

Molecular screening of vector-borne pathogens in European badgers (*Meles meles*) in Lithuania

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Abstract

Vector-borne pathogens are increasingly recognised in European wildlife, but little is known about their occurrence in European badgers (*Meles meles*), particularly in the Baltic region. Badgers may play a role in maintaining and transmitting pathogens relevant to animal and human health. This study aimed to investigate the presence and diversity of selected vector-borne pathogens in European badgers from Lithuania. Spleen samples from 24 badgers were screened for eight pathogens (*Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Borrelia* spp., *Hepatozoon* spp., *Mycoplasma* spp., *Neoehrlichia mikurensis*, and *Rickettsia* spp.) using PCR assays. Seventeen of 24 badgers (70.8%) were infected with at least one pathogen. *Babesia* spp. was the most prevalent, detected in 15 individuals (62.5%), followed by *Anaplasma phagocytophilum* (3/24; 12.5%) and *Mycoplasma* spp. (3/24; 12.5%). No evidence of infection with *Bartonella* spp., *Borrelia* spp., *Hepatozoon* spp., *Neoehrlichia mikurensis*, or *Rickettsia* spp. was found. Phylogenetic analysis confirmed that the detected sequences corresponded to *Babesia* sp. type A, *A. phagocytophilum* and *Mycoplasma* spp. This study provides the first molecular evidence of vector-borne pathogens in European badgers from Lithuania. The results suggest that badgers may contribute to the maintenance of multiple vector-borne pathogens in natural ecosystems.

Keywords: badgers; *Meles meles*; pathogens; vector-borne; Lithuania

Introduction

The European badger (*Meles meles*) is a medium-sized, opportunistic, omnivorous mammal widely distributed across Europe and western Asia (Kurek et al. 2022). This species is highly adaptable and can thrive in diverse habitats, including forests, mountainous regions, and urbanised areas (Graham et al. 2020). Despite ongoing urbanisation, European badger populations have increased in recent years, largely due to their ecological flexibility (Geiger et al. 2018, Szekeres et al. 2025).

European badgers are well known for constructing extensive burrow systems, called setts, which can be used not only by conspecifics but also by other carnivores, such as the red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) (Wodecka et al. 2016, Guardone et al. 2020, Tieri et al. 2024). Such burrow sharing and close spatial overlap may facilitate contact with a wide range of ectoparasites and the pathogens they transmit (Do Linh San 2007, Guardone et al. 2020). In addition, the omnivorous diet and foraging behaviour of badgers increase their exposure to various sources of infection, potentially positioning them as important hosts in the epidemiology of several zoonotic and wildlife diseases (Silk et al. 2017, Guardone et al. 2020).

Badgers have been found to carry a variety of pathogens, including bacteria, protozoa, and viruses, some of

which are of significant veterinary and public health concern (Battisti et al. 2020, Guardone et al. 2020, Lindhorst et al. 2024). Among bacterial agents, *Mycobacterium bovis*, the causative agent of bovine tuberculosis, is the most extensively studied due to its impact on livestock and potential zoonotic transmission (Blanco Vázquez et al. 2021, Tieri et al. 2024). Molecular studies have detected *Babesia* spp. and *Hepatozoon* spp., protozoan parasites transmitted mainly by ticks (Bartley et al. 2017, Guardone et al. 2020, Szekeres et al. 2025). *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato, agents responsible for granulocytic anaplasmosis and Lyme borreliosis, have been identified in badger blood and associated ticks (Gern and Sell 2009, Wodecka et al. 2016, Szewczyk et al. 2019). Other vector-borne pathogens, such as *Bartonella* spp. and *Rickettsia* spp., have also been detected in European badgers (Gerrikagoitia et al. 2012, Jurczyk et al. 2022).

In Lithuania, the European badger population has increased markedly over the past decades (Zoomuziejus 2025). However, despite its ecological relevance, this species remains poorly studied, and data on the occurrence of vector-borne pathogens are lacking. This study aimed to investigate the occurrence of vector-borne pathogens in European badgers from Lithuania. Understanding the presence and distribution of these pathogens in local badger

populations will contribute to a broader knowledge of their epidemiology in the Baltic region and help assess potential risks to domestic animals and humans.

Material and methods

Sample collection and DNA extraction

Spleen samples were collected from 24 European badgers that were either legally hunted or found road-killed in four districts of Lithuania (Klaipėda, Marijampolė, Telšiai and Šiauliai) in 2024. Genomic DNA was extracted from the spleen tissue using a Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's instructions. The purity and concentration of the extracted DNA were measured using a anoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Lithuania), and the DNA was diluted to a final concentration of 100 ng/μL. All DNA samples were stored at -20°C until further molecular analyses.

Pathogen DNA amplification

Screening for the presence of eight vector-borne pathogens (*Anaplasma* spp., *Babesia* spp., *Bartonella* spp., *Borrelia* spp., *Hepatozoon* spp., *Mycoplasma* spp., *Neoehrlichia mikurensis*, and *Rickettsia* spp.) was performed using different PCR assays.

Three multiplex real-time PCR reactions were applied: the first assay targeted *Anaplasma* spp. (*23S rRNA*), *Borrelia* spp. (*23S rRNA*), and *N. mikurensis* (*groEL*). The second multiplex assay was designed to detect *Babesia* species using two genetic targets, *gltA* and the internal transcribed spacer (ITS) region. The third multiplex real-time PCR assay simultaneously targeted *Bartonella* spp. (*ssrA*) and *Rickettsia* spp. (*gltA*). Detailed information on

primer and probe sequences, and target genes is provided in Table 1. Real-time PCR reactions were performed in a final volume of 15 μL, comprising 7.5 μL 2× Sensi Mix™ II Probe No-ROX (Bioline, England), 0.3 μL primer–probe mixtures (containing 1 pM of each primer and 0.5 pM of each probe), 6.2 μL nuclease-free water, and 1 μL of template DNA. Thermal cycling conditions included an initial denaturation step at 95°C for 10 min, followed by 45 amplification cycles consisting of denaturation at 95°C for 20 s, annealing at 60°C for 1 min, and extension at 72°C for 20 s. Samples were considered positive if reproducible amplification was obtained with threshold cycle (Ct) values below 38.

In addition, conventional PCR assays were applied for the detection of *Hepatozoon* spp. and *Mycoplasma* spp., targeting the *18S rRNA* and *16S rRNA* genes, respectively. *Hepatozoon* spp. DNA amplification was carried out using the primers HepF (ATACATGAGCAAATCTCAAC) and HepR (CTTATTATTCCATGCTGCAG) (Inokuma et al. 2002). PCR reactions were performed in a final volume of 20 μL containing 1× DreamTaq Green Master Mix (Thermo Fisher Scientific, Lithuania), primers at a final concentration of 0.5 μM each, nuclease-free water, and 2 μL of extracted DNA. Thermal cycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 53°C for 30 s, and extension at 72°C for 60 s, with a final extension at 72°C for 10 min. The expected amplicon size was approximately 625 bp.

Mycoplasma spp. amplification was performed with the primers 322s (GCCCATATTCCTACGGGAAGCAGCAGT) and 938as (CTCCACCACTTGTTTCAGGTCCCGTC), as previously described by Varanat et al. (2011). PCR reactions were carried out in a total volume

Table 1. Primers and target genes used for multiplex real-time PCR assays

Pathogen	Target gene	Primers	Sequence 5'→3'	Reference
<i>Anaplasma</i> spp.	<i>23S rRNA</i>	forward	TGACAGCGTACCTTTTGCAT	Dahmani et al. 2015, Dahmani et al. 2018
		reverse probe	GTAACAGGTTCCGGTCTCCCA CTTGGTTTCGGGTCTAATCC	
<i>Babesia</i> spp.	<i>18S rRNA</i>	forward	CAGCTTGACGGTAGGGTATTGG	Radzijeuskaja et al. 2008
		reverse probe	TCGAACCCTAATCCCCGTTA CGAGGCAGCAACGG	
	<i>ITS</i>	forward	CTCACACAACGATGAAGGACGCA	Azagi et al. 2021
		reverse probe	AACAGAGGCAGTGTGTACAATACATTGAGA GCA+GAATTTAG+CAAAT+CAACAGG	
<i>Bartonella</i> spp.	<i>ssrA</i>	forward	AGTTGCAAATGACAACTATGCCG	Mardosaitė-Busaitienė et al. 2019
reverse probe	AAGGCTTCTGTTGCCAGGYG ACCCCGCTTAAACCTGCGACGGTT			
<i>Borrelia</i> spp.	<i>23S rRNA</i>	forward	GCTTCAGCCTGGCCATAAATAG	Sakalauskas et al. 2019
		reverse	AGCGAGTCTTAAAAGGGCGATTTAGT	
		probe	TCACTCGGSTTCGGGTCTACCACATCT	
<i>Neoehrlichia mikurensis</i>	<i>groEL</i>	forward	GCAAATGGAGATAAAAACATAGGTAGTAAA	Jenkins et al. 2019
		reverse probe	CATACCGTCAGTTTTTCAACTTCTAA TTACAGTTGAGGAAAGTAAGGGA	
<i>Rickettsia</i> spp.	<i>gltA</i>	forward	TGCMGAYCATGAGCACAATGCTTC	Sakalauskas et al. 2019
		reverse	CCCAAAGTGAKGCAATACCCGT	
		probe	TGCCGGCTCATCYGGAGCTAACCC	

Table 2. Primers and PCR protocols used for nested PCR assays

Pathogen	Target gene	Primer sequence 5'→3'	PCR conditions	Reference
<i>Anaplasma</i> spp.	<i>groEL</i>	HS1: AITGGGCTGGTAITGAAAT	PCR1: 3× (94°C 1 min, 48°C 2 min, 70°C 1 min 30 s), then 37× (88°C 1 min, 52°C 2 min, 70°C 1 min 30 s), 68°C 5 min PCR2: 3× (94°C 1 min, 48°C 2 min, 70°C 1 min 30 s), then 37× (88°C 1 min, 55°C 2 min, 70°C 1 min 30 s), 68°C 5 min	Sumner et al. 1997, Liz et al. 2002
		HS6a: CCICCGGIACIAIACCTTC		
		HS43: ATWGCWAARGAAGCATAGTC		
		HSVR: CTCAACAGCAGCTCTAGTAGC		
<i>Babesia</i> spp.	<i>18S rRNA</i>	BS1: GACGGTAGGGTATTGGCCT	PCR1: 94°C 3 min, 35× (94°C 60 s, 58°C 60 s, 72°C 90 s), 72°C 3 min PCR2: 94°C 3 min, 35× (94°C 60 s, 64°C 60 s, 72°C 90 s), 72°C 3 min	Armstrong et al. 1998
		BS2: ATTCACCGGATCACTCGATC		
		PiroA: AATACCCAATCCTGACACAGGG		
		PiroC: CCAACAAAATAGAACCAAAGTCCTAC		

of 25 µL containing 5× MyTaq reaction buffer (Bioline Reagents Ltd., London, UK), MyTaq DNA polymerase (1 U), primers at a final concentration of 1 µM each, nuclease-free water, and 2 µL of extracted DNA. The thermal cycling protocol included an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 20 s, annealing at 68°C for 25 s, and extension at 72°C for 30 s, with a final extension step at 72°C for 3 min. The expected amplicon size was approximately 600 bp.

Samples that tested positive for *Anaplasma* spp. and *Babesia* spp. in the initial screening were subsequently subjected to nested PCR (Table 2). For *Anaplasma* spp., the *groEL* gene was amplified in two successive rounds using the primer pair HS1a/HS6a and HS43/HSVR; the resulting amplicon was approximately 1300 bp. Reactions were carried out in a final volume of 25 µL containing 1× DreamTaq Green Master Mix (Thermo Fisher Scientific, Vilnius, Lithuania), primers at a final concentration of 0.4 µM each, nuclease-free water, and 2 µL template DNA. For primary amplification, 2 µL of extracted DNA was used, whereas 1 µL of the first-round PCR product served as a template in the second round.

For *Babesia* spp., a fragment of the *18S rRNA* gene was amplified using the primer sets BS1/BS2 and PiroA/PiroC in the first and second amplification rounds, respectively. PCR reactions were performed in a final volume of 20 µL using 1× MyTaq Mix (Bioline, USA), primers at a final concentration of 0.5 µM each, nuclease-free water, 2U DNA polymerase, and 2 µL of template DNA for the first round, or 2 µL of the first-round PCR product for the second amplification.

Positive and negative controls were included in each PCR run. Nuclease-free water was used as a negative control, while DNA of the respective pathogen, previously confirmed by sequencing, served as a positive control.

Pathogen DNA sequencing

PCR products obtained by conventional and nested PCR assays from the positive samples were subsequently sequenced. PCR products were separated on a 1.5% agarose gel stained with ethidium bromide. All positive

samples were purified from agarose gel using the GenJet PCR purification kit (ThermoFisher Scientific, Vilnius, Lithuania) and sent to Macrogen (Amsterdam, The Netherlands) for sequencing.

Data analysis

The obtained DNA sequences were edited using MEGA X software, (Kumar et al. 2018) and aligned with available sequences in GenBank using BLASTn programme. Phylogenetic trees were constructed using the Maximum Likelihood method and bootstrap analysis with 1000 replicates. Representative sequences obtained in this study were submitted to the GenBank database under the accession numbers PX210510 for the *18S rRNA* of *Babesia* sp., PX216856 for the *groEL* gene of *Anaplasma phagocytophilum*, and PX227288 for the *16S rRNA* of *Mycoplasma* sp.

Results

Prevalence

Of the 24 European badgers examined, 17 (70.8%) were infected with at least one of the screened pathogens. *Babesia* spp. (15/24; 62.5%) infection was the most prevalent, followed by *Anaplasma* spp. (3/24; 12.5%) and *Mycoplasma* spp. (3/24; 12.5%). *Babesia* spp.-positive individuals originated from the Telšiai (9/9), Šiauliai (3/5) and Klaipėda (3/5) districts, while no *Babesia* spp. infections were recorded in Marijampolė (0/5). *Anaplasma* spp. and *Mycoplasma* spp. infections were detected exclusively in badgers collected in the Telšiai district (in both cases, 3 individuals out of 9). Co-infections with two pathogens were observed in four individuals: two were infected with *Babesia* spp. and *Anaplasma* spp., and two with *Babesia* spp. and *Mycoplasma* spp. All co-infections were detected in badgers collected in Telšiai. *Bartonella* spp. or *Hepatozoon* spp. were not detected in any of the animals examined.

Sequence analysis

Babesia spp.

All *Babesia*-positive samples were successfully sequenced. The 15 partial *18S rRNA* gene sequences obtained in this study were identical to each other and showed 100% identity with *Babesia* sp. type A sequences previously reported from badgers in France (PP621229), Spain (PP979585 and MW578973), Italy (MK742771), the United Kingdom (KX528553) and China (MG799845) (Figure 1). A 100% match was also observed with sequences derived from a tick and red fox in Spain (MW829613 and

KT223484), as well as from a tick in the United Kingdom (OR730825).

Anaplasma spp.

All three *Anaplasma*-positive samples had identical *groEL* sequences, showing 100% identity with *A. phagocytophilum* sequences previously reported from humans in Poland (MG570466 and KF015601), horses in Sweden and Germany (AY529490 and AF482760), and wild boar and red fox in the Czech Republic (MT498616). In the phylogenetic tree, they grouped with high bootstrap support (98%) within the *A. phagocytophilum* cluster,

Figure 1. Phylogenetic tree of partial sequences of the *18S rRNA* gene of *Babesia* spp. Samples isolated from European badgers in this study are marked with “•”. The number of samples represented by the sequence is given in parentheses (n x)

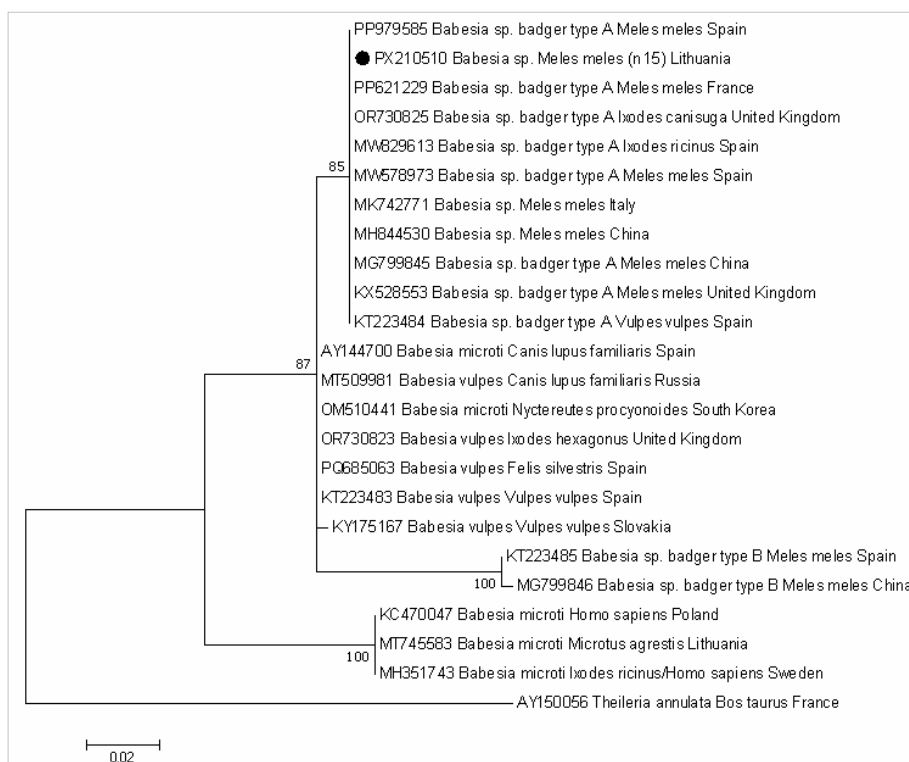
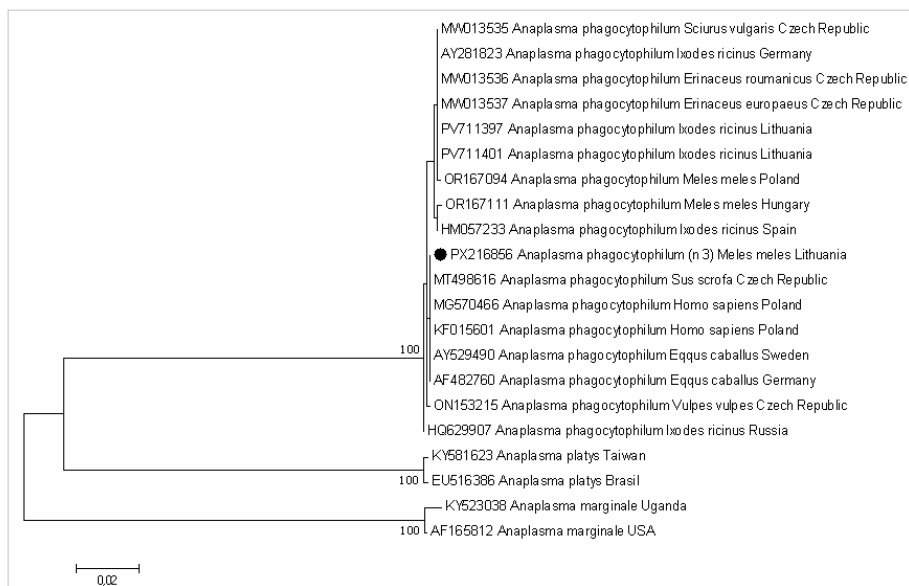


Figure 2. Phylogenetic tree of the *Anaplasma phagocytophilum groEL* gene

Samples isolated from European badgers in this study are marked with “•”. The number of samples represented by the sequence is given in parentheses (n x)



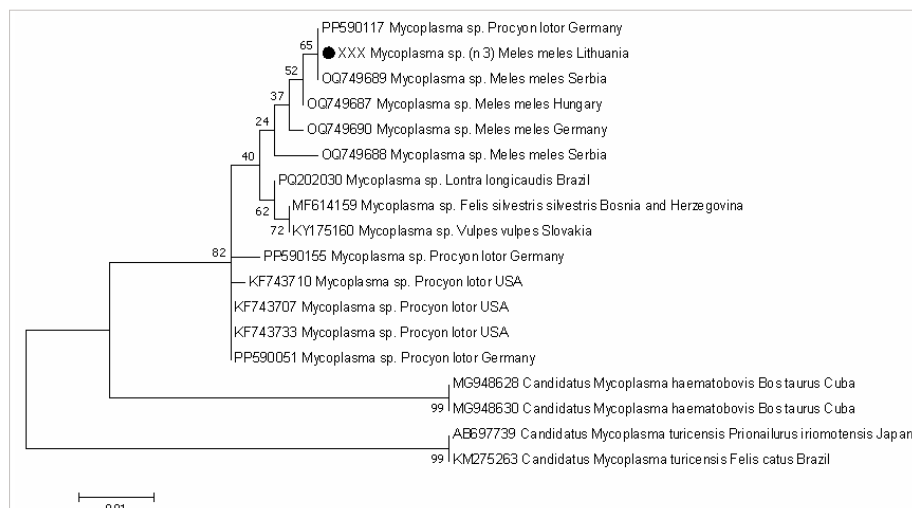


Figure 3. *Mycoplasma* spp. phylogenetic tree of the 16S rRNA gene

Samples isolated from European badgers in this study are marked with “•”. The number of samples represented by the sequence is given in parentheses (n x)

together with sequences previously reported from badgers in Poland (OR167094) and Hungary (OR167111), as well as from other wild and domestic hosts in Europe (Figure 2).

Mycoplasma spp.

All three *Mycoplasma*-positive samples had identical 16S rRNA sequences and clustered with *Mycoplasma* sp. strains previously detected in European badgers from Serbia (OQ749689), Hungary (OQ749687), and Germany (OQ749690), as well as in other carnivores such as raccoons (*Procyon lotor*) in Germany (PP590117) (Figure 3).

Discussion

This study provides the first molecular evidence of vector-borne pathogens in European badgers from Lithuania. Among the eight pathogens screened, only *Babesia* spp., *A. phagocytophilum*, and *Mycoplasma* spp. were detected. Co-infections of *Babesia* with *A. phagocytophilum* or *Mycoplasma* were also observed. These findings highlight the potential role of badgers as hosts for multiple vector-borne pathogens and contribute to the expanding knowledge of evidence on pathogen diversity in European wildlife.

Many infections (62.5%) detected in this study were caused by *Babesia* spp., making it the dominant pathogen in the examined European badgers. The same prevalence of *Babesia* spp. in badgers was reported in other European countries, suggesting that these parasites are widely distributed across the range of the species (Bartley et al. 2017, Santoro et al. 2019, Guardone et al. 2020, Lindhorst et al. 2024, Szekeres et al. 2025). The 18S rRNA sequences obtained in this study were identical to those of *Babesia* sp. type A. In addition, *Babesia* types B and C have been identified in badgers from several European countries (Bartley et al. 2017, Santoro et al. 2019, Lindhorst et al. 2024), indicating that a genetically conserved lineage circulates among badger populations across different geographical

regions. Infections with other *Babesia* genotypes have also been reported in previous studies (Bartley et al. 2017, Santoro et al. 2019). The high prevalence of *Babesia* spp. in badgers raises questions about their role as reservoir hosts. Although the pathogenicity of *Babesia* sp. type A in badgers remains unclear, related *Babesia* species are known to cause disease in domestic and wild carnivores, and cross-species transmission cannot be excluded (Paulauskas et al. 2014, Zygnier et al. 2021, Fayos et al. 2025). Therefore, the detection of a widely distributed lineage in Lithuanian badgers contributes to the understanding of the ecology of *Babesia* spp. in European wildlife and highlights the need for further research.

Anaplasma phagocytophilum is a well-recognised tick-borne pathogen with a broad host range, including wild and domestic mammals, and is the causative agent of human granulocytic anaplasmosis (Stuenkel et al. 2013, de la Fuente et al. 2016). In this study, *A. phagocytophilum* was detected less frequently (12.5%) compared with *Babesia* spp. Reports from other European countries indicate that badgers may serve as hosts for this bacterium (Szewczyk et al. 2019, Guardone et al. 2020, Lindhorst et al. 2024, Szekeres et al. 2025). The co-occurrence with *Babesia* spp. observed in this study may reflect overlapping transmission routes through shared tick vectors, particularly *Ixodes* spp., which are known to transmit both pathogens (Bown et al. 2008). Further research with larger sample sizes and vector investigations is needed to clarify the role of badgers in the epidemiology of *A. phagocytophilum*.

Mycoplasma spp. DNA was detected in several individuals in this study. Haematropic *Mycoplasma* species are increasingly recognised in wildlife, yet their taxonomy and pathogenic potential remain poorly understood (Millán et al. 2021). Previous studies have reported these bacteria in badgers from several European countries (Millán et al. 2018, Lindhorst et al. 2024, Tieri et al. 2024). In Lithuania, *Mycoplasma* spp. has previously been detected in domestic cats and their ectoparasites (fleas and ticks) (Razgūnaitė et

al. 2024). The detection of *Mycoplasma* spp. in Lithuanian badgers adds to the evidence of their wide distribution across European wildlife. However, further studies are needed to clarify species identity, host specificity, and possible health impacts in both wildlife and domestic animals.

In this study, no evidence of infection with *Bartonella* spp., *Borrelia* spp., *Hepatozoon* spp., *Neoehrlichia mikurensis*, or *Rickettsia* spp. was found in the examined badgers. However, these pathogens have previously been reported in Lithuania in a range of hosts and vectors, including wildlife, domestic animals, ticks, lice and fleas (Paulauskas et al. 2008, Lipatova et al. 2015, Radzijeuskaja et al. 2018, Baltrūnaitė et al. 2020, Aleksandravičienė et al. 2021, Razgūnaitė et al. 2021, Snegiriovaitė et al. 2025a, 2025b). In other European countries, these pathogens have occasionally been identified in badgers, for example, *Bartonella* spp. DNA was detected in badgers in Spain (Millán et al. 2021), whilst *Borrelia* spp. was reported in individuals from Switzerland and Poland (Gern and Sell 2009, Wodecka et al. 2016). Infection induced by *Hepatozoon* spp. was detected in badgers in Spain and Hungary (Ortuño et al. 2022, Szekeres et al. 2025), and *Neoehrlichia mikurensis* was recently detected in tissues of badgers from Central Europe (Hornok et al. 2017). In addition, *Rickettsia* spp. DNA was revealed in badgers in Poland (Jurczyk et al. 2022). These findings suggest that although our study did not reveal infections with these agents, badgers in other parts of Europe may serve as incidental or potential reservoir hosts, reflecting regional variation in pathogen circulation and vector distribution.

Conclusions

This study is the first report exhibiting a relatively high prevalence of vector-borne pathogens in European badgers in Lithuania. The detection of *Babesia* spp., *Anaplasma phagocytophilum*, and *Mycoplasma* spp., including cases of co-infection, demonstrates that badgers may play a role in the maintenance of multiple pathogens within natural ecosystems. Moreover, as badger populations can reach high local densities and frequently overlap with human and domestic animal habitats, their role in the epidemiology of vector-borne diseases deserves further attention. Further research is required to investigate other potential pathogens that European badgers may harbour, as their role in the epidemiology of vector-borne diseases may extend beyond the pathogens identified in this study. Larger sample sizes and integrated analyses of vectors are needed to clarify the reservoir competence of badgers and to assess the potential risks of cross-species transmission to domestic animals and humans.

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