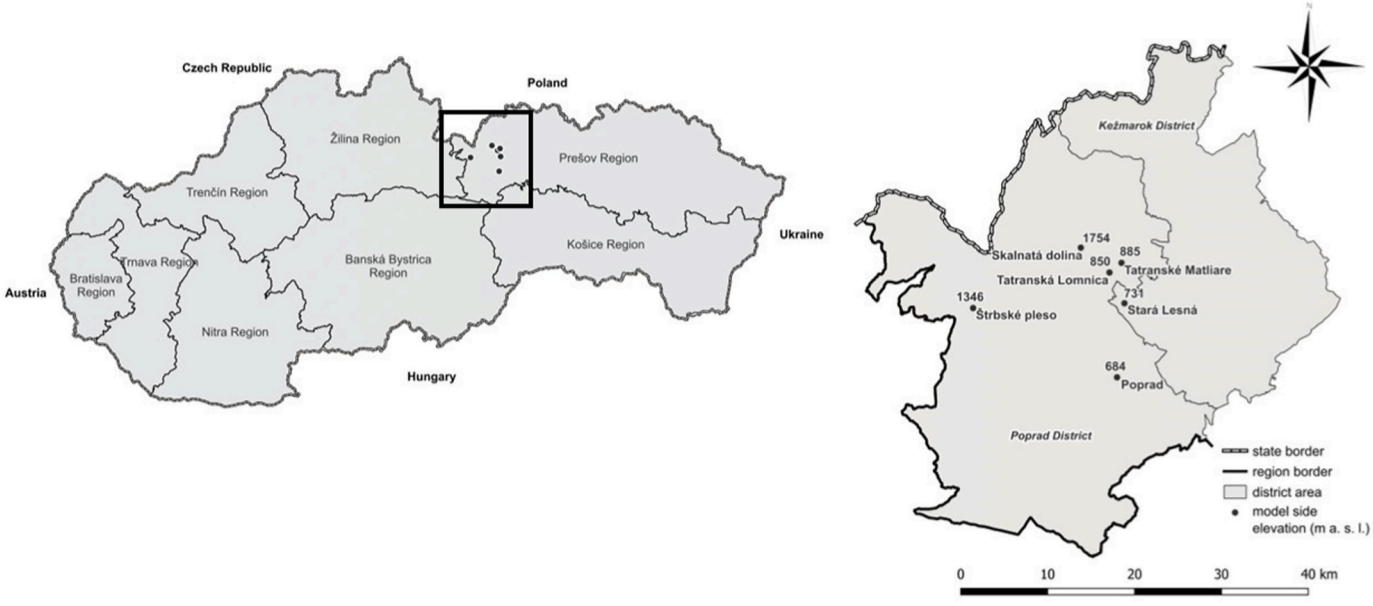

Fig. 1. Map of Slovakia with sites in the High Tatra Mountains region where small mammals were collected (circles); names of collection locations and altitudes are also provided.

Table 1
Primers used for the detection of pathogen DNA.

Pathogen	Target gene	Amplicon length (bp)	Primer name	Primer sequence (5′-3′)	Annealing temperature	Reference
Bacteria						
A. phagocytophilum	*p44/msp2*	334	MSP2f	CCAGCGTTTAGCAAGATAAGAG	55 °C	Eberts et al. (2011)
			MSP2r	GCCCAGTAACATCATAAGC		
Borrelia spp.	*rrfA-rrlB*	222–255	IGSa	CGACCTTCTTCGCCTTAAAGC	57 °C	Derdáková et al. (2003)
			IGSb	AGCTCTTATTCGCTGATGGTA		
Bartonella spp.	*ssrA*	257	ssrA-F	GCTATGGTAATAAATGGACAATGAAATAA	60 °C	Diaz et al. (2012)
			ssrA-R	GCTTCTGTTGCCAGGTG		
Unicellular parasites						
Babesia ssp.	18S rRNA	433–489	BJ1	GTCTTGTAATTGGAATGATGG	58 °C	Casati et al. (2006)
			BN2	TAGTTTATGGTTAGGACTACG		

Table 2
Prevalence of tick-borne pathogens in small mammals from different sampling sites in the High Tatra Mountains.

Sampling site	Elevation (m. a.s.l.)	Host	*N*	*Babesia* spp.	*Anaplasma* spp.	*Bartonella* spp.	*Borrelia* spp.
Poprad	684	*Apodemus flavicollis*	8	12.5% ($n = 1$)	0	87.5% ($n = 7$)	12.5% ($n = 1$)
		Clethrionomys glareolus	3	0	0	100% ($n = 3$)	0
		Mus musculus	5	0	0	40.0% ($n = 2$)	0
		Rattus norvegicus	1	0	0	0	0
		Sorex araneus	1	0	0	0	0
		Sorex minutus	1	0	0	0	0
		Total	19	5.3% ($n = 1$)	0	63.2% ($n = 12$)	5.3% ($n = 1$)
Stará Lesná	731	*Apodemus flavicollis*	11	0	9.1% ($n = 1$)	81.8% ($n = 9$)	9.1% ($n = 1$)
		Clethrionomys glareolus	11	0	0	90.9% ($n = 10$)	9.1% ($n = 1$)
		Microtus arvalis	3	0	0	100% ($n = 3$)	0
		Total	25	0	4.0% ($n = 1$)	88.0% ($n = 22$)	8.0% ($n = 2$)
Tatranská Lomnica	850	*Apodemus agrarius*	8	0	0	100% ($n = 8$)	28.6% ($n = 2$)
		Apodemus flavicollis	2	0	0	50.0% ($n = 2$)	0
		Clethrionomys glareolus	3	0	0	66.7% ($n = 2$)	33.3% ($n = 1$)
		Mus musculus	5	0	0	0	0
		Total	18	0	0	61.1% ($n = 11$)	16.6% ($n = 3$)
Tatranské Matliare	885	*Apodemus agrarius*	4	0	0	100% ($n = 4$)	0
		Apodemus flavicollis	13	15.4% ($n = 2$)	0	46.15% ($n = 6$)	30.8% ($n = 4$)
		Clethrionomys glareolus	8	0	0	25.0% ($n = 2$)	12.5% ($n = 1$)
		Mus musculus	1	0	0	0	0
		Rattus norvegicus	3	0	0	0	0
		Total	29	6.9% ($n = 2$)	0	41.4% ($n = 12$)	17.2% ($n = 5$)
Štrbské pleso	1346	*Apodemus flavicollis*	2	0	0	100% ($n = 2$)	50.0%) ($n = 1$)
		Clethrionomys glareolus	10	10.0% ($n = 1$)	10.0% ($n = 1$)	40.0% ($n = 4$)	10.0% ($n = 1$)
		Mus musculus	2	0	0	0	0
		Crocidura suaveolens	1	0	0	0	0
		Total	15	6.7% ($n = 1$)	6.7% ($n = 1$)	40.0% ($n = 6$)	13.3% ($n = 2$)
Skalnatá dolina	1754	*Apodemus flavicollis*	24	0	4.17% ($n = 1$)	70.8% ($n = 17$)	8.3% ($n = 2$)
		Clethrionomys glareolus	13	0	0	46.15% ($n = 6$)	23.1% ($n = 3$)
		Microtus sp.	2	0	0	50.0% ($n = 1$)	0
		Mus musculus	10	10.0% ($n = 1$)	0	30.0% ($n = 3$)	0
		Rattus norvegicus	1	0	0	0	0
		Total	50	2.0% ($n = 1$)	2.0% ($n = 1$)	54.0% ($n = 27$)	10.0% ($n = 5$)

Abbreviation: N, number of examined small mammals.

confirmed the presence of *Borrelia afzelii* in all tested samples (GenBank: PQ408973, PQ420929 and PQ420930).

The overall prevalence of *Babesia* spp. (3.2%) and *A. phagocytophilum* (1.9%) in tested animals was comparatively lower. *Babesia microti* was confirmed in *A. flavicollis* and *M. musculus*. The nucleotide sequences (GenBank: PQ423045 and PQ423047) obtained from PCR-positive samples were 100% identical with each other and showed a minimum of 99.2% similarity with at least 100 homologous sequences (according to the results of the BLAST search in GenBank) from ticks or small mammals studied elsewhere in the world, including Slovakia (e.g. GenBank: PP086648, MN355504, MH351731, MH628094, AY144692, and others).

Babesia sp. 18S rRNA gene nucleotide sequences (GenBank: PQ423046 and PQ423049) from *A. flavicollis* and *C. glareolus* showed 99.7–100% (100% query coverage) similarity with the following DNA sequences: *B. canis* isolates from the blood of red foxes (*Vulpes vulpes*) in Poland (GenBank: MK872807 and MN134074), from ticks (*D. reticulatus*) in Kazachstan (GenBank: MK070118), from a bat (*Nyctalus noctula*) in Hungary (GenBank: KP835549), "SR5" isolate from a dog in Slovakia (GenBank: MK508870), and from *D. reticulatus* ticks in Poland and Hungary (GenBank: MW362500 and OM913540).

Babesia sp. (GenBank: PQ423048), associated with cervids, was found in a DNA sample isolated from the spleen of *A. flavicollis*. This sequence is particularly highly similar to the "*Babesia* sp.- deer clade" (e. g. GenBank: MG344773 and MG344775) and *Babesia* cf. *odocoilei* (GenBank: KU351828, KU351827 and PP512757) extracted from blood or ticks of wild cervids.

Anaplasma phagocytophilum was confirmed in three samples (1.9%) from the spleen biopsies of two yellow-necked mice (*A. flavicollis*) and one bank vole (*C. glareolus*). The nucleotide sequences from wild small mammals were identical (GenBank: PQ408972 and PQ420800). A minimum of 98.77% (99% query coverage) similarity with sequences from GenBank was observed, for instance, with KZA2 and KZ-A1 strains from human patients in South Korea (GenBank: MH734192 and CP035303), small mammals and ticks in Florida, USA (GenBank: JQ063009), *A. phagocytophilum* JM strain from a meadow jumping

Table 3
Prevalence of tick-borne pathogens in small mammals from the High Tatra Mountains according to host species.

Host	*N*	*Babesia* spp.	*Anaplasma* spp.	*Bartonella* spp.	*Borrelia* spp.
Apodemus agrarius	12	0	0	100% (*n* = 12)	16.7% (*n* = 2)
Apodemus flavicollis	60	5.0% (*n* = 3)	3.3% (*n* = 2)	70.0% (*n* = 42)	15.0% (*n* = 9)
Clethrionomys glareolus	48	2.1% (*n* = 1)	2.1% (*n* = 1)	56.25% (*n* = 27)	14.6% (*n* = 7)
Microtus arvalis	3	0	0	3/3	0
Microtus sp.	2	0	0	1/2	0
Mus musculus	23	4.35% (*n* = 1)	0	21.7% (*n* = 5)	0
Rattus norvegicus	5	0	0	0	0
Crocidura suaveolens	1	0	0	0	0
Sorex minutus	1	0	0	0	0
Sorex araneus	1	0	0	0	0
Total	156	3.2% (*n* = 5)	1.9% (*n* = 3)	57.7% (*n* = 90)	11.5% (*n* = 18)

Abbreviation: N, number of examined small mammals.

mouse (*Zapus hudsonius*) trapped at Camp Ripley (GenBank: CP006617), or North American human strain HZ2 (GenBank: CP006616).

4. Discussion

This study was conducted in the High Tatras region, primarily a protected area of the Tatra National Park. Individuals of ten small mammal species, the most common of which were *A. flavicollis* and *C. agrarius*, were tested for the presence of selected pathogens. These abundant rodent species host a variety of ectoparasites and are an essential part of the life cycles of diverse pathogens, such as *Borrelia* spp., *Anaplasma phagocytophilum*, *Babesia* spp., "*Candidatus* Neoehrlichia mikurensis", and *Rickettsia* spp. (Rizzoli et al., 2014). *Bartonella* spp. were the most prevalent pathogens detected in wild small mammals, especially in *A. flavicollis*, *C. glareolus*, and *M. musculus*.

In European rodents, four species of the genus *Bartonella*, namely *B. grahamii*, *B. taylori*, *B. birtlesii*, and *B. rochalimae* are widespread, with a prevalence range of 3.3–65.8% (Gutiérrez et al., 2015; Szewczyk et al., 2021; Krügel et al., 2022). Seven species, the yellow-necked mouse (*A. flavicollis*), wood mouse (*A. sylvaticus*), striped field mouse (*A. agrarius*), bank vole (*C. glareolus*), common vole (*Microtis arvalis*), field vole (*Microtus agrestis*), and root vole (*Microtus oeconomus*) contribute significantly to the maintenance and spread of *Bartonella* spp. infections (Buffet et al., 2013; Gutiérrez et al., 2015).

The overall prevalence of *Bartonella* spp. in small mammals included in our study (57.6%; 90/156) aligns with results previously published from other parts of Slovakia (Špitalská et al., 2017). Exceptionally high prevalence was observed in *A. agrarius* (100%) and *A. flavicollis* (70%). The ecological niche, behavior, or immune system traits probably make these species particularly susceptible to *Bartonella* spp. infection. The most common species of *Bartonella* detected in our study were *B. grahamii* and *Bartonella* sp. Špitálska et al. (2017) investigated the prevalence and diversity of *Bartonella* spp. in the spleens of small mammals of six species (*A. sylvaticus*, *C. glareolus*, *M. arvalis*, *M. subterraneus*, *M. minutus,* and *Talpa europaea*) from different habitats in south-western and central Slovakia and identified four different *Bartonella* spp. clusters, including *B. taylorii*, *B. rochalimae*, *B. elizabethae*, and *B. grahamii*, as well as an unidentified *Bartonella* sp. (wbs11) with the highest total prevalence of 69% in *C. glareolus* and the lowest in *M. arvalis* (61%). Kraljik et al. (2016) detected *Bartonella* genotypes in 9% of examined striped field mice (*A. agrarius*) and identified five clades of bacteria: *B. grahamii*, *B. taylorii*, *B. birtlesii*, *B. clarridgeiae/B. rochalimae*, and *B. elizabethae/B. tribocorum.*

Bartonella grahamii is highly prevalent in small mammals in Slovakia, with the overall prevalence range of 56–73.8%, depending on habitat type. This bacterium was identified in several rodent species, including *A. flavicollis* (prevalence of 63%), *C. glareolus* (prevalence of 69%), and *M. arvalis* (prevalence of 61.1%) (Špitálská et al., 2017). In our study, the genotyping confirmed the presence of *B. grahamii* in *C. glareolus* and *Bartonella* sp. showing BLAST similarity with *B. rochalimae* (92.0%/99% query coverage; e.g. GenBank: MK780191) and/or *B. coopersplainsensis* (99.41%/99 query coverage; e.g. GenBank: KT355809, MF765616 and MF765638) in house mice (*M. musculus*). This synanthropic species represents a significant reservoir of *Bartonella* spp., posing a public health risk, especially considering its close association with human habitats and the surrounding environment.

Various authors have repeatedly published on the significant role of rodents as reservoir hosts of spirochetes (e.g. Gern et al., 1998; Humair et al., 1999). The prevalence of *Borrelia* spp., specifically *Borrelia burgdorferi* (*sensu lato*), in rodent populations across Europe varies depending on the region, habitat, rodent species, season, and tick density. Studies have reported varying infection rates in rodent populations, commonly reaching up to 60% (Nyman, 2017; Kalmár et al., 2019; Paulauskas et al., 2022). In our study, the overall prevalence of *B. burgdorferi* (*s.l.*) in tested rodents reached 11.5% (18/156). A single species, *B. afzelii*, was confirmed in all PCR-positive DNA samples from three species of the genera *Apodemus* (*A. flavicollis* and *A. agrarius*) and *Clethrionomys* (*C. glareolus*). Hanincová et al. (2003) recorded the presence of *B. afzelii* in 18% and 29%, of tested *C. glareolus* and *A. flavicollis* trapped in lowland areas of western Slovakia, respectively. In the study of Štefančíková et al. (2008), the prevalence of *Borrelia* spp. in *Apodemus* spp. trapped in the eastern and north-eastern parts of the country reached 4.5% (5/110). The results reported by Hamšíková et al. (2016) are similar to the data from our study. These authors recorded an overall prevalence of 11.9% (72/605) in rodents from sampling sites in south-western Slovakia, with *M. arvalis*, *C. glareolus*, and *Apodemus* spp. being predominantly infected; *Borrelia afzelii* prevailed (72.2%; 52/72) among *Borrelia* genospecies in tested rodents.

Babesia microti is the most studied species in rodents, particularly prevalent in European vole populations such as *Microtus* (Siński et al., 2006; Hong et al., 2014; Blaňarová et al., 2016; Galfsky et al., 2019; Azagi et al., 2022). The zoonotic "Jena/Germany" *B. microti* strain is the predominant genotype in rodent reservoirs in some regions, underscoring the importance of rodents in human babesiosis epidemiology in Europe. Our study revealed an overall prevalence of 3.2% (5/156) of *Babesia* spp. in small mammals. Hamšíková et al. (2016) detected *B. microti* in 1.3–4.2% of 606 rodents examined in south-western Slovakia, with positive samples primarily from *A. flavicollis* (47.1%) *M. arvalis* (47.1%), and *M. glareolus* (5.8 %). Blaňarová et al. (2016) confirmed *B. microti* presence in rodent ear (0.6%) and spleen biopsies (1.9%) in rodent embryos (3.8%) as well as in feeding larval (5.2%) and nymphal (8.7%) *Ixodes ricinus* ticks. The prevalence of piroplasms in questing nymphal and adult *I. ricinus* ticks ranged from 0.3 to 0.5%. In our study *B. microti* was found in *A. flavicollis*, *C. glareolus*, and *M. musculus*, with nucleotide sequences showing 100% identity and at least 99.2% similarity with over 100 homologous *B. microti* 18S rRNA gene sequences in GenBank. This indicates a high genetic consistency among *B. microti* strains and potential cross-species transmission between wild and synanthropic rodents. Several experimental studies indicated some resistance of the house mouse (*M. musculus*) to *B. microti* infection and these mice are not natural reservoirs for the pathogen. Experimental infections in laboratory *M. musculus* strains are usually transient, suggesting inherent resistance mechanisms (Clark and Allison, 1974; Cox and Young, 1969; Barnard et al., 1993). Social factors such as group size, aggressive behavior, and stress-related hormonal changes also impact mice immune response and infection resistance (Du Preez et al., 2020). The risk of pathogen spillover increases in environments where wild and synanthropic rodents cohabitate, as wild rodents may introduce *B. microti* strains to synanthropic rodent populations, facilitating transmission to humans and animals. This pathogen exchange

between different rodent species, particularly through vectors like ticks or fleas, poses significant public health risks, especially in protected areas like the High Tatra National Park, where wildlife and human activities overlap. In our study, *Babesia* sp. closely related to *B. canis*, and strains found in wild ungulates were identified in *A. flavicollis* and *C. glareolus*.

Ixodes ricinus is the primary vector of zoonotic *Babesia* spp. in Europe (Hildebrandt et al., 2021). Zoonotic *B. microti* strains have been detected in questing *I. ricinus* ticks in Slovakia with typically low prevalence (1–2%) (Blaňarová et al., 2016), and specific data on the incidence or prevalence of human infections are lacking. To accurately assess the burden of human babesiosis caused by *B. microti* strains, more targeted epidemiological studies are essential. These should include research on protozoan presence in small mammal populations that act as carriers and reservoirs (Ebani et al., 2011; Kjelland et al., 2011; Jahfari et al., 2014; Kallio et al., 2014; Chastagner et al., 2016; Tołkacz et al., 2017; Tufts and Diuk-Wasser, 2018).

In Europe, the prevalence of *A. phagocytophilum* infection in rodents varies widely, with rates reaching up to 23% in some studies (Liz et al., 2000; Ebani et al., 2011; Kjelland et al., 2011; Jahfari et al., 2014; Kallio et al., 2014; Chastagner et al., 2016). Biotic and abiotic factors, such as host diversity and abundance, seasonal variations, and habitat type influence the prevalence. Susceptibility to *A. phagocytophilum* also differs markedly among rodent species. For instance, the bank vole (*C. glareolus*) and the yellow-necked mice (*A. flavicollis*) are recognized as key reservoirs of this pathogen. The prevalence and transmission dynamics of *A. phagocytophilum* are driven by the interactions between host species and environmental elements, including vector dynamics and habitat characteristics. In our study, the observed prevalence of *A. phagocytophilum* in tested rodents was low, with only three positive individuals (two *A. flavicollis* and one *C. glareolus*), corresponding to an overall prevalence of 1.9%. This aligns with previously reported data from other regions in Slovakia, which indicate prevalences between 0.5% and 2.2% (Spitalská et al., 2008; Štefančíková et al., 2008; Svitálková et al., 2015; Blaňarová et al., 2014; Víchová et al., 2014). Genetic variants of *A. phagocytophilum* have been detected in wildlife in Slovakia including rodents (Kazimírová et al., 2024). Notably, distinct genetic variants circulate between *I. ricinus* and wild ungulates and between endophilic *I. trianguliceps* ticks and small rodents (Blaňarová et al., 2014). While the "ruminant" strain appears non-pathogenic, the "rodent" strain is considered a potential risk to human health (Bown et al., 2009; Blaňarová et al., 2014; Jahfari et al., 2014; Kazimírová et al., 2024).

The results of our study confirm the presence of *A. phagocytophilum* in the High Tatra Mountains region, but the low prevalence observed suggests that small mammals may not serve as the primary reservoirs for bacteria in this area. Different genetic variants of the bacterium in rodents, particularly strains potentially pathogenic for humans, underscores the importance of continued monitoring and investigation to better understand the epidemiology of *A. phagocytophilum* in wildlife and assess its public health implications.

5. Conclusions

Most of the High Tatra Mountains area is a protected landscape zone, significantly limiting the possibilities for research on pathogens in animals due to legal restrictions. Nevertheless, this study provides evidence of the significant role of rodents as reservoirs of vector-borne pathogens in these protected areas. A high prevalence of bacterial vector-borne pathogens was confirmed in the studied animals, with *Bartonella* spp. being the most common. The results presented here are a rare opportunity to update data on the epidemiological situation and clarify the current role of small mammals in the circulation of disease agents within this protected territory. Further research, particularly on epidemiological studies, host-pathogen relationships, and transmission routes of the pathogens, is necessary to fill the current gaps in knowledge about vector-borne pathogens in both the protected area and Slovakia in general. These investigations would enhance our understanding of the ecology of these pathogens in the region and support a robust theoretical basis for developing measures to reduce public health risks from these infections.

CRediT authorship contribution statement

Bronislava Víchová: Conceptualization, Supervision, Writing – original draft, Investigation, Methodology. **Michal Stanko:** Writing – review & editing, Supervision, Methodology. **Martina Miterpáková:** Writing – review & editing, Project administration, Funding acquisition. **Zuzana Hurníková:** Methodology, Investigation, Writing – review & editing. **Yaroslav Syrota:** Methodology, Investigation, Writing – review & editing. **Patrícia Schmer-Jakšová:** Investigation, Methodology, Writing – review & editing. **Petronela Komorová:** Investigation, Methodology, Writing – review & editing. **Lucia Vargová:** Investigation, Methodology, Writing – review & editing. **Veronika Blažeková:** Investigation, Methodology, Writing – review & editing. **Dana Zubriková:** Investigation, Methodology, Writing – review & editing. **Klaudia Mária Švirlochová:** Methodology, Investigation, Writing – review & editing. **Gabriela Chovancová:** Methodology, Investigation, Writing – review & editing.

Ethical approval

All carcasses have been handled following the authorization by the Ministry of Environment of the Slovak Republic under permit No. 498/2018–6.3 and further transported to the Laboratory of Molecular Ecology of Vectors (Institute of Parasitology SAS).

Statement on the use of AI-assisted technologies

While preparing this article, the authors used Grammarly (https://www.grammarly.com/) to correct grammatical errors and improve readability. After using this tool, the authors reviewed and edited the content as needed. The authors take full responsibility for the content of the published article.

Data availability

The data supporting the conclusions of this article are included within the article. Representative sequences were deposited in the GenBank database under the accession numbers PQ429062 and PQ429063 (*Bartonella grahamii ssrA* gene), PQ408971 and PQ408970 (*Bartonella* sp. *ssrA* gene), PQ408972 and PQ420800 (*Anaplasma phagocytophilum p44/msp2* gene), PQ408973, PQ420929 and PQ420930 (*Borrelia afzelii* 5S-23S ITS), and PQ423045-PQ423049 (*Babesia microti* and *Babesia* sp. 18S rRNA gene).

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Declaration of competing 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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